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INVESTIGATOR CERTIFICATION

FOR THE CLINICAL INVESTIGATION OF

NC NEXUS

I, Cynthia M. Powell, agree to participate as the Principal Investigator in the clinical investigation of the NC NEXUS study.

I have been provided a copy of the following Food and Drug Administration (FDA) regulations: 21 CFR Part 812, Investigational Device Exemptions; 21 CFR Part 50, Protection of Human Subjects; and 21 CFR Part 54, Financial Disclosure by Clinical Investigators.

I agree and/or certify that:

1) *All investigators who will participate in the investigation have signed the enclosed Investigator Agreement*

2) The list of investigators includes all the investigators participating in the investigation

3) No investigator will be added to the investigation until they have signed the agreement.

SIGNATURE OF PRINCIPAL INVESTIGATOR

As the Principal Investigator of this research, I have read the foregoing and agree to be bound by its terms.

Cynthia M. Powell
Signature of Principal Investigator

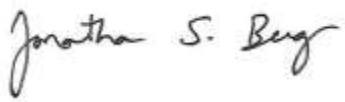
11/4/2015
Date

CERTIFICATION OF ALL PARTICIPATING INVESTIGATORS

PHYSICIAN CO-INVESTIGATORS (i.e., physicians participating as co- or sub-investigators on this clinical investigation under supervision of the Principal Investigator): A current CV or statement of relevant experience and a completed Certification of Financial Interest form and, if applicable, Financial Interest Disclosure form is required to be submitted to the sponsor (sponsor-investigator) for each physician co-investigator listed below.

As a physician co-investigator for this investigation, I have read the foregoing and agree to be bound by its terms.

Jonathan S. Berg
Name of Physician Co-Investigator (please print or type)



_____ __11/13/15___
Signature Date

Bradford Powell
Name of Physician Co-Investigator (please print or type)

_____ _____11/13/15_____
Signature Date

Karen E. Weck
Name of Physician Co-Investigator (please print or type)

_____ _____11/13/15_____
Signature Date

Kirk C. Wilhelmsen
Name of Physician Co-Investigator (please print or type)

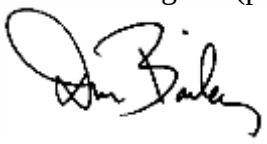
11/13/15

Signature Date

NON-PHYSICIAN CO-INVESTIGATORS (i.e., non-physicians participating as co- or sub-investigators on this clinical investigation under supervision of the Principal Investigator): A current CV or statement of relevant experience and a completed Certification of Financial Interest form and, if applicable, a Financial Interest Disclosure form is required to be submitted to the sponsor (sponsor-investigator) for each non-physician co-investigator listed below.

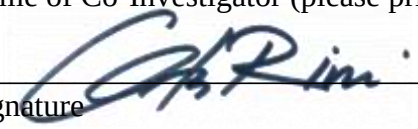
As a non-physician co-investigator for this investigation, I have read the foregoing and agree to be bound by its applicable terms.

Don B. Bailey
Name of Co-Investigator (please print or type)



Signature 11/3/15
Date

Christine M. Rini
Name of Co-Investigator (please print or type)



Signature 11/3/15
Date

Myra I. Roche
Name of Co-Investigator (please print or type)

_____ 11/3/15____
Signature Date

Form Approved: OMB No. 0910-0616. Expiration Date: 2/28/2018. See PRA Statement on page 2.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Certification of Compliance

Under 42 U.S.C. § 282(j)(5)(B), with Requirements of ClinicalTrials.gov Data Bank (42 U.S.C. § 282(j))

(For submission with an application/submission, including amendments, supplements, and resubmissions, under §§ 505, 515, 520(m), or 510(k) of the Federal Food, Drug, and Cosmetic Act or § 351 of the Public Health Service Act.)

SPONSOR / APPLICANT / SUBMITTER INFORMATION

1. Name of Sponsor/Applicant/Submitter
Cynthia M. Powell, MD

2. Date of the Application/Submission Which This Certification Accompanies

3. Address

Address 1 *(Street address, P.O. box, company name c/o)*
CB# 7487 Medical School Wing E, Room 117

Address 2 *(Apartment, suite, unit, building, floor, etc.)*
The University of North Carolina at Chapel Hill

City
Chapel Hill

State/Province/Region
NC

Country
United States of America

ZIP or Postal Code
27713

4. Telephone and Fax Numbers *(Include country code if applicable and area code)*

(Tel): 919-966-4202

(Fax): 919-966-3025

PRODUCT INFORMATION

5. **For Drugs/Biologics:** Include Any/All Available Established, Proprietary and/or Chemical/Biochemical/Blood/Cellular/Gene Therapy Product Name(s).
For Devices: Include Any/All Common or Usual Name(s), Classification, Trade or Proprietary or Model Name(s) and/or Model Number(s)

NC NEXUS: North Carolina Newborn Exome Sequencing for Universal Screening

Continuation Page for #5

APPLICATION / SUBMISSION INFORMATION

6. Type of Application/Submission Which This Certification Accompanies

☐ IND ☐ NDA ☐ ANDA ☐ BLA ☐ PMA ☐ HDE ☐ 510(k) ☐ PDP ☒ Other

7. Include IND/NDA/ANDA/BLA/PMA/HDE/510(k)/PDP/ Other Number *(If number previously assigned)*
000000

If BLA was selected in item 6, provide Supplement Number

8. Serial Number Assigned to Application/Submission Which This Certification Accompanies
00000

CERTIFICATION STATEMENT / INFORMATION

9. Check only one of the following boxes *(See instructions for additional information and explanation)*

☐ A. I certify that the requirements of 42 U.S.C. § 282(j), Section 402(j) of the Public Health Service Act do not apply because the application/submission which this certification accompanies does not reference any clinical trial.

☒ B. I certify that the requirements of 42 U.S.C. § 282(j), Section 402(j) of the Public Health Service Act do not apply to any clinical trial referenced in the application/submission which this certification accompanies.

☐ C. I certify that the requirements of 42 U.S.C. § 282(j), Section 402(j) of the Public Health Service Act apply to one or more of the clinical trials referenced in the application/submission which this certification accompanies and that those requirements have been met.

Certification Statement / Information section continued on page 2

CERTIFICATION STATEMENT / INFORMATION *(Continued)*

10. If you checked box C, in number 9, provide the National Clinical Trial (NCT) Number(s) for any "applicable clinical trial(s)," under 42 U.S.C. § 282(J)(1)(a)(i), section 402(j)(1)(a)(i) of the Public Health Service Act, referenced in the application/ submission which this Certification accompanies. *(Add continuation page as necessary.)*

NCT Number(s): _____ _____ _____ _____ _____

Continuation Page for #10

The undersigned declares, to the best of her/his knowledge, that this is an accurate, true, and complete submission of information. I understand that the failure to submit the certification required by 42 U.S.C. § 282(j)(5)(B), section 402(j)(5)(B) of the Public Health Service Act, and the knowing submission of a false certification under such section are prohibited acts under 21 U.S.C. § 331, section 301 of the Federal Food, Drug, and Cosmetic Act.

Warning: A willfully and knowingly false statement is a criminal offense, U.S. Code, title 18, section 1001.

11. Name and Title of the Person who Signs Number 15

Name	Title
Cynthia M. Powell, MD	Professor of Pediatrics and Genetics, Director, Med. Genetics Residency Program

12. Address

Address 1 *(Street address, P.O. box, company name c/o)* CB# 7487 Medical School Wing E, Room 117		
Address 2 *(Apartment, suite, unit, building, floor, etc.)* University of North Carolina at Chapel Hill		
City Chapel Hill	State/Province/Region NC	
Country United States of America		ZIP or Postal Code 27713

13. Telephone and Fax Numbers *(Include country code if applicable and area code)*

(Tel): 919-966-4202

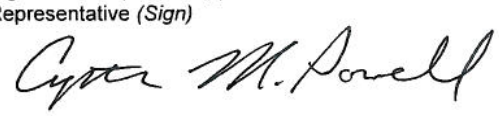
(Fax): 919-966-3025

14. Date of Certification

10/30/2015

15. Signature of Sponsor/Applicant/Submitter or an Authorized Representative *(Sign)*

Sign



This section applies only to requirements of the Paperwork Reduction Act of 1995.

*****DO NOT SEND YOUR COMPLETED FORM TO THE PRA STAFF EMAIL ADDRESS BELOW.*****

The burden time for this collection of information is estimated to average 15 minutes and 45 minutes (depending on the type of application/ submission) per response, including the time to review instructions, search existing data sources, gather and maintain the data needed and complete and review the collection of information. Send comments regarding this burden estimate or any other aspect of this information collection, including suggestions for reducing this burden to:

Department of Health and Human Services
Food and Drug Administration
Office of Chief Information Officer
Paperwork Reduction Act (PRA) Staff
PRAStaff@fda.hhs.gov

"An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB number."

November 20, 2015

Food and Drug Administration
Center for Devices and Radiological Health
Document Mail Center - WO66-G609
10903 New Hampshire Avenue
Silver Spring, Maryland 20993

Re.: Original IDE Application

Dear Madam/Sir:

Pursuant to 21 CFR 812, I am submitting an original, Sponsor-Investigator IDE application for NC NEXUS. Enclosed are one original paper copy and two e-copies for your review.

Device Information:

Device name:	NC NEXUS (North Carolina Newborn Exome Sequencing for Universal Screening)
IDE Number:	Q140207
Intended use of device:	The NC NEXUS study will evaluate the use of exome sequencing as a potential means to augment newborn screening (NBS). The main technical outcome will be to examine the sensitivity and specificity of this technology in detecting conditions that are currently screened for in newborns. Another technical outcome will be to examine the capacity of exome sequencing to detect other conditions that would be beneficial to identify at an early age in children but for which there is currently no available diagnostic method.
Sponsor-Investigator Contact Information:	Cynthia M. Powell, MD Department of Pediatrics University of North Carolina at Chapel Hill Chapel Hill, NC 27599-4202 Phone: 919-966-4202 Fax: 919-966-3025 Email:powellcm@med.unc.edu

Investigator Contact Information:	Jonathan S. Berg, MD, PhD Department of Genetics University of North Carolina Chapel Hill, NC 27599-4202 Phone: 919-966-7040 Fax: 919-843-0291 Email: jsberg@med.unc.edu
Regulatory Support Information:	Gregory J. Gatto, PhD Drug Development Regulatory Project Leader RTI International and The North Carolina Translational and Clinical Sciences (NC TraCS) Institute at UNC at Chapel Hill 3040 E Cornwallis Rd Research Triangle Park, NC 279909-2194 Phone: 919-541-5930 Fax: 919-485-5600 Email: ggatto@rti.org
Manufacturer Information:	Not Applicable
Additional Information:	Pre-submission Number: Q14027 Pre-submission IDE meetings and communications Date: May 02, 2014 Purpose: NC NEXUS teleconference with the FDA for clarification/feedback of study protocol Pre-submission number: Q140207 *Meeting minutes are provided in Attachment 1 of this letter* Date: July 02, 2014 Purpose: Pre-submission IDE review request Pre-submission number: Q140207/S001 FDA contact person: Kellie B. Kelm, PhD Date: July 14, 2014 Purpose: Addendum to the pre-submission IDE (Q140207/S001) Pre-submission number: Q140207/S002 FDA contact person: Sunita J. Shukla, MPH, PhD

Date: August 27, 2014
Purpose: FDA responses following review by the Office of In-Vitro Diagnostics and Radiological Health
Pre-submission number(s): Q140207/S001 and Q140207/S002
FDA Lead Reviewer: Sunita J. Shukla, MPH, Ph.D.
FDA responses are provided in Attachment 2 of this letter

Date: September 22, 2014
Purpose: Email from Jonathan Berg to Sunita Shukla with questions about the IDE and requesting guidance.
Pre-submission number: Q140207/S002
FDA contact person: Sunita J. Shukla, MPH, PhD

Date: December 2, 2014
Purpose: Email from Jonathan Berg to Sunita Shukla with questions about the IDE and requesting guidance.
Pre-submission number: Q140207/S002
FDA contact person: Sunita J. Shukla, MPH, PhD

Date: December 10, 2014
Purpose: FDA responses (in red) to the questions that UNC emailed on 12/2/14.
Pre-submission number: Q140207/S002
FDA contact person: Sunita J. Shukla, MPH, PhD
FDA responses are provided in Attachment 3 of this letter

Date: May 21, 2015
Purpose: FDA draft document review for NC NEXUS.
Pre-submission number: Q140207/S003
FDA contact person: Sunita J. Shukla, MPH, PhD
Communications are provide in Attachment 4 of this letter. Please note that the documents provided to the FDA for review are located

in the Appendix (Section 14) of the IDE application.

Waiver requests: No waivers are being requested

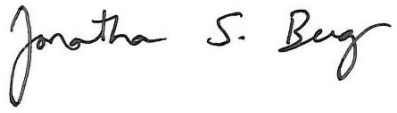
Referenced files: Not applicable

eCopy Statement: The eCopy is an exact duplicate of the paper copy

If you have any questions about the material included in this IDE, please do not hesitate to contact me as per above.

Please accept my thanks, in advance, for the FDA's review and consideration of this original IDE application.

Sincerely,



Cynthia M. Powell, MD

Jonathan S. Berg, MD, PhD

ATTACHMENT 1

Minutes of the meeting between NC NEXUS and FDA held on May 02, 2014

NC NEXUS teleconference with the FDA - Q140207 Presubmission (1-U19-HD077632-01)
May 2, 2014, 11am -12pm

Attendees: Sunita Shukla (Scientific Reviewer, FDA CDRH/OIR/DCTD), Cynthia Powell (PI, UNC), Jonathan Berg (PI, UNC), Laura Milko (Project Manager, UNC), Courtney Lias (Division Director, FDA CDRH/OIR), Denise Johnson-Lyles (Toxicology Branch Chief, FDA CDRH/OIR/DCTD), David Litwack (Personalized Medicine, FDA CDRH/OIR), Zivana Tezak (Personalized Medicine, FDA), Kelliey Kelm (Scientific Reviewer, FDA CDRH/OIR/DCTD), Tiina Urv (NIH/NICHD), Anastasia Wise (NIH/NHGRI), Jonathan Gitlin (NIH/NHGRI)

Agenda:

- Sign-in and introduction (11:00 – 11:05 am)
- Presentation by Dr. Jonathan Berg (11:05 – 11:25 am)
- Clarification of study protocol for FDA feedback (11:25 – 11:50 am)
- Summary of FDA's feedback and action items (11:50am – 12pm)

Minutes:

- Clarification of study protocol for FDA feedback
    - **FDA Question: How and what results would be returned to the healthy newborn cohort?**

      NC NEXUS Answer: A significant effort of this project is to identify a core set of genes (referred to as the NGS-NBS panel) for return to all study participants. We expect that very few (1-3%) of the healthy group would receive positive results from the NGS-NBS. All healthy newborns will receive standard of care newborn screening (NBS) results from the State of North Carolina as well. Some parents of children in the healthy newborn cohort (and affected cohort) will also be randomized to an "experimental" arm in which parents will make decisions about additional information (from three categories: childhood-onset non-medically actionable, adult-onset medically actionable, and carrier status) that they would want to learn.

    - **FDA Question: Is NC NEXUS only sequencing a subset of genes?**

      NC NEXUS Answer: No. The process would best be described as "exome sequencing with focused informatics analysis." We will sequence exomes based on commercially available reagents (we are currently using Agilent SureSelect Human All Exon version 5, but will explore other options based on coverage, cost, and ease of use) and perform focused informatics analysis on a subset panel (NGS-NBS) that all study participants will receive. For the diagnosed cohort, we will also return indication-related diagnostic results; this is the only case where we would return VUS.

    - **FDA Question: A.) What access will the study participants have to genetic counseling? B.) What information will study participants receive to help them to make informed decisions about receiving results and placing them in the medical record and will this information be separated out for childhood vs. adult onset diseases?**

NC NEXUS Answer: A.) Parents of all participants will receive standard of care genetic counseling by board certified (American Board of Medical Genetics (ABMG) or American Board of Genetic Counseling (ABGC)) genetic counselors and medical geneticists during decision-making and return of results. One of the PI's (Dr. Powell) in addition to being a board-certified clinical geneticist is also a board certified genetic counselor. Additional genetic counselors are part of our study team and will participate in return of results sessions with study participants. This discussion will include the risks, benefits, and limitations of exome sequencing with focused informatics analysis. For example, parents will be told about the Genetic Information Nondiscrimination Act (GINA) and the types of insurance (eg. life and long-term care) that are not covered. Parents will be given information about the likelihood of any given type of results and the follow-up plan that would be put in place if such a result was present. Finally, parents will be educated about the limitations of the process, including the nuances of what a "negative" result would mean.

B.) A "decision aid" will be provided to parents that clearly outlines the pros and cons of choosing to learn different types of results, and there will be ample opportunities for questions. A similar decision aid was developed and used previously by Dr. Powell in the context of a Fragile X study. The NC NEXUS decision aid will be shown to focus groups to confirm clarity and ease of understanding before being given to the parents of NC NEXUS participants. The decision aid and counseling sessions will be divided up by the categories of choices that may be available. First, the choice to participate in the NC NEXUS study and receive the NGS-NBS panel, and second, the additional three categories of information that parents may choose to learn if they are randomized into the experimental arm of the study (as described above). Information will be provided in the decision aid that describes the legal consequences of placing results in the electronic medical record. Since all results will be subject to confirmation, clinical interpretation, and reporting by ABMG-certified molecular geneticists in the hospital's CLIA lab, such results could be made part of the official medical record. We do not yet have a determination from the Institutional Review Board as to whether parents will be required to sign an additional consent in order to place positive results in the medical record, or whether the consent to participate in the study will be sufficient.

- **FDA Question: Will parents of participants who choose only to get pathogenic results be able to get more information (e.g. a file of the full dataset of variants)?**

  NC NEXUS Answer: No. We do not have any plans to release complete variant datasets to parents. If parents disagree with the method of analysis they can elect not to participate in the study.

- **FDA Question: Will people understand that they may be getting back unanticipated results?**

NC NEXUS Answer: Yes, this will be clearly stated in the consent documents that parents of subjects will be given prior to enrollment in any part of the study. If they do not wish to receive any unanticipated results they will have the option of not participating in the study. In the group randomized to decide whether or not to learn additional results (beyond those related to their child's condition and/or those that are included in the NGS-NBS panel results) the decision aid tool that is being developed as part of the research study will enable them to learn more about these options and decide what, if any, additional results they wish to learn.

- **FDA Question: How will negative results be reported?**

  NC NEXUS Answer: We propose to return negative results to parents in the form of a "research report" that will summarize the test process, including coverage metrics, genes tested, aggregate information about the number of variants, and a disclaimer about the limitations of whole exome sequencing. The research report would not be placed in the medical record.

- **FDA Question: Is Cohort 2 already diagnosed with a medical condition?**

  NC NEXUS Answer: Yes, Cohort 2 will have been clinically diagnosed with a genetic disorder (eg. via biochemical assay or other clinical work-up). Parents will already be aware that their child is affected with a medical condition, even though the genetic etiology (specific gene mutations) may not be known. One important aspect of the NC NEXUS project is evaluating the performance of NGS-NBS in predicting and diagnosing the causal genetic factor underlying rare disorders as well as disorders with known genetic and environmentally-induced components (e.g. hearing loss).

- **FDA Question: How does Cohort 3 factor into the ELSI research about decision-making by parents?**

  NC NEXUS Answer: The healthy newborn cohort provides information about parental decision-making in a "real world" setting, as would be expected for pregnant couples who are at no increased risk for genetic disorders. The ELSI component of the NC NEXUS project is comprised of a series of questionnaires and surveys as part of a longitudinal study to look at hopes, expectations, and anxieties associated with making choices about the return of NGS-NBS results.

- **FDA Question: How are threshold cut-offs and actionability scores determined?**

  NC Nexus Answer: The actionability score will be based on an algorithm, which incorporates factors such as severity and knowledge of the disease and intervention. The actionability score along with age of onset will help to determine which bin the disease will be categorized in to. Along with informatics, an expert panel will also help set the threshold and decision making process. For example, Duchene's Muscular Dystrophy would be

categorized in the childhood onset non-medically actionable bin, however treatment can delay the onset of disease, thus this may be categorized in the NGS-NBS bin.

- Summary of FDA's feedback and action items

NC NEXUS provided an informative ~~satisfactory level of information to~~ presentation to help clarify some of the issues in the "Q140207 Memo to Sponsor – FINAL" that arose from the presubmission questions below. Further internal discussions within the FDA will be required before answers can be provided. NC NEXUS will send draft meeting minutes within two weeks of the teleconference. The FDA will request any additional information that is necessary, and conduct internal meetings to provide updated ~~answers~~ responses to the NC NEXUS presubmission questions. The FDA will respond in the context of the presentation and make specific suggestions about what, if any, aspect(s) of the proposed study might trigger a determination of significant risk. The FDA will also provide specific feedback about any changes, if necessary, that would ameliorate the need for an IDE and provide helpful information for future studies. NC NEXUS and the FDA will maintain open communication via email and teleconference(s), as needed, in a timely fashion. ~~The FDA expects to be able to get back to NC NEXUS fairly soon.~~ Some additional discussion points regarding the study and associated risk are noted below:

1. **What level of risk is involved in the proposed study?**
   - More specific details on the information/reports that will be returned to parents and placed in the medical file.
   - Examples of diagnostic methods besides Sanger sequencing that may be needed to confirm certain types of mutations, such as large deletions, that may be detected by WES were provided to the FDA. NC Nexus stated that the~~These~~ confirmatory tests will also be done in a CLIA-approved laboratory.

2. **Will our proposed study require an IDE?**
   a. Which results will be returned to parents?
   b. Which results will be placed in patient's electronic file and associated ethical implications regarding the children?
   - Other confirmatory methods besides Sanger
   - Examples of diseases in the distinct binning categories ~~(~~FDA requested a list of all diseases, if available, that will be included in the study).

3. **What modifications of the protocol are recommended by the FDA?**
   - More information on the proposed study, including ethical implications regarding how data generated in the study will be used is needed for the FDA to understand the risks of the study and the mitigations that could be put in place to address these risks. The addition of mitigations is an example of protocol modifications. ~~Modifications based on feedback about reporting negative results~~

4. **During the course of the study, what changes to the protocol or IRB would require additional review by the FDA?**

a. Changes to study (e.g. software) reviewed by both the PI and the IRB

ATTACHMENT 2

FDA responses following review by the Office of In-Vitro Diagnostics and Radiological Health of Q140207/S001 and Q140207/S002 dated August 27, 2014

DEPARTMENT OF HEALTH & HUMAN SERVICES **Public Health Service**

Food and Drug Administration
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FROM: Sunita Shukla, PhD
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FDA/CDRH/OIR/DCTD
Sunita.Shukla@fda.hhs.gov
301-796-6406

RE: Q140207/S001 and Q140207/S002
DEVICE: NC NEXUS
DATED: Q140207/S001: 7/02/14
Q140207/S002: 7/14/14
RECEIVED: Q140207/S001: 7/02/14
Q140207/S002: 7/14/14

DATE: August 27, 2014

Dear Dr. Milko,

Thank you for submitting the follow-up requested information for our review. The pre-submissions noted above seek FDA input regarding your clinical protocol.

This is an informal communication that represents the best judgment of the Office of In Vitro Diagnostics and Radiological Health staff and consultants who reviewed the protocols. It does

not constitute an advisory opinion and does not bind or otherwise obligate or commit the agency to the views expressed, as per 21 CFR 10.85(k). We have provided an evaluation of your proposed studies below.

Proposed Intended Use/Indications for Use (excerpted from the submission):

The NC NEXUS study will evaluate the use of exome sequencing as a potential means to augment newborn screening (NBS). The main technical outcome will be to examine the sensitivity and specificity of this technology in detecting conditions that are currently screened for in newborns. Another technical outcome will be to examine the capacity of exome sequencing to detect other conditions that would be beneficial to identify at an early age in children but for which there is currently no available diagnostic method.

Aim of Study (excerpted from the submission):

Carry out Whole Exome Sequencing (WES) from various target populations (see below) using DNA collected from buccal swabs (using Oragen Discover (OGR-250) sample collection kits. Key aim of study is how to divide the broad range of genomic variants into categories that will allow parents to make well-informed decisions about a) whether or not to pursue exome sequencing for their newborn; and b) what types of genomic information they are interested in learning. The study proposes a "binning" method of the results based on clinical validity and clinical actionability (see below) and the development of a standardized procedure for categorizing genomic loci into the binned categories (below). To assess the impact of non-medically actionable WES findings, parents will be randomized into 2 groups: control group (will receive all medically actionable results) and experimental group (will be asked to decide what, if any, of the non-medically actionable information they choose to learn about their child. A decision aid (see below) will be used to help parents make an informed decision about study participation and parental preference for return of results.

Binning Categories (excerpted from the submission):

- **Bin 1**: Findings that provide medically actionable incidental results, including conditions screened for in the current NBS context as well as other medical conditions that are not currently included in current NBS protocols. This category will represent the core results from NGS-NBS.
- **Bin 2**: Findings related to a childhood health condition with no specific medical intervention (non- medically actionable). (These findings, along with Bin R, will be returned to parents randomized to have the opportunity to learn them, if they request them after making an informed decision to do so)
  - Bin 2a: Findings selected by as likely to cause people very little distress
  - Bin 2b: Findings selected as likely to cause some people moderate distress
  - Bin 2c: Findings selected as likely to cause most people a considerable amount of stress
- **Bin R**: Findings about reproductive risks, likely to cause little to moderate stress
- **Bin X**: Findings related to untreatable adult-onset health conditions (not to be returned to parents)
- **Bin 3**: Findings that have no clear association with any genetic disorder (not to be returned to parents)

Decision Aid: You state that your study team has extensive experience in health communication, consent, health literacy, NBS and informed consent. Drawing on this experience the study team will develop and test an electronic Decision Aid tool that will explain the complexities of WES to parents and their options for return of results. The Decision Aid tool will be utilized by parents during the consent process and by those participating in a longitudinal study to investigate the acceptability of Next-Generation Sequencing (NGS)/WES for their children. The control group will be given access to a version of the online decision aid that provides information about sequencing and the NGS-NBS panel, and helps them decide whether they want to agree to sequencing. The experimental group will be given access to a version of the online Decision Aid that provides information about sequencing, the NGS-NBS panel, and the categories of additional information they can request to learn. Parents can opt to learn some, all, or none of the additional categories of information. More information regarding the target populations is shown in the table below:

Target Populations (excerpted from submission):

NC NEXUS Study Population and Recruitment Estimates				
	Cohorts	Estimated numbers of subjects available for recruitment		
		Current Patients (Age 0-5 years)	New cases* or births/yr	Total
Disorders currently detected through NBS	PKU	33	5-7	60
	MCADD	28	5-7	60
	CF confirmed	65	12-22	155
	CF with false positive NBS	N/A	130	500
	CRMS	10	1-3	20
	Hearing Loss	1800	200	2600
Disorders that currently cannot be detected by NBS	Other patients in Genetics & Metabolism Clinic, Neurology Clinic	20	5	50
	PCD	20	5	45
	Well Child	N/A	3500	5080

Disease cohorts are ascertained using current standard newborn screening methods

* New cases are identified in the newborn period and enrolled by 6 months of age

Confirmation of Results (excerpted from the submission):

Many of the variants, including rare variants, will be confirmed using Sanger sequencing. However, it is possible that WES may identify mutations for which clinical testing is currently available but for which Sanger sequencing is not ideal. If Sanger is not optimal, gold standard molecular diagnostics tests will be performed (for example, the Qiagen Pyromark MD (pyrosequencing) and Affymetrix GeneChip system (expression, copy number variation, etc) will be available). Clinical reports regarding any positive findings will be generated by the CLIA-based lab after confirmation through Sanger sequencing, which will then be provided to parents and placed in the electronic medical record. Research reports, describing the aggregate exome

sequencing results such as total number of variants identified in different categories (but no specific variant details), will be provided to all parents, but will NOT be placed in the electronic medical record.

Bioinformatics: You propose to develop and evaluate various bioinformatics approaches for the utilization in NGS-NBS. You also state that you will determine the types of variants that can reliably be detected using your current pipeline, and you will explore novel methods that promise to detect types of variants not readily detectable by current approaches to WES. Specifically, you propose to explore thresholds for selecting variants to be further analyzed in an effort to optimize the performance characteristics. In order to enhance the sensitivity of your approach, you will compare methods for calling single nucleotide variants and explore methods of detecting certain types of variants to determine those types that can or cannot be reliably detected. In order to enhance the specificity of your approach, you will investigate the application of a gene-specific mutational burden metric to help adjudicate and re-classify genetic variants (which may result in re-classification of bins for various incidental findings).

Revised Results: You state that over the course of time, association of more genes with diseases and the development of prevention or treatment will result in reassignment of loci and lead to changes in the interpretation of WES findings. When such reassignment occurs, parents will be re-contacted if the results they have received change during the period of the Project.

Specific questions for FDA:

UNC requests FDA feedback on the following questions:

1. *What level of risk is involved in the proposed study?*

- **FDA Response:** Based on the information provided, FDA has determined that your proposed clinical investigation is a Significant Risk device study and you will need to submit an IDE application for this investigation**.** This risk assessment is based on the following rationale:

  1) In your proposal (Q140207/S001), you have stated the following, "*The risk of parental anxiety due to return of unexpected incidental findings raises new and challenging human subjects issues. Further complicating the return of incidental findings is their heterogeneity with potential psychological and clinical impact on patients ranging from trivial to profound……How to handle the return of incidental findings is a central challenge to genomic medicine and will be particularly important in the use of WES and other forms of whole genome sequencing in children*." Thus, your study proposes to evaluate the risk associated with the return of incidental (investigational) findings (of varying degrees) to parents and the psychological impact this will have upon the parents and children over a given period of time. FDA agrees that this is an important study objective. In addition to the potential psychological risks, we also point out that there may also be physical and social risks to the children depending on what parents choose to do as a result of the research. The probability and magnitude of these risks cannot be quantified, especially in the cohort where children are not currently experiencing symptoms. Therefore, we cannot determine that these risks are non-significant.

As a mitigation for the risk, on page 155 you have stated that the binning strategy will "*allow for a systematic approach to parent education and informed consent as it relates to newborn screening…..Finally, the manner in which incidental findings are delivered (if parents so choose) will also be category-driven and risk-calibrated to protect them and their offspring from harm.*" Although the decision aid tools will take the nature of the incidental findings into account, given the aim of your study, the risks of sharing all types of incidental findings cannot be fully anticipated. For example, parents may view children as "sick" or especially vulnerable as a result of the research findings, even if the incidental results have no known medical significance. Such unforeseen or unpredictable consequences for patients may warrant an ongoing relationship between the parents, researchers, and child advocates.

2) You state that: *Many of the variants, including rare variants, will be confirmed using Sanger sequencing. However, it is possible that WES may identify mutations for which clinical testing is currently available but for which Sanger sequencing is not ideal. If Sanger is not optimal, gold standard molecular diagnostics tests will be performed (for example, the Qiagen Pyromark MD (pyrosequencing) and Affymetrix GeneChip system (expression, copy number variation, etc) will be available).* We acknowledge that you state that investigational test results will be confirmed; however you also state that test results will be revised over time based on evolving bioinformatics approaches that you will develop. In such cases, this re-categorization of information will result in changes in the interpretation of WES findings, and will lead to re-contacting of parents to notify them of these changes. Since the bioinformatics approaches involved in potentially revising investigational results will be developed, modified, and evaluated throughout the course of the study, the probability and magnitude of the risk of re-analysis of results cannot be defined. Therefore, we cannot determine that such risk is non-significant.

3) We also point out that, as a result of this research, detailed information will be available in the child's medical record that may have long-term effects that are difficult to predict. The Agency is aware of circumstances where genetic information obtained in research has affected insurability, has been discoverable in legal proceedings or has otherwise been used against the research participant or a member of his/her family. We are particularly concerned because this information will be obtained about children who cannot consent or refuse for themselves. This risk is significant, and may not be mitigable.

2. *Will our proposed study require an IDE?*

- **FDA Response:** As outlined in our response to #1 above, the proposed study will require an IDE.

3. *What modifications of the protocol are recommended by the FDA?*

- **FDA Response:** We would like to emphasize that we believe you have proposed a study that may answer some important questions in the evolving field of NBS. FDA herein offers to work with you as your study evolves in order to suggest ways of mitigating potential risks and to expedite the IDE process. For example, review of informed consent forms is a part of the IDE process. Thus, it may be helpful to provide this information (as a supplement to this pre-submission) to us in advance of your IDE submission so that we can provide any suggested modifications at that time.

The following modifications are also recommended at this time:

1. Please provide details in your IDE submission or as a supplement to your pre-submission that outline the duration of follow-up of parents and children after parents are informed of research results. In addition, please provide information about who will be providing this follow-up, how often, and how this follow-up may help to mitigate any medical or social risks that may occur as a result of the research. Information on what actions will be taken to maintain contact with parents, and procedures for parents who wish to drop out of the study, should also be provided. In particular, it may be helpful to have the binning committee (or another body of experts) suggest additional precautions or safeguards for oversight before and after parents have been informed of the investigational findings.

   *Please note that additional mitigations and safeguards may be recommended by FDA during the IDE process and/or as we continue to discuss your study proposal with you in any subsequent pre-submissions.

4. During the course of the study, what changes to the protocol or IRB would require additional review by the FDA?

- **FDA Response:** Due to the evolving nature of the study components (such as binning categories, informatics changes, etc), FDA will provide further guidance regarding which modifications would result in a need for an IDE supplement prior to proceeding. We can discuss this in more detail in our upcoming teleconference on 8/28/14 from 1-2 PM ET.

Note that any revisions that you would like to submit in response to this letter (after the meeting) or new protocols for FDA feedback (called a pre-submission supplement) should be submitted as an eCopy[1] to the address below and should reference the pre-submission number above (Q140207) in the cover letter to facilitate processing.

U.S. Food and Drug Administration
Center for Devices and Radiological Health
Document Control Center – WO66-G609
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002

If you have any questions or comments regarding this review, please contact Sunita Shukla, at (301) 796-6406 or at sunita.shukla@fda.hhs.gov

Sunita J. Shukla -S
2014.08.26 16:05:46
-04'00'

Branch Concurrence:
Toxicology Branch Chief

Denise Johnson-lyles -S

ATTACHMENT 3
Communications regarding FDA responses to the questions that UNC emailed on 12/2/14

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Dear Dr. Berg,

Please find attached FDA responses (in red) to the questions that you emailed on 12/2/14. Please let me know if you have any further questions. Thank you, Sunita

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From: Shukla, Sunita
Sent: Tuesday, December 02, 2014 3:52 PM
To: 'Berg, Jonathan'
Cc: Milko, Laura V.; Powell, Cynthia M.; Bailey, Don
Subject: RE: IDE questions

Dear Dr. Berg,

Thank you for your email and questions. I am going to review your questions and go over these with our review team. I will email you our feedback prior to 12/11/14. Thank you, Sunita

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From: Berg, Jonathan [mailto:jonathan_berg@med.unc.edu]
Sent: Tuesday, December 02, 2014 10:59 AM
To: Shukla, Sunita
Cc: Milko, Laura V.; Powell, Cynthia M.; Bailey, Don
Subject: IDE questions

Hi Sunita,

We have made some progress here at UNC in preparing our IDE, but we have lots of questions! We've had meetings with both the IRB and the regulatory specialist within the CTSA here at UNC, and really no one has much experience with this type of submission. We are planning a meeting with the regulatory specialists at RTI to see if they can provide any additional assistance.

For now, could you please have a look at the following questions and provide guidance?

1. Report of prior investigations:
What constitutes "prior investigations" if this is a new device specific to this research project?
- We have experience with preparation of the exome sequencing libraries through other ongoing projects but these projects are not directly related to NC NEXUS. ***How much detail do we need to provide about the library production process?***
- The location where sequencing will occur is a core facility at UNC and is not under our direct control. The core facility has generated large amounts of sequence data for other projects, including for example the TCGA. ***What level of detail do we need to provide about the core facility's capabilities and previous sequence output?***
- Our group has broad experience with psychosocial research in children and adults, as well as the development of educational materials, both of which will be part of the ELSI aspect of this project, but these prior projects do not directly relate to NC NEXUS. ***How much detail do we need to provide about previous psychosocial research?***

2. Labeling:
How shall we "label" our device, if the "device" includes the technical and psychosocial aspects of the project?
- From a technical standpoint we would describe the "next-generation sequencing newborn screening" platform as "exome sequencing with focused informatics analysis and Sanger confirmation of positive results." The FDA has determined that the decision aid that will be developed and the psychosocial research that will be conducted are also part of the "device" that is being evaluated. In that case, the "device" would be described as "informed parental decision-making aided by an electronic decision aid regarding their acceptance of next-generation sequencing newborn screening for their child, with follow-up surveys and questionnaires regarding the psychosocial impact of the screening." ***Can you provide us with guidance about how we would label this device, and what that "labeling" would entail? How do we provide "copies of all labeling for the device"?***

3. Manufacturing information:
What constitutes the "manufacture" of our "device"?
- As described above, our "device" does not naturally adhere to what is described in the regulatory guidance: "A description of the methods, facilities, and controls used for the manufacture,

processing, storage, and, where appropriate, installation of the device, in sufficient details so that a person generally familiar with good manufacturing practice can make a knowledgeable judgment about the quality control used in the manufacture of the device." ***If we are not manufacturing a product for any kind of distribution, how do we respond to this section of the IDE application?***

4. Investigational plan/protocol:
Is there a standard format for the protocol?
- The three examples that you sent us are very different in terms of structure and level of detail. Since we are currently working on the IRB submission for this project, we would like to avoid duplicating effort. ***Would it be reasonable to submit the IRB protocol, assuming that it covered the study design, patient selection, procedures, safety monitoring, and analysis plan? If so, can you please send us a list of subcategories from the main template that need to be covered for a "device" such as the NC NEXUS project?***

Thank you for your answers. We have a meeting next Thursday morning (12/11/14) to discuss the IDE application and would appreciate your responses by then.

Sincerely,
Jonathan

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FDA responses (in red) to the questions that UNC emailed on 12/2/14

1. Report of prior investigations:
What constitutes "prior investigations" if this is a new device specific to this research project?
If there have not been any prior investigations using your device (which would include laboratory/animal studies and reports of prior publications), you should state this in your IDE application.
- We have experience with preparation of the exome sequencing libraries through other ongoing projects but these projects are not directly related to NC NEXUS. ***How much detail do we need to provide about the library production process?*** Although your experience with the preparation of the exome sequencing libraries are not related directly to the NC NEXUS project, please provide relevant information regarding the preparation of the library that will be used for the current study. Relevant information would include: an SOP/written protocol describing the preparation of the exome sequencing library and its components and properties (such as reagents, stability, etc), instrumentation to be used, enrichment of exon targets, verification of library quality and other quality control steps that are performed during library preparation.
- The location where sequencing will occur is a core facility at UNC and is not under our direct control. The core facility has generated large amounts of sequence data for other projects, including for example the TCGA. ***What level of detail do we need to provide about the core facility's capabilities and previous sequence output?*** Please provide a description of the core facility where the sequencing will occur and its role. For example, please indicate the facility's role in the preparation of the sequencing libraries, evaluation of the quality of the libraries, sample extraction/storage/handling, sequence output, etc. Please note that if the sample extraction and library preparation are occurring outside of the core facility, please indicate where these will take place. You may include a brief description of the core facility's relevant prior experience with sequencing and other aspects that are similar to your study.

- Our group has broad experience with psychosocial research in children and adults, as well as the development of educational materials, both of which will be part of the ELSI aspect of this project, but these prior projects do not directly relate to NC NEXUS. ***How much detail do we need to provide about previous psychosocial research?*** Since the ELSI component of your study represents a unique aspect of the IDE application with regard to study risk, please include a brief description of the past relevant experience that will be applicable to the development of the ELSI component described in the proposed IDE study. Relevant information should also include past experience regarding follow-up, mitigation of risks and other safeguards related to the investigational findings.

2. Labeling:
<u>How shall we "label" our device, if the "device" includes the technical and psychosocial aspects of the project?</u>

- From a technical standpoint we would describe the "next-generation sequencing newborn screening" platform as "exome sequencing with focused informatics analysis and Sanger confirmation of positive results." The FDA has determined that the decision aid that will be developed and the psychosocial research that will be conducted are also part of the "device" that is being evaluated. In that case, the "device" would be described as "informed parental decision-making aided by an electronic decision aid regarding their acceptance of next-generation sequencing newborn screening for their child, with follow-up surveys and questionnaires regarding the psychosocial impact of the screening." ***Can you provide us with guidance about how we would label this device, and what that "labeling" would entail? How do we provide "copies of all labeling for the device"?*** Please note that for the purposes of the current IDE study, device labeling is not applicable. However, as provided in prior FDA feedback, please ensure that the relevant information regarding the device/study and ELSI components, for exam ple, are provided as part of the IDE (which will also include the study protocol and informed consent documents).

3. Manufacturing information:
<u>What constitutes the "manufacture" of our "device"?</u>

- As described above, our "device" does not naturally adhere to what is described in the regulatory guidance: "A description of the methods, facilities, and controls used for the manufacture, processing, storage, and, where appropriate, installation of the device, in sufficient details so that a person generally familiar with good manufacturing practice can make a knowledgeable judgment about the quality control used in the manufacture of the device." ***If we are not manufacturing a product for any kind of distribution, how do we respond to this section of the IDE application?*** Due to the nature of your device, this section will not be applicable to your IDE application.

4. Investigational plan/protocol:
<u>Is there a standard format for the protocol?</u>

- The three examples that you sent us are very different in terms of structure and level of detail. Since we are currently working on the IRB submission for this project, we would like to avoid duplicating effort. ***Would it be reasonable to submit the IRB protocol, assuming that it covered the study design, patient selection, procedures, safety monitoring, and analysis plan? If so, can you please send us a list of subcategories from the main template that needs to be covered for a "device" such as the NC NEXUS project?*** The information contained within the IRB protocol may be appropriate for the IDE application. Based on the above noted feedback and the example IDE content that was emailed to you on 9/22/14, it is acceptable to state “Not applicable” for

sections of the IDE application that do not apply to your device. The example below illustrates a few potential subsections regarding your device (please note that the subsections below are examples and may not be inclusive of other information you will include in your IDE, such as safety monitoring and ELSI). Please note that any additional information may be requested interactively during the review of your IDE.

3.2 Internal Validation of Performance
3.2.1 Accuracy
3.2.5 Potential Interfering Substances
3.2.6 Reagents and Stability.
3.2.8 Sample to Sample Carry-over
4.4 Description of Device
4.4.2 Instrument
4.4.3b Software
4.4.4 Data Analysis
4.4.5 Anticipated Changes
4.5 Monitoring Procedures .(QC)

ATTACHMENT 4
Communications regarding FDA draft review of NC NEXUS' documents provided to the FDA on May 21, 2015

****

From: Berg, Jonathan [mailto:jonathan_berg@med.unc.edu]
Sent: Wednesday, May 27, 2015 6:21 PM
To: Shukla, Sunita <Sunita.Shukla@fda.hhs.gov>; Milko, Laura V. <laura_milko@med.unc.edu>
Cc: Powell, Cynthia M. <powellcm@med.unc.edu>; Bailey, Don <dbailey@rti.org>
Subject: Re: FDA draft document review for NC NEXUS

Sunita,

Thank you for your input. We have been working with our regulatory groups on the IDE submission but do not have a target submission date yet. We will update you with our expected timeframe when it seems clearer.

-Jonathan

****

From: <Shukla>, Sunita <Sunita.Shukla@fda.hhs.gov>
Date: Wednesday, May 27, 2015 at 4:46 PM
To: "Berg, Jonathan" <jonathan_berg@med.unc.edu>, "Milko, Laura V." <laura_milko@med.unc.edu>
Cc: "Powell, Cynthia M." <powellcm@med.unc.edu>, "Bailey, Don" <dbailey@rti.org>
Subject: RE: FDA draft document review for NC NEXUS

Dear Dr. Berg,

Thank you for submitting the documents containing the decision aid information. Based on a cursory review of the documents, we have the following general questions/suggestions for your IDE:

1. Please provide a timeframe for when you will be submitting your IDE. This will allow for us to coordinate our efforts, workload, timelines and set up any necessary meetings with you prior to the receipt of the IDE (especially since we will only have a 30 day review clock for the IDE). This will also allow for us to provide any appropriate background for internal review team members prior to the receipt of the IDE.
2. Although we may not have specific comments on the documents you provided on 5/21/15, there may be additional comments once we have received the full IDE package. Please note that we will work interactively with you throughout the review of the IDE to work through any issues.
3. Although you have provided the online decision aid content in the 5/21/15 email, please note that it will be useful for you to provide the associated screenshots that will be

viewed by the study participants. This will help us evaluate the content, presentation of material, and other aspects that will be seen by the study participants.

4. As with the feedback that was provided to you during the review of Q140207 (and related Supplements), please ensure that you provide your plan/SOP for how you will address any triggers/changes to your analytical processes (such as those that would affect binning of results, etc) or ELSI components. Please also describe what risks such changes will be associated with and how these changes/risks will be conveyed to study participants.

We look forward to hearing back from you regarding your timeframe of your IDE submission. Please let me know if you have other questions. Thank you, Sunita

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From: Berg, Jonathan [mailto:jonathan_berg@med.unc.edu]
Sent: Tuesday, May 26, 2015 10:53 PM
To: Shukla, Sunita; Milko, Laura V.
Cc: Powell, Cynthia M.; Bailey, Don
Subject: Re: FDA draft document review for NC NEXUS

Hi Sunita,

The documents we sent are only for a preliminary review. The complete IDE will contain much more detail about our project.

We were under the impression that it would be helpful for you to see the consent forms and the decision aid content so that you could provide feedback before the full IDE is submitted. Was that incorrect?

Thanks,

-Jonathan

****

From: <Shukla>, Sunita <Sunita.Shukla@fda.hhs.gov>
Date: Tuesday, May 26, 2015 at 6:01 PM
To: "Milko, Laura V." <laura_milko@med.unc.edu>
Cc: "Powell, Cynthia M." <powellcm@med.unc.edu>, "Berg, Jonathan" <jonathan_berg@med.unc.edu>, "Bailey, Don" <dbailey@rti.org>
Subject: RE: FDA draft document review for NC NEXUS

Dear Ms. Milko,

We will be discussing your documents internally, however I wanted to check if the documents you emailed on 5/21/15 are part of the IDE submission (and there will be other documents describing the test, etc) or are the documents you emailed going to be the entirety of the IDE submission you plan on submitting? Thank you, Sunita

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From: Milko, Laura V. [mailto:laura_milko@med.unc.edu]
Sent: Thursday, May 21, 2015 7:32 AM
To: Shukla, Sunita
Cc: Powell, Cynthia M.; Berg, Jonathan; Bailey, Don
Subject: FDA draft document review for NC NEXUS

Dear Dr. Shukla,

Prior to finalizing the IDE application for the NC NEXUS study, we have several draft documents that we'd like to provide for informal review and feedback. These documents, once finalized, will be important for establishing the timeline, goals, and endpoints for the study, and we'd appreciate your suggestions for ways to mitigate potential risks and expedite the IDE process. Please find the following attached:

- Study flows for the 'well-child and 'diagnosed' cohorts. Abbreviations: T1 Q, T2 Q, and T3 Q refer to questionnaires that will be given at three different time points. NGS-NBS refers to the select group of conditions that we determine to be similar to current RUSP conditions; these are childhood onset medically actionable conditions, and positive findings will be confirmed and returned to all participants. RoR is a return of results encounter. AI refers to "additional information" that parents randomized to the decision arm will be asked to decide about whether to learn after viewing the

second part of the online decision aid; categories include adult onset medically actionable, childhood onset non-medically actionable, and carrier status.

- NC NEXUS Recruitment Decision Aid – This will be in the form of a brochure that is given to prospective parent participants prior to enrollment in the study.
- Decision Aid "shooting script" files - These documents show our working shooting scripts for the online decision aid, part 1 (whether to have their child undergo NGS-NBS) and part 2 (whether to learn additional information). It shows the content for each group (single parent versus couple) or for parents with a newborn versus child with a diagnosed condition. In addition the columns show the narration, the text that would appear on screen, any animation or data capture. There may be small changes to wording during the development process to accommodate suggested by the team programming the decision aid or by the NEXUS steering committee as the decision aid takes shape.

- Well-Child cohort information sheet to get verbal consent for parent(s) to participate in the study (Phase I)
- Diagnosed cohort information sheet to get verbal consent for parent(s) to participate in the study (Phase I)
- Well-Child cohort parental consent for their child to have genomic sequencing for Phase II
- Diagnosed cohort parental consent for their child to have genomic sequencing for Phase II

We look forward to hearing from you. Please contact us if you have any questions.

Best,
Laura

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ORIGINAL INVESTIGATIONAL DEVICE EXEMPTION APPLICATION

November 20, 2015

IDE APPLICATION TITLE: NCNEXUS (North Carolina Newborn Exome Sequencing for Universal Screening)

IDE NUMBER: Q140207

SPONSOR-INVESTIGATOR: Cynthia M. Powell, MD
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List of Abbreviations

Abbreviation	Definition
ABMG	American Board of Medical Genetics
ACA	Affordable Care Act
AHRQ	Agency for Healthcare Research and Quality
AI	Indication-based analysis
ALS	Amyotrophic lateral sclerosis
BAM	Binary form of Sequence Alignment/Map
BRCA	BReast CAncer
BSP	BioSpecimen Processing
CF	Cystic Fibrosis
CLIA	Clinical Laboratory Improvement Amendments
CRMS	CFTR-related syndrome
dbSNP	Single Nucleotide Polymorphism database
DA	Decision aid
DM	Disease mutation
DNA	Deoxyribonucleic acid
EC	Ethical Committee
EHR	Electronic health record
ELSI	Ethical, legal, and social implications
ePSS	Electronic Preventive Services Selector
ExAC	Exome Aggregation Consortium
FDA	Food and Drug Administration
FXS	Fragile X Syndrome
GATK	Genome Analysis Toolkit
HBOC	Hereditary breast and ovarian cancer syndrome
HGMD	Human Gene Mutation Database
HIPPA	Health Insurance Portability and Accountability Act
HTSF	High-Throughput Sequencing Facility
ID	Identification number
IDE	Investigational Device Exemption
IRB	Institutional Review Board
IT	Information technology
KP	Known pathogenic
LP	Likely pathogenic
MAF	Mutation Annotation Format
MCADD	Medium chain acylCoA dehydrogenase deficiency
MGL	Molecular Genetics Laboratory
MaPSeq	Massively Parallel Sequencing
NBS	Newborn screening
NCBI	National Center for Biotechnology Information

Abbreviation	Definition
NCNEXUS	North Carolina Newborn Exome Sequencing for Universal Screening
NC TraCS	The North Carolina Translational and Clinical Sciences Institute
NGS	Next generation sequencing
NHGRI	National Human Genome Research Institute
NHLBI	National Heart, Lung, and Blood Institute
NICHD	National Institute of Child Health and Human Development
NSIGHT	Newborn Sequencing In Genomic medicine and public HealTh
OMIM	Online Mendelian Inheritance in Man
PCR	Polymerase chain reaction
PHI	Protected health information
PKU	Phenylketonuria
Polyphen	Polymorphism Phenotyping
QC	Quality control
QA	Quality assurance
REDCap	Research Electronic Data Capture
RefSeq	Reference Sequence
RENCI	Renaissance Computing Institute
RNA	Ribonucleic acid
rRNA	Ribosomal RNA
RoR	Return of Results
RTI	RTI International
RUSP	Recommended Uniform Screening Panel
SMA	Sequence Alignment/Map
SNP	Simple nucleotide polymorphism
T1Q	Time 1 questionnaire
T2Q	Time 2 questionnaire
T3Q	Time 3 questionnaire
T4Q	Time 4 questionnaire
UTR	Untranslated region
WES	Whole exome sequencing
UNC	University of North Carolina
VCF	Variant Call Format
VUS	Variant of uncertain clinical significance

LIST OF FIGURES

LIST OF TABLES

1. NAME AND ADDRESS OF THE SPONSOR INVESTIGATOR

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2. REPORT OF PRIOR INVESTIGATIONS

2.1 General

There are no prior investigations or outcomes of the NC NEXUS device, laboratory/animal studies, or prior publications. However, investigators and other personnel have extensive experience with similar workflows through previous and ongoing projects that are not directly related to the NC NEXUS device. Per the Q140207 12 2014 UNC email – FINAL correspondence, we utilize our prior experience with the preparation, sequencing, and analysis of the exome libraries to describe in Sections 2.1.1 and 2.1.2 the workflows that will be used for NC NEXUS. We will utilize a robust infrastructure for pre-analytic specimen handling that is completely operational, highly flexible, and fully integrated with external databases and with other systems at UNC, including the BioSpecimen Processing (BSP) Facility, the High-Throughput Sequencing Facility (HTSF), and the UNC Hospitals CLIA-certified Molecular Genetics Laboratory (MGL).

In addition, investigators in the NC NEXUS project have successfully executed other projects that examine the psychosocial aspects of genomic technologies. Examples of these include a Fragile X Newborn Screening pilot study that included development of a Decision Aid and evaluation of distress levels in mothers of screen-positive infants, responses of patients and family members to diagnostic whole exome sequencing, and development of a website to educate potential research participants about screening for a select set of rare medically actionable conditions.

2.1.1 Laboratory methods

Upon enrollment, DNA will be collected non-invasively by swabbing the inside of subjects' mouths using Oragen OC-175 sample collection kits (DNA Genotek, Ontario, Canada) (ORAcollect for pediatrics (OC-175) instructions: see Appendix 1). Duplicate samples will be labeled with unique participant sample identifier barcodes to ensure sample integrity. One sample will be sent to the BSP and the other to the MGL. The BSP barcodes all samples and uses highly automated procedures for DNA isolation (MSMI DNA extraction from OC-175 collection systems: see Appendix 2). The BSP has processed approximately 100,000 samples and generated almost 1 million aliquots for 76 different projects in the last 4 years. The MGL routinely isolates DNA from thousands of samples per year for clinical genetic testing using automated methods validated for quality, including cheek swab samples (Mol Gen Newborn Saliva Extraction by BioRobot EZ1: see Appendix 3). The strategy of obtaining duplicate samples at the time of the enrollment encounter ensures rigorous quality control by identity screening using an efficient and cost effective panel of eight common SNPs that are genotyped in the MGL and compared to exome sequence variant calls at those positions. This approach has been employed in an ongoing research study that uses the same pipeline, which has resulted in the identification and correction of four sample swaps that occurred at different stages of the exome sequencing process of 600+ samples. If there is a

discrepancy between the research exome sequence data and the Sanger sequencing data generated in the MGL, testing will be repeated in both labs using the independent samples. If DNA isolated by the BSP and MGL appears to have come from different subjects, a new sample will be obtained, subjected to repeat testing, and the source of error determined. Duplication of cheek swab samples in the BSP and MGL will provide additional confirmation of accuracy and patient identity for results that are reported clinically.

Exome library production will be performed using the same workflow that is routinely performed in the laboratory of co-PI, Jonathan Berg, by highly skilled research technicians and laboratory managers under stringent quality control conditions (Exome Library Production protocols: see Appendix 4). Whole exome libraries are prepared using the Agilent Technologies Bravo A liquid handling platform (Santa Clara, CA) in conjunction with the Agilent SureSelect XT Target Enrichment System Kit (Agilent SSEL Automated Target Enrichments: see Appendix 5). Our research group has successfully generated over 600 high quality exome sequencing libraries for use in other studies at UNC. All protocols are followed rigorously to ensure a high level of reproducibility between samples.

- **Exome library preparation:** Our current protocol for library preparation utilizes the SureSelect XT Human All Exon V5 library (Agilent Technologies Inc. Santa Clara, CA). We have established methods for automated low-input protocols employing a 96-well format on an Agilent Bravo A instrument programmed for use with Agilent protocols. Enriched libraries are tested for QC/QA for size distribution and concentration using an Agilent 2200 Tapestation. Index barcodes are used in order to pool samples (currently four per pool).

- **Massively parallel sequencing*:*** Pools of samples will be subjected to massively parallel sequencing using either Illumina HiSeq 2000 or Illumina HiSeq 2500 sequencers that are housed, maintained, and operated by the UNC HTSF, which has provided high quality raw sequence for several large sequencing initiatives at UNC (High-throughput DNA Sequencing on the Illum…HiSeq 2500: see Appendix 6). Currently, the HiSeq platform can produce more than enough sequence to generate 50-100x average coverage exome data when pooling four samples per lane. The HTSF operates under stringent quality control (QC) conditions: (1) DNA/RNA concentration is estimated based on fluorescent detection, (2) library quality is verified using the LabChip LX automated electrophoresis system (Caliper), providing information related to size of the inserts and level of contamination, and (3) analysis of sequencing data (e.g. sequence coverage, presence of adapter sequence, rRNA gene contamination).

2.1.2 Bioinformatics pipeline

The Renaissance Computing Institute (www.renci.org) has been integrally involved in the development of an integrated pipeline for variant calling and analysis. Through a

combination of existing and adapted computing tools coupled with traditional analysis tools, the bioinformatics pipelines are able to: (1) perform large scale computations including alignment and variant calling, (2) coordinate a pipeline of such calculations, (3) store reads, assemblies, variants, and annotations, (4) provide data sets to researchers, and (5) provide for efficient query of a large variant database. The RENCI team has built an infrastructure that integrates the technologies necessary to achieve these goals. Bioinformatic processing and the provenance of data and analyses are managed and logged using the MaPSeq workflow manager (Reilly et al. 2014).

- **Initial informatics analysis (mapping, alignment, variant calling):** The early bioinformatics steps required to generate sample-specific reads from multiplexed flow cells are performed using Casava. The resulting fastq files are then further processed using BWA to align reads to the current reference sequence. In addition to its considerable performance characteristics, BWA operates on paired-end reads, performs gapped alignments, and creates output in SAM format. Resulting SAM files of aligned reads are sorted, indexed, and converted to binary BAM files using Picard and SAMtools. Post-alignment optimization, including PCR duplicate removal, realignment of reads, and quality score recalibration are performed using The Genome Analysis Toolkit (GATK). Genetic variants are called from BAM files using the GATK Unified Genotyper.

- **Variant annotation**: VCF files are annotated using a variant database, developed by RENCI, that calculates and stores annotations about all known and newly observed variants including those generated by several federated projects at UNC and external data such as the 1000 Genomes Project, NHLBI GO Exome project, and the Exome Aggregation Consortium (ExAC) (Owen et al. 2014) . The database utilizes built-in scripts to perform routine updating of information from external sources and applies annotations to novel variants, with information such as transcript location, whether the variant affects a splice site, and type of mutation (e.g. missense, nonsense, or indel). These scripts will automatically import and archive new genome builds or reference transcript sets, and translate all data to the new reference system. The database can retrieve variant information based on any previously used reference sequence and integrate summary incidence data from sources that used different builds. An archival version of all data sources are kept such that it is possible to reconstruct a view of the data as it existed at any point in the past. Functional annotation of exome sequence variants leverages diverse types of information, including dbSNP identifier, occurrence in the Human Gene Mutation Database, ClinVar, or other disease-specific mutation databases, frequency in control populations, and other annotations related to gene structure and protein effects of the variant. Further analysis, including protein structure information, sequence conservation, motif conservation, or other context-specific predictors are collected and calculated.

Quality metrics are captured at all stages of processing to determine if outputs can be used for analysis. Metrics include checks on input file correctness, distributions of

nucleotide and quality scores, percent of reads aligned, read gap distributions, percent of reads with pairs, metrics on coverage across the genome and from targeted regions, and metrics from GATK on called variants. Automated procedures are in place at key milestones in the sequencing analysis processes to inform study personnel when data quality metrics are not being met so that appropriate action can be taken.

2.1.3 Pilot study to investigate non-invasive assisted saliva collection

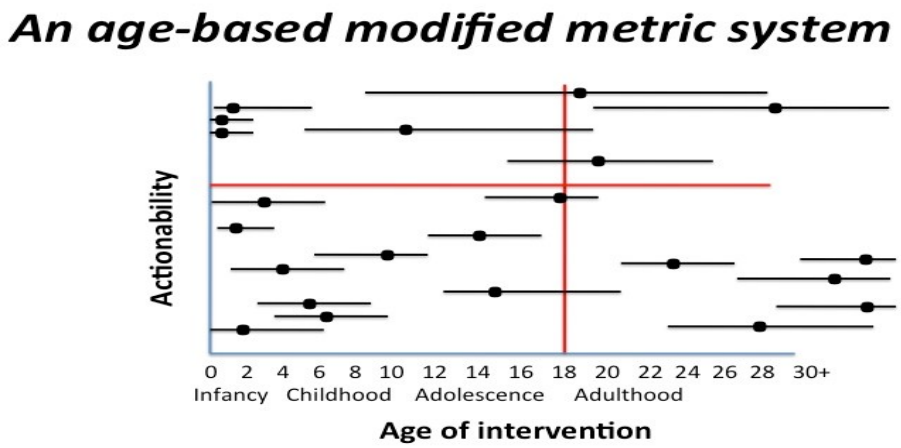
A pilot study was conducted to investigate whether non-invasive assisted saliva collection kits from DNA Genotek (Ontario, CA) will consistently yield enough high quality DNA from newborn babies to successfully perform next generation sequencing for newborn screening (NGS-NBS), and to test the laboratory methods and bioinformatics pipeline described above in Sections 2.1.1 and 2.1.2. Dual DNA samples were collected from eight newborns at the UNC Newborn Nursery using either the Oragene DISCOVER OGR-250 kit and CS1 accessory kit (n=3), or the ORAcollect for Pediatrics kit (n=5). One sample from each pair was isolated at the UNC BSP facility using the MSMI Automated DNA extraction protocol (MSMI DNA extraction from OC-175 collection systems: see Appendix 2). Total DNA concentration and quality were measured using a NanoDrop spectrophotometer and PicoGreen Assay for dsDNA (Thermo Fisher Scientific, Wilmington DE); mean sample yield was 4.24 ug. Purified DNA samples were enriched for the human exome using the low-input protocol developed for the commercially available Agilent SureSelect XT automated library prep and capture system (Appendences 3 and 4). Whole exome sequencing (WES) was performed on an Illumina HiSeq 2500 (High-throughput DNA Sequencing on the Illum…HiSeq 2500: see Appendix 6). The average depth of coverage for all participants across the entire region targeted for enrichment was 101.8x with a mean q30 Yield Passing Filtering at 89.28x. A set of eight pre-determined polymorphic single nucleotide polymorphisms (SNPs) with no clinical implications were used to confirm and troubleshoot unbiased sequence coverage of the genome using the bioinformatics pipeline described in Section 2.1.2. The second sample was isolated at the MGL facility using the Qiagen Biorobot EZ1 protocol (Mol Gen Newborn Saliva Extraction by BioRobot EZ1: see Appendix 3) in preparation for Sanger sequencing (Mol Gen Custom DNA Sequencing, see Appendix 7) of the same eight pre-determined SNPs. Results of the eight SNP comparison between exome and Sanger sequencing were 100% concordant. No data on disease-associated variants was generated and no results were returned to participants.

2.1.4 Semi-quantitative metric for assessment of clinical actionability

A semi-quantitative metric was developed for a previous study to systematically assess aspects of clinical actionability (a measure of the likelihood and severity of disease outcomes, and the efficacy and potential harms of interventions) and provide a standardized mechanism to classify different types of potential genomic findings to guide informed decision-making, analysis and return of results (Berg et al. 2015)

This semi-quantitative has been expanded for use in the NC NEXUS study by developing a quadrant-based framework, in which the continuum of clinical actionability is represented on the Y axis and the age of onset of disease or age of implementation of preventative intervention is represented on the X axis. By setting a threshold for clinical actionability and using 18 as the threshold for age of onset, we can define medically actionable childhood onset conditions (upper left quadrant), medically actionable adult onset conditions (upper right quadrant), non-medically actionable childhood conditions (lower left quadrant), and non-medically actionable adult onset conditions (lower right quadrant). (see Figure 1 and Section 3.1.3.1).

Fig. 1: An age-based modified metric system



2.1.5 Development of decision aids

The NC NEXUS group at RTI International has demonstrated expertise with the development of educational materials for informed decision-making (Bailey et al 2013a; Bailey et al 2013b). Development of an electronic Decision Aid will be one of the main ELSI objectives of this project. The following previous projects do not directly relate to NC NEXUS but provide examples of the types of resources developed by RTI.

- **Decision Aid to support clinical trial involvement for Fragile X Syndrome (FXS):** Advances in understanding the molecular basis of Fragile X Syndrome (FXS) have led to a new generation of treatments and clinical trials are under way using a variety of novel compounds. The possibility of side effects and the potential for significant changes in behavior and ability elevate to a new level the importance of obtaining meaningful informed consent, not only from parents, but also from individuals with FXS. Little is known about the extent to which individuals with FXS can be or are involved in decisions about research participation. RTI holds a National Institute of Child Health and Human

Development-funded R01 grant to develop a tablet-based decision aid and evaluate its effect on participation in the consent process for a hypothetical clinical trial (Bailey et al 2013a; Bailey et al 2013b). This research will assist researchers and clinicians in maximizing decisional capacity and consent. To date, we have completed content development and are developing the tablet-based application. We will test our decision aid in a randomized controlled trial to evaluate its efficacy compared to standard practice of informed consent.

- **Dissemination and implementation of pediatric cardiovascular risk reduction:** RTI also developed an implementation package to facilitate adoption of the National Heart Lung and Blood Institute's Integrated Guideline for Pediatric Cardiovascular Risk Reduction. This guideline focuses on the seven leading risk factors for cardiovascular disease from birth to young adulthood in a comprehensive 400-page document, which is too cumbersome for routine use by providers. The goal of this project was to make clinical implementation of the recommendations easy and sustainable for pediatric care providers by providing tools and nontraditional educational strategies for providers and patients, centered on a smartphone clinical decision support application. Implementation was evaluated through an 18-month cluster randomized trial in which use of decision support materials were associated with greater adherence to the clinical guideline.

- **BRCA decision aid:** Women with hereditary breast and ovarian cancer syndrome (HBOC) face a higher risk of earlier, more aggressive cancer. Because of HBOC's rarity, screening is recommended only for women with strong cancer family histories. However, most patients do not have accurate history available and struggle to understand genetic concepts. RTI developed *Cancer in the Family*, an online clinical decision support tool, which calculates women's HBOC risk and promotes shared patient–provider decisions about screening. A pilot evaluation (n = 9 providers, n = 48 patients) assessed the tool's impact on knowledge, attitudes, and screening decisions. Patients entered complete family histories (67%), calculated personal risk (96%), and shared risk printouts with providers (65%). HBOC knowledge increased dramatically for patients and providers, and many patients (75%) perceived tool results as valid. The tool prompted patient–provider discussions about HBOC risk and cancer family history (88%).

- **Electronic Preventive Services Selector (ePSS):** In October 2006, the Agency for Healthcare Research and Quality (AHRQ) launched the Electronic Preventive Services Selector (ePSS) for primary care clinicians. This interactive tool is designed to provide real-time decision support for clinicians as they identify appropriate preventive service(s) for patients. In partnership with Healthwise, RTI developed: 1) A set of "Patient Information Prescriptions (Ix)," which is a virtual prescription pad that enables clinicians to share information about a health topic with a patient and provides links to other relevant health information and patient education materials for specific recommendations. 2) The content for the

Patient-Clinician Communication Support material, which is titled "Partner With Your Patients: Shared Decisionmaking for Better Preventive Care." 3) Actionable recommendations for ePSS enhancements including user interface redesign, adaptive content management guidance, and integration of additional content to support informed decision-making and patient-clinician communication based on Web analytics, user sentiment assessments, and field notes from ethnographic observations of clinician users during patient encounters.

2.1.6 Psychosocial research methods

The UNC research team has extensive experience in the conduct of longitudinal observational behavioral research and in studies that include randomization (e.g., randomized controlled trials evaluating interventions in patient populations). We also have specific training and expertise in measurement and psychometrics, which we applied when developing a measurement protocol for this study. We have met regularly to finalize and refine the measures for NC NEXUS. The selection of measures was guided by the research literature, our own scientific and clinical expertise, and consultation with outside experts as necessary. We use validated measures from the literature when possible. When necessary, measures are adapted for the purpose of the study or developed specifically for these projects. The constructs utilized in NC NEXUS include genomic testing knowledge, health literacy, decision regret, test-specific distress, perceived stress, social support, and emotional responses in addition to demographic information and health-related questions. In addition, we are developing measures to assess the decision making process in couples, relationship quality, parental bonding, and perceptions of tested children.

2.2 Specific Content

2.2.1 Other unpublished information

The decision aid in development for NC NEXUS is informed by best practices supported by the International Patient Decision Aids Standards. Our extensive experimental and formative work with parents will ensure that their needs are represented.

We conducted qualitative, open-ended interviews with 33 couples with a current pregnancy, recent birth, or a child under 5 years of age that had gone through genetic testing. The interviews were aimed at understanding parental knowledge and values for the types of information that might be returned in the NC NEXUS project. We also asked couples to make mock decisions about the types of results they would want from genomic sequencing, and tested draft materials to be used in the Decision Aid. We transcribed and analyzed the data from these interviews using a Framework Analysis approach, which systematically identified the important themes and conclusions across the data.

Some parents thought that more knowledge of their child's genetic information would be helpful, and would opt to have testing done, even if there was no medical utility. This was expressed as the personal utility of knowing information, which some parents perceived as their obligation to know and viewed as being important for ensuring quality of life for their child and family. On the other hand, some parents had questions about the validity of these tests and what they meant, how privacy is ensured, and had concerns about labeling their child. In general, parents welcomed the opportunity to participate in research if it could benefit other children, even if it did not benefit their own child. Parents viewed the preliminary Decision Aid materials as helpful and provided useful feedback on how to make the materials more relevant, preferring less technical language and simpler explanations for complicated terms.

We conducted a discrete choice experiment with 1,289 adults in the United States using an online panel. We examined how parents view various attributes related to genetic disorders, and assessed preferences for the types of information parents would prefer to know. When deciding what kinds of genetic test results are most important to know, parents showed preference for conditions with a high degree of penetrance, that begin earlier in life, progress rapidly, have severe mental and/or physical symptoms, and are characterized by a shortened lifespan. The relative importance scores show that the most important attribute as a whole was the likelihood that a given condition would develop, if given a true-positive test result, which accounted for 38.5% of the differences in profile selection.

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3. INVESTIGATIONAL PLAN

Since newborn screening (NBS) began in the 1960's, technological advances have resulted in its use for an increasing number of disorders. Recent developments in whole genome and whole exome sequencing (WES), now afford the opportunity to comprehensively define the variation within an individual's genome in a rapid and affordable manner.

Many challenges arise with the clinical application of genome-scale sequencing and in deriving practical benefit to infants and children. Its utility in NBS has yet to be demonstrated and its application in the pediatric population requires special examination, not only for potential clinical benefits, but also for the unique ethical challenges it presents. In this study we utilize a highly interdisciplinary approach to identifying, confronting and overcoming the major challenges that must be met in order to implement deep sequencing technology to enhance current newborn screening in a diverse pediatric population.

3.1 Purpose of the Investigation

3.1.1 Name of the Investigational Device

North Carolina Newborn Exome Sequencing for Universal Screening (NC NEXUS)

3.1.2 Intended Use of the Investigational Device

The intended use of the NC NEXUS device is to investigate the potential of genome scale next generation sequencing to augment and extend current newborn screening in a diverse pediatric population.

The NC NEXUS study will evaluate the use of exome sequencing as a potential means to augment newborn screening (NBS). The main technical outcome will be to examine the sensitivity and specificity of this technology in detecting conditions that are currently screened for in newborns. Another technical outcome will be to examine the capacity of exome sequencing to detect other conditions that would be beneficial to identify at an early age in children but for which there is currently no available diagnostic method. In addition to the examination of technical outcomes, the NC NEXUS project includes a highly integrated set of research aims that will address the ethical/legal/social implications (ELSI) aspects of exome sequencing in newborns.

3.1.3 Objectives of the clinical investigation

The objective of the NC NEXUS study is to investigate the potential of genome scale next generation sequencing to augment and extend current newborn screening in a diverse pediatric population. There are three key scientific objectives:

1. Evaluate how next generation sequencing newborn screening (NGS-NBS) can extend the utility of current NBS.
2. Devise and evaluate a clinically oriented framework for analysis of NGS-NBS based on principles of ethics and evidence-based medicine.

3. Develop best practices for incorporating NGS-NBS into clinical care by exploring the ethical, legal and social issues (ELSI) involved in informed decision-making and return of results after testing.

To investigate the potential of next generation sequencing (NGS) to extend newborn screening (NBS), two cohorts will be recruited at UNC Hospitals clinics.

1. The "Diagnosed Cohort" consisting of ~200 children with a confirmed NBS disorder or a condition eligible for NBS and their parents.
2. The "Well-Child Cohort" consisting of ~200 pregnant couples and their healthy newborns.

Parents who elect to enroll in the study will use an electronic decision aid to decide whether they are interested in NGS-NBS for their child, and will meet with a genetic counselor to provide informed consent for sequencing.

Duplicate saliva samples will be labeled with study ID numbers and delivered to the BioSpecimen Processing (BSP) Facility and the Molecular Genetics Laboratory (MGL) for DNA extraction and storage. Exome capture and massively parallel sequencing will be used to generate sequence data, which will be analyzed using targeted informatics analyses. Clinically significant variants will be confirmed in the MGL by Sanger sequencing (Mol Gen Custom DNA Sequencing, see Appendix 7) and reported to parents in a genetic counseling session.

"NGS-NBS" will include genes associated with disorders that are currently in the Recommended Uniform Screening Panel (RUSP) which are conditions that are recommended and approved by the Secretary of Health and Human Services to be screened for in newborns by all states in the U.S. (See http://www.hrsa.gov/advisorycommittees/mchbadvisory/heritabledisorders/recommendedpanel/index.html). NGS-NBS will also include conditions similar to those included in traditional newborn screening but which have not been recommended for inclusion in the RUSP due to lack of an available screening method (such as familial hypercholesterolemia, Wilson disease, and ornithine transcarbamylase deficiency). These conditions will be defined using the semi-quantitative metric (described in Sections 3.1.3.1 and 3.2.3.6.5 below) and will include those with onset in infancy or childhood for which there is medical actionability (i.e. treatment, monitoring and/or medical management to improve outcomes).

Results will be returned for diagnostic findings ("Diagnosed Cohort" only) and medically actionable disorders of childhood (both cohorts). Two-thirds will be randomly assigned to a "decision group" that will be eligible to request additional genomic findings. These parents will utilize the electronic decision aid to learn about the categories of additional findings and indicate their interest in having their child's genomic data analyzed for any results in these categories. The other one-third will be assigned to the "control group" with respect to the impact of other, non-medically actionable genomic findings.

All parents will complete questionnaires to assess the study impact. We will analyze the impact of NGS-NBS in all participants and we will be able to compare the impact of learning about additional non-medically actionable genomic findings between the "decision group" and the "control group."

Upon completion of this project, we aim to have established a practical and ethical infrastructure by which to apply genomic sequencing in NBS for the tangible benefit of patients. We are optimistic that by addressing central challenges facing the clinical implementation of genome-scale analysis in children, we will contribute significantly to the establishment of best practices as NBS moves into the genomic era.

3.1.3.1 Primary objectives

The NC NEXUS project has three (3) primary objectives:

Primary Objective 1: Evaluate how next generation sequencing newborn screening (NGS-NBS) can extend the utility of current NBS. From a technical standpoint, our overarching aims are to evaluate the technical possibilities and limitations of NGS-NBS using exome sequencing as a platform for generating sequence variant data. We will use focused indication-based analysis for children in the "Diagnosed Cohort" and focused analysis of lists of genes assigned to distinct categories based on clinical actionability and age of onset or intervention (see Primary Objective 2 and NC NEXUS_Actionability Scores: see Appendix 8).

We will examine the sensitivity and specificity of WES for conditions included in the "Diagnosed Cohort" since these individuals are already known to have specific health conditions. These conditions will include phenylketonuria (PKU), medium chain acyl-CoA dehydrogenase deficiency, cystic fibrosis, and congenital hearing loss. We will include in this cohort children diagnosed with medical conditions that are not currently screened for on the recommended uniform screening panel, in order to evaluate future potential candidates for NBS. Such conditions will include Wilson disease, lysosomal storage disorders, and Menkes disease (see Section 3.2.3.6.4)

We will also explore the yield of findings related to medically actionable disorders of childhood through analysis of the "Well-Child Cohort". These individuals will likely be asymptomatic for any conditions identified, and we will follow any newborns with positive findings for development of disease manifestations and will monitor clinical actions taken by providers.

Primary Objective 2: Devise and evaluate a clinically oriented framework for analysis of NGS-NBS based on principles of ethics and evidence-based medicine. We will develop strategies to guide clinicians, clinical laboratories and patients/families in their decisions regarding the genomic findings that will be

detected by sequencing in ways that respect the child and protect his/her future autonomy, while also respecting parental interests and rights.

We will adapt a previously developed semi-quantitative metric for assessing clinical actionability (30) for use in the NC NEXUS project by adding the dimension of age of onset or age of intervention. This method allows us to assign gene-disease pairs into four categories (see Section 3.2.3.6.4):

1. Medically actionable childhood onset conditions (which we refer to as "NGS-NBS"). See Section 3.2.3.6.4.1
2. Medically actionable adult onset conditions, such as those that confer risk of cancer, aortic dissection, etc. See Section 3.2.3.6.4.2
3. Non-medically actionable childhood onset conditions such as those that cause intellectual disability, muscular dystrophies, or neurodegeneration. See Section 3.2.3.6.4.2
4. Non-medically actionable adult onset conditions such as early-onset Alzheimer disease. No participants in the NC NEXUS project will receive this category of information. See Section 3.2.3.6.4.3

A major goal of the NC NEXUS project is to systematically curate all known gene-disease pairs and assign them to one of these categories. This is an ongoing aspect of the project and one of the primary endpoints.

We will also evaluate communication of results to parents, including both negative results and positive results. Board-certified medical geneticists and genetic counselors will return all primary NGS-NBS results.

Primary Objective 3: Develop best practices for incorporating NGS-NBS into clinical care by exploring the ethical, legal and social issues (ELSI) involved in informed decision-making and return of results after testing. We will develop novel decision support tools and evaluate their usefulness in parental decision-making as this new technology is deployed in this vulnerable population.

We will evaluate informed parental decision making aided by an electronic decision aid regarding acceptance of 1) NGS-NBS for their child, and 2) categories of additional non-medically actionable genomic findings (for those randomized to the "decision group"). We will utilize questionnaires regarding the psychosocial impact of the screening. **Please note that in some of the informational material attached to this application you will see references to the use of survey(s). In all cases, survey(s) are the questionnaire(s).**

3.1.3.2 Secondary objectives

Planned secondary analyses will investigate (a) consequences of having the decision to obtain additional genomic results (comparing the decision and control groups' test-related

distress), (b) couples' agreement on decisions (using accepted methods for dyadic data analyses), (c) choices regarding return of non-medically actionable genomic results, (d) reasons for decisions about results, (e) predictors of test-related distress among parents who accept NGS-NBS or additional genomic results, and (f) outcomes for partners/fathers. We recognize the potential for other secondary analyses, given the exceptionally rich data we will collect in the study. Our planned secondary analyses will address questions relevant to real-world use of NGS-NBS and further development of approaches to support decision-making in our populations. Other analyses may also be performed.

3.1.4 Anticipated duration of the clinical investigation

Project Period (based on NIH award: 09/05/2013 – 08/31/2018). Recruitment of the "Well-Child Cohort" will take 2.5 years, and the recruitment of the "Diagnosed Cohort" will take 2.25 years.

3.2 Clinical Protocol

3.2.1 Title of Clinical Protocol

Short Name: The NC NEXUS Study
Long Name: The North Carolina Newborn Exome Sequencing for Universal Screening Study

3.2.2 Study Design

3.2.2.1 General Study Design

The NC NEXUS project is an exploratory longitudinal cohort study, with randomization of enrolled parents into two groups with different opportunities for decision-making about categories of non-medically actionable genomic information.

3.2.2.1.1 Cohorts:

We will enroll parents and their eligible child into the following 2 cohorts:

- The "Diagnosed Cohort": Our goal is to sequence 200 children from this cohort including infants and children up to age 5 who have metabolic disorders such as phenylketonuria (PKU) and medium chain acyl-CoA-dehydrogenase deficiency (MCADD); cystic fibrosis (CF) and *CFTR*-related metabolic syndrome (CRMS); congenital hearing loss; primary ciliary dyskinesia, and a variety of other rare conditions.

- The "Well-Child Cohort": Our goal is to sequence 200 infants whose parents enroll prenatally. The expectant mothers must be pregnant with an intrauterine pregnancy of 18 weeks or greater, have no pending or positive prenatal diagnostic test results for congenital malformations or chromosomal abnormalities and have been identified by medical personnel in the obstetrics clinic as possible candidates.

3.2.2.1.2 Randomization:

In order to assess the impact of the additional non-medically actionable genomic findings available upon request, parents will be randomized in a 2:1 ratio to "Decision" or "Control" groups, respectively. Both groups will learn any childhood onset medically actionable results ("NGS-NBS") from genomic sequencing at their return of result visit.

3.2.2.1.3 Study design narrative:

The NC NEXUS project will utilize a system of tiered informed consent by which parents will participate in a study of informed decision-making about whether they would accept NGS-NBS for their child or infant, and (in the case of those randomized to the "Decision" group) whether they wish to learn about other additional categories of genomic information.

Potential participants will be approached by a recruiter during a regularly scheduled clinic visit and provided with an informational brochure about the NC NEXUS study (NEXUS Recruitment Brochure for the Diagnosed cohort: Appendix 9 and NEXUS Recruitment Brochure for the Well-child cohort: Appendix 10).

- If they are interested in learning more about the study, contact information will be obtained and they will be given a study brochure and consent form.
  There will be no further interactions with parents who decline to learn more about the study
- Parents who expressed interest in the study will receive a telephone call during which the informed consent for initial participation will be reviewed and consent given verbally (NCNEXUS_information_sheet_Phase_I_Diagnosed cohort: Appendix 11 and NCNEXUS_information_sheet_Phase_I_WC cohort: Appendix 12).
- Those who agree to participate will be given access to the online decision aid, which will provide information about genomic sequencing and the potential types of results that would be included in the "NGS-NBS" analysis (NC NEXUS Decision aid overview: Appendix 13; NEXUS Online DA Decision 1 Shooting Script: Appendix 14; NEXUS Online DA Shooting Script QA content: Appendix 15).
- Those who are not interested in participating will complete an exit questionnaire for decliners.

After receiving additional information through the electronic decision aid, parents who are interested in obtaining sequencing for their child will be scheduled for an in-person study visit ("Visit 1") with a genetic counselor to obtain formal consent for sequencing (NC NEXUS_consent_phase II_diagnosed cohort: Appendix 16 and NC NEXUS_consent_phase II_Well-Child cohort: Appendix 17). Cheek swab samples will

be delivered to the BSP and MGL for processing. Exome sequencing will be performed, with focused informatics analysis depending on the cohort (described below).

The randomization status will be revealed to the parents when they are scheduled for their return of results visit. Parents who are randomized to the "decision" group will be given access to additional content in the electronic decision aid prior to their second in-person study visit (NEXUS_Online DA_Decision 2_Shooting script: Appendix 18). This information will include a description of the additional categories of non-medically actionable information.

All participants will have a second in-person study visit ("Visit 2") with a board certified medical geneticist and genetic counselor for return of results from NGS-NBS and (in the case of the "Diagnosed Cohort") the indication-based analysis.

- Parents who are randomized to the "control" group will receive their primary results but will not be eligible for additional categories of information.
- Parents who are randomized to the "decision" group will receive their primary results and will have the opportunity to discuss any questions they have regarding additional categories of non-medically actionable information. They will then be eligible to request analysis of any, all, or none of the additional information.

  Parents randomized to the "decision" group will have up to one additional visit with a medical geneticist and/or genetic counselor ("Visit 3") depending on the additional categories of non-medically actionable information they have requested.

  Parents will be asked to complete questionnaires at defined time points during the study (see study design schematic below). Parents who complete the questionnaires will be paid $20 for each questionnaire completed. Payment will be mailed immediately upon completion of a questionnaire. We have found that this amount of money recognizes their time and effort but is not coercive. Measures included in these questionnaires are shown in NC NEXUS Project 3 Longitudinal Study Measure (see Appendix 19). Slight changes or adjustments based on feedback from user testing may lead to minor changes (e.g., to reduce burden by removing some items or measures, change wording if any is found to be confusing to users, or add measures if users suggest we are missing a key construct), in which case the FDA will be provided with a 5-day notice of any updates or changes in the measures.

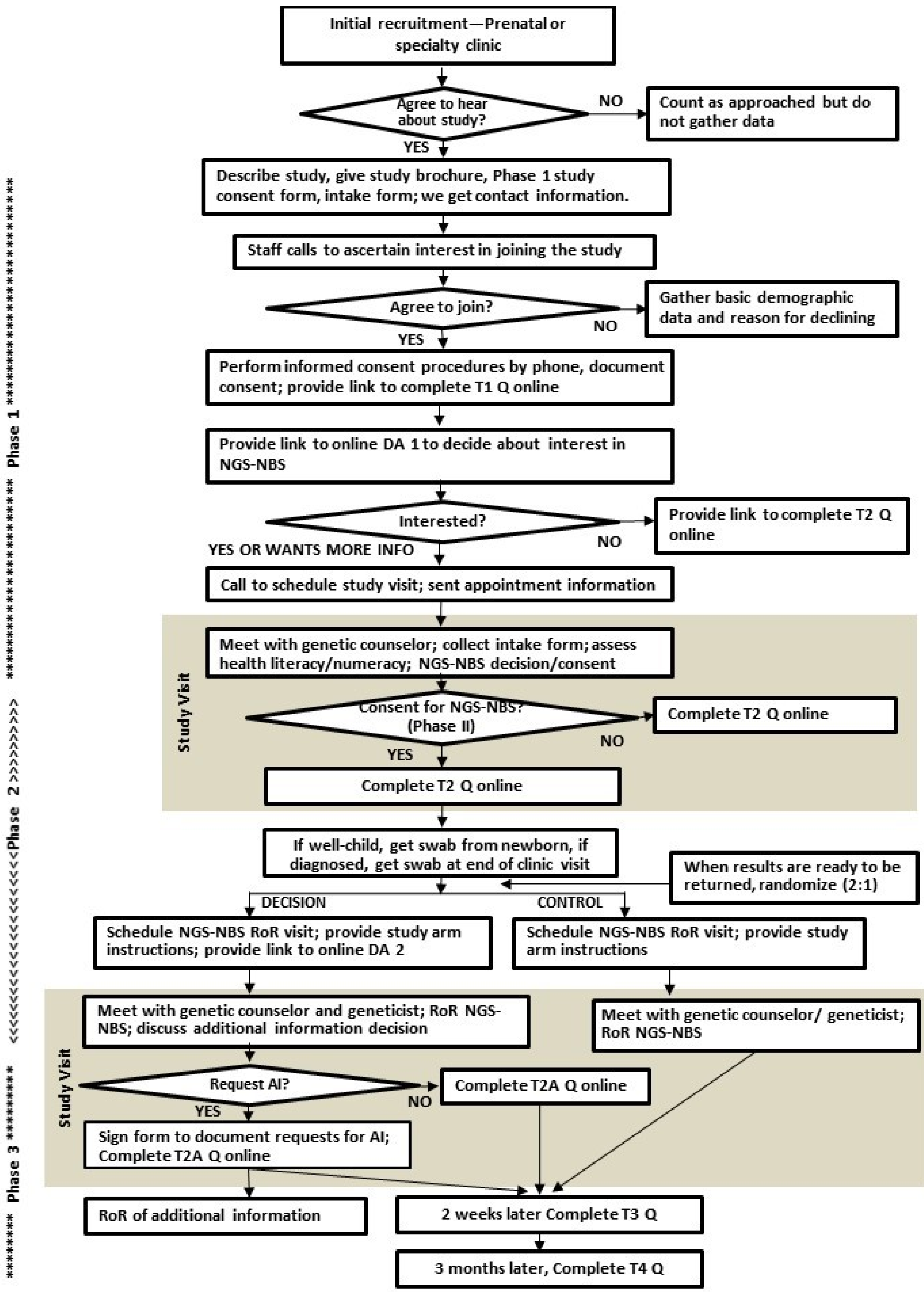
- **Intake Form (Draft version will be provided upon request):** The intake form collects information about demographics, previous experience with genetic testing, knowledge about genomic sequencing, and (for the "Well-Child" Cohort) pregnancy anxiety. Parents will be asked to complete this form and bring it with them to the initial study visit, if they agree to a visit.

- **Time 1 questionnaire (Draft version upon request)**: After parents decide to proceed with an in-person study visit (either to consent to NGS-NGS or to get more information about it in an in-person consultation) or not to proceed with an in-person visit (declining further participation in the study), they will be given access to the Time 1 questionnaire, which will provide valuable information about differences between parents who are and who are not interested in NGS-NBS.

- **Time 2 questionnaire (Draft version will be provided upon request)**: All parents who attend the initial in-person study visit (whether or not they have accepted NGS-NBS) will complete the Time 2 questionnaire, which gathers data about their decision-making process, mood, and knowledge about the project. In addition, parents in the "decision" group will be asked to complete a brief post-decision questionnaire (the Time 2A questionnaire) after they decide whether or not to request additional genomic findings to assess the consequences of having this option.

- **Follow up Assessments (Time 3 and Time 4 questionnaires) (Draft versions will be provided upon request)**: All parents will complete two follow up questionnaires following the return of result visit: a Time 3 questionnaire completed within 2 weeks of the visit and a Time 4 questionnaire completed 3 months after the visit. These questionnaires will assess the short- and longer-term consequences of decision-making and results disclosure.

3.2.2.2 Study Design Schematic



3.2.3 Study Procedures

3.2.3.1 Participant Selection

Participants can be either sex and of any race or ethnicity, but parents must be fluent in English or Spanish. For mothers who are married or in a marriage-like relationship, their partners must also consent to participate. Participants will self-identify their preferred language (English or Spanish)

Although this study will focus on newborns, in order to increase our numbers of prospective subjects in the "Diagnosed Cohort," we will enroll children up to age 5 years. In order to obtain more meaningful data from WES analysis, particularly for conditions such as PKU, including determining any genotype-phenotype correlations or influence of other genetic factors, it is important to obtain clinical data in children beyond infancy. The "Well-Child Cohort" will consist of newborns in order to more accurately reflect the typical age of NBS.

Pregnant women and their partners will be recruited from the Prenatal clinics at UNC and verification of their pregnant status will be by self-identification and by determining that they are being followed in the prenatal clinics and have had their pregnancies verified as part of standard prenatal care.

3.2.3.2 Anticipated Number of Research Subjects

Recruitment will use established channels at UNC, and we expect that ~80% of mothers we approach for recruitment will have a partner who is reasonably available and who therefore would be approached for recruitment.

For the "Diagnosed Cohort," there are 560 current pediatric patients under age 5 with the selected conditions being followed at UNC, and an additional 330 infants under 6 months old are diagnosed each year. We estimate that ~80% of parents of children in the "Diagnosed" cohort will elect to have their child undergo sequencing. Thus, in order to sequence 200 children in this cohort, we anticipate enrolling 250 family units (couples or single parents, and their child).

For the "Well-Child Cohort," there are approximately 3,500 expectant mothers at UNC per year who will be eligible, providing a large population from which to recruit. In our prior study on newborn screening for fragile X syndrome (FXS), 64% of couples agreed to join the study and accepted screening. We therefore estimate that ~64% of parents approached for recruitment to the "Well-Child Cohort" will agree to join it, although this estimate is conservative because joining our study will not necessitate also accepting NGS-NBS. We project a 5% dropout rate between Times 1–3 (when parents are actively involved in making decisions and receiving results) and an additional 5% dropout at Time 4. In order to sequence 200 children in this cohort, we anticipate enrolling 350 eligible family units.

Based on these estimates, we will approach 48 parents per month over 20 to 21 months for recruitment to yield a sample of 400 completing the study over approximately 30 months (200 each in the diagnosed cohort and well-child cohort).

3.2.3.3 Inclusion Criteria

3.2.3.3.1 "Diagnosed Cohort"

Parents meeting the following criteria:

1. Parents of a child who meets the criteria below AND
2. At least 18 years old.
3. For mothers who are married or in a marriage-like relationship, their partners must also consent to participate. Mothers who are not married or not in a marriage-like relationship will be able to participate individually.
4. Must be able to provide informed consent for their child and for themselves
5. Must be fluent in English or Spanish

Children meeting the following criteria:

1. Infants and children from 0-5 years
2. Diagnosed with known or suspected monogenic disorder, such as:
   - Phenylketonuria
   - Medium chain acyl-CoA-dehydrogenase deficiency (MCADD)
   - Cystic fibrosis or *CFTR*-related metabolic syndrome.
   - Congenital hearing loss
   - Other rare disorders such as primary ciliary dyskinesia or mucopolysaccharidosis

   OR
   - Those with positive newborn screens but non-confirmatory follow-up testing ("false positives")
3. Medically stable

3.2.3.3.2 "Well-Child Cohort"

Parents meeting the following criteria:

1. Pregnant with an intrauterine pregnancy of 18 weeks or greater
2. At least 18 years old
3. For mothers who are married or in a marriage-like relationship, their partners must also consent to participate. Mothers who are not married or not in a marriage-like relationship will be able to participate individually.
4. Must be able to provide informed consent for their child and for themselves
5. Must be fluent in English or Spanish
6. Have no pending or positive prenatal diagnostic test results for congenital malformations or chromosomal abnormalities
7. Have been identified by medical personnel in the OB clinic as possible candidates.

Newborns:

1. Have no complications at the time of birth or unexpected medical problems; however, depending on their clinical course, those whose parents have previously consented to the study may have DNA sampling once stabilized and discharged from the Neonatal Intensive Care Unit, if the parents agree.

3.2.3.4 Exclusion Criteria

Parents:

1. Younger than 18 years old
2. Unwilling to complete study procedures
3. Have cognitive or other impairments that preclude them giving informed consent
4. Disagree about their child's participation
5. Transfer their prenatal care to another institution
6. Are not fluent in English or Spanish

Children:

1. Do not meet diagnostic criteria as above
2. Medically unstable
3. Medical care transferred to another institution
4. Not born at UNC Hospitals in Chapel Hill, NC

3.2.3.5 Recruitment and enrollment procedures

After having been identified by their clinician as eligible, couples will be contacted by a study recruiter, and asked if they would like to hear about the study. Fathers will also be recruited if they are reasonably available. Thus, these procedures discuss the involvement of "parents." However, when fathers are not reasonably available, mothers may participate on their own. For those in a couple relationship parents must be concordant in their decisions to consent or the couple will not be eligible.

3.2.3.5.1 The "Well-Child Cohort":

Initial Contact: The recruiter will approach those pregnant couples in the UNC prenatal clinic who have been identified as potentially eligible and who have been informed about the study by their clinician.

- **Not eligible:** We will thank them for their time, not gather any information, and not contact them again.
- **Decliners**: We will thank them for their time, not gather any information, and not contact them again.
- **Accepters**: The recruiter will briefly describe the study and give the couple a **study brochure** (NEXUS Recruitment Brochure for the Well-child cohort: Appendix 10) that describes study procedures, genomic sequencing, the kinds of conditions that could be identified, the randomization process, and a decision aid

to help them make a decision about joining the study. They will also be given a copy of the consent form (NEXUS Recruitment Brochure for the Well-child cohort: Appendix 10) about joining the study to read and the intake form for each member of the couple (or just for mothers if they are participating without a partner). The intake form collects information about demographics, previous experience with genetic testing, knowledge about genomic sequencing, and pregnancy anxiety. Couples will be asked to complete this form and bring it with them to the study visit.

The couple will be asked if they will agree to being contacted by phone by the study scheduler to ascertain their interest in joining the study. Couples who agree will be asked for their phone number and contact information, including email address, and their language preference, which will be entered into the study database (REDCap) along with the clinic from which they were recruited and gestational age of the pregnancy. The recruiter will provide a timeframe for the call (e.g., a week) and ask that both members of the couple read the study brochure and informed consent form before that time and be present on the call.

Recruitment phone call: The study scheduler will call the couple and ask if they would like to join the study.

- **Decliners**: Couples who indicate they are not interested in joining the study will be asked to provide basic demographic data and a reason for declining. Completion of this step will end their participation and their identifying information will be shredded.
- **Accepters:** We will obtain the **couple's verbal consent** for Phase 1; joining the study.

If both parents have not read the study brochure and the consent form by the time the scheduler calls, they will be given additional time to read it, agreeing on how much time that will be.

Parents who have access to an Internet-enabled computer will then be provided with a link to access the Time 1 questionnaire. Each member of the couple will complete the questionnaires in the study independently.

After completing the Time 1 questionnaire, they will be given access to the online electronic decision aid. The decision aid will provide information about sequencing and the types of results available, the frequency of such findings, the risks and benefits of study participation, and guide parents thru the decision-making process. Parents will be encouraged to view the decision aid before the study visit and can use it to indicate their choice of one of three options:

(1) not interested and do not wish to schedule a visit, or
(2) interested and want to schedule a study visit, or
(3) undecided and want to schedule a study visit to learn more.

Thus, couples are **not** expected to make a decision at this time and will have ample time to gather information and consider their options.

Parents who do not have internet access will be mailed a copy of the Time 1 questionnaire with instructions to return the completed copy in a pre-paid envelope. They will then view the decision aid at the study visit.

Those who agree to a study visit will be scheduled and sent information about the appointment time, date and location of the visit.

- **Non-compliant decliners:** Couples who verbally consent to the study but who do not successfully schedule a study visit or who fail to appear for their scheduled study visit will be asked if they would be willing to reschedule their visit. If not, they will be considered to be decliners. Decliners will be asked to provide basic demographic information (for comparison to the accepters) and their reasons for declining**.** Completion of this step will end their participation in the study.

Study Visit Activities: Couples who schedule a study visit will meet in person with a genetic counselor who will collect their completed intake form, assess their health literacy and numeracy with a validated brief interview, and discuss any issues generated by the electronic decision aid about accepting or declining sequencing of their child. The counselor will answer questions and assess their understanding of main points of informed consent including the range of results that could be returned, the planned use and storage of genetic data as well as the risks and benefits of genomic sequencing**. Consent (for Phase II; sequencing) will be obtained** from both members of the couple except in cases where the father is not reasonably available. In those cases only the mother will be consented.

- **Sequencing consenters**: After consenting, couples will complete the Time 2 questionnaire about their decision, mood, and knowledge about the project. They will also be asked to provide consent for access to their child's state newborn screening results and pertinent medical records.
- **Sequencing decliners**: Parents who decline sequencing will be asked to complete the Time 2 questionnaire and will exit the study.
- **Couples who are unsure about their sequencing decision:** The couple can defer their decision about sequencing and defer scheduling a study visit. This option will provide additional time for parents to view the decision aid and make a decision about genomic sequencing prior to giving birth (which normally occurs around 40 weeks gestation). In order to facilitate the time couples have to read the informed consent forms and view the electronic decision aid, we are providing access to these prior to and after the study visit. They will have ample time to have their questions answered, and confer with others before providing consent (e.g., family members, healthcare providers).
- If and when a deferring couple decides to proceed, they will contact the study office and a study visit will be scheduled (see above: study activities). The genetic counselor will review the information and obtain consent. After providing consent

for sequencing, couples will complete the Time 2 questionnaire as described above. They will also be asked to provide consent for access to their child's state newborn screening results and pertinent medical records.

Couples can change their decision from yes to no before the sample is obtained after the baby is born. They will be asked about their decision, complete the Time 2 questionnaire again and exit from the study.

3.2.3.5.2 The "Diagnosed Cohort"*:*

Many of these families live long distances from UNC and we would like to coordinate the study visit with an upcoming clinical visit which necessitates that we contact them before their child's appointment. All parents will have first been introduced to the study by their child's clinician either in person, by phone or by a letter accompanying the study pamphlet.

Initial Contact: Parents will be contacted by mail before their upcoming clinic visit and sent the study brochure and a letter from their child's clinician inviting them to participate with an opt-out postcard to return if they wish to decline. They will also be sent a copy of the consent form to join the study. If parents do not opt out, they will be contacted 2-4 weeks later by a telephone call or recruited at the time of their child's appointment.

Phone call recruitment and study visit scheduling: as described above

In-Person Recruitment: Some parents may not be reachable by phone before their child's clinic visit. In these cases, a study recruiter will ask them if they want to join the study at the time of their child's clinic visit. When possible, they will have already been sent the recruitment brochure and the consent form to join the study. If they wish to join, they can give verbal consent for Phase I (joining the study) and complete the intake form. They will then complete the Time 1 questionnaire (online) and the rest of the study visit activities as described above. After they complete the Time 1 questionnaire, they will be provided with a link to the electronic decision aid.

Parents who attend a study visit (including those who are recruited in person) will meet with a genetic counselor to discuss any questions they have about NGS-NBS and about accepting or declining sequencing of their child as described for the "Well-Child" cohort above. Consent for Phase II (consent for sequencing) will be obtained from those who agree.

Decliners: As described above.
Sequence consenters: As described above.
Sequence decliners: As described above.

3.2.3.6 Study treatment and/or diagnostic procedures

3.2.3.6.1 Sample Collection and DNA isolation:

Saliva samples will be collected by trained study personnel using Oragene sponge collection kits. Sponges will be swabbed inside the cheeks and along the gums of the infant (Appendix 1).
Need to describe the procedures of collection of saliva (any known inferences such for foreign substances) and tracking/linking samples with participate.

- In the "Well-Child Cohort," arrangements will be made to contact us when the baby is born to obtain the sample. The PIs will be notified through EPIC at the time of the infant's delivery. The buccal swab will be obtained during the baby's stay at UNC Hospitals following his or her delivery or at a future well-baby appointment at a UNC clinic or at a postpartum clinic visit for the mother.

- In the "Diagnosed Cohort," the buccal swab will be obtained after parental consent to sequencing is obtained which could occur at the time of study visit or at a future in-person visit).

DNA will be isolated from duplicate samples using standard procedures in the UNC BSP and the CLIA-certified Molecular Diagnostic Laboratory (MSMI DNA extraction from OC-175 collection systems: see Appendix 2 and Mol Gen Newborn Saliva Extraction by BioRobot EZ1: see Appendix 3).

3.2.3.6.2 Exome Sequencing:

An aliquot from each uniquely coded DNA sample will be transferred by the BSP to the lab of Dr. Jonathan Berg, MD, PhD, Associate Professor in the Department of Genetics at UNC. Samples will be subjected to NGS using whole exome sequencing (WES) as described in section 2.1.1 (Agilent SSEL Automated Target Enrichments: see Appendix 5). Sequencing libraries will be transferred to the HTSF for massively parallel sequencing using the Illumina HiSeq 2000 or HiSeq 2500 platform (Appendix 6). Changes in technology may alter the choice of target capture or sequencing platform, which might affect the yield of positive results but would not affect the nature of results returned.

3.2.3.6.3 Bioinformatics:

Raw sequence data from the HTSF will be analyzed using standard bioinformatics methods to map sequence fragments and align them to the reference human genome. Genetic variants will be identified using a custom pipeline that has been developed in collaboration with colleagues in the Department of Genetics and the Renaissance Computing Institute (RENCI). The current pipeline is as follows:

- Fragments are aligned against an indexed reference human genome (NCBI 37.1 / hg19) using BWA
- Resulting SAM files are sorted, indexed, and converted to binary BAM files using Picard and SAMtools.
- Post-alignment optimization, including PCR duplicate removal, realignment of reads, and quality score recalibration are performed using The Genome Analysis Toolkit (GATK).
- Single nucleotide variants and small insertions and deletions are called using the GATK Unified Genotyper.
- Quality metrics will be incorporated so that coverage with quality scores can be assessed for any given nucleotide.

Genetic variants identified (approximately 100,000 per individual exome) will be deposited in a dedicated database and will be extensively annotated and subjected to *in silico* analysis. Annotations that will be applied to the variants and reviewed by the analysts who interpret the variants are as follows:

- RefSeq transcripts, with protein effects
- 1000 genomes project and Exome Aggregation Consortium (ExAC) variant frequency data
- Human Gene Mutation Database (HGMD) mutations and ClinVar pathogenicity assertions
- Additional annotations are possible, such as dbSNP entry, OMIM identifiers, evolutionary conservation, Polyphen and other protein prediction algorithms.
- Curated references from the biomedical literature.

3.2.3.6.4 Categorization of possible genomic information:

In order to evaluate the range of genomic findings, we utilize a framework for "binning" genes into categorical lists that will facilitate informed decision-making. We will utilize the following categories of genomic results in this study:

1. ***Next-Generation Sequencing Newborn Screen (NGS-NBS):*** Medically actionable childhood conditions, representing the core results that will be returned to all participants in the study. The NGS-NBS includes genes implicated in conditions that are currently screened for in standard state newborn screens, including metabolic disorders, endocrine disorders, and hearing loss. In addition, we will include other medically actionable conditions that are not amenable to current screening methods but can be detected using genetic sequencing (e.g. hereditary cancer susceptibility with onset or initiation of screening protocols in childhood). The criteria for determining which genes to include in the NGS-NBS are part of the overall aims of the research project (see below). These findings represent the default set of results that would be returned with every sequencing report. All parents consenting to sequencing of their child will learn if their child has one or more variants in this category that are determined to indicate with high likelihood that the child has or will likely develop a particular genetic disorder.

2. ***Additional genomic findings:*** Conditions that do not meet the threshold for inclusion in the NGS-NBS. Only parents randomized to the "decision" group will be asked to decide if they wish to learn any, all or none of these additional findings. Human curation and analysis of these variants will **not** be performed until parents request them. This analysis would **not** be done for children in the control group (see randomization procedure below). These additional findings fall into the following categories:

   A. *Medically-actionable adult onset conditions:* These disorders would be similar to the kinds of results described in NGS-NBS (above) but are related to conditions in which the onset or initiation of screening protocols occurs in adulthood, such as Hereditary Breast and Ovarian Cancer gene mutations.

   B. *Non-medically actionable childhood onset conditions:* The findings in this group relate to childhood health conditions that have no specific medical interventions. This category includes genes implicated in genetic disorders for which no specific preventive measure or treatment has been shown to mitigate morbidity. Examples include Rett syndrome and Angelman syndrome, conditions associated with intellectual disability in childhood for which there is no medical treatment, but for which early identification and initiation of therapy services are beneficial.

   C. *Carrier status:* This category relates to findings that have reproductive implications, such as carrier status for recessive disorders such as cystic fibrosis and Fanconi anemia.

3. ***Excluded genomic findings*** *(Non-medically actionable adult onset conditions):* In keeping with ethical norms in the field and to protect a child's ultimate autonomy, we have defined a process for choosing genes that would be excluded from analysis and would not be returned, regardless of the randomization status. Thus, **no participants will receive genomic results related to non-medically actionable adult onset conditions**. This category is exemplified by conditions such as amyotrophic lateral sclerosis (ALS).

3.2.3.6.5 Defining Clinical Actionability:

We have developed a semi-quantitative metric for scoring the actionability of gene-disease pairs in order to facilitate their assignment into "bins" used to guide the return of results (30). This framework has been adopted by the NC NEXUS project, and we have assembled a diverse group of experts and stakeholders to systematically assess genes implicated in Mendelian disease. This method assesses each gene-disease pair through the following five questions:

1) What is the nature of the threat to health for an individual carrying a pathogenic allele of the given gene? (Ranging from sudden death to no phenotypic impact)
2) What is the chance that this threat will materialize? (Related to penetrance)
3) How effective are interventions for preventing harm? (A critical component of medical actionability)
4) How acceptable are the interventions in terms of the burdens or risks placed on the individual? (Reflecting the possible hazards and downsides of medical intervention)
5) What is the knowledge-base regarding the nature of the disorder and its management in pre-symptomatic individuals?

Each gene-disease pair receives a score from 0 to 15, and we will determine a threshold level that indicates a level of medical actionability that justifies inclusion in the NGS-NBS.

In addition to the actionability score, each condition will be characterized in terms of the typical age of onset and age at which interventions would be initiated. Thus, we can generate a two-dimensional representation of the age-based actionability that can be used to define the four categories described above.

- Conditions that have an actionability score that exceeds the threshold and have onset of disease or interventions before age 18 would be considered candidates for NGS-NBS.
- Conditions that have an actionability score that exceeds the threshold but have onset of disease or interventions after age 18 would be included in the adult-onset medically actionable category. Many genetic conditions may have variable ages of symptom onset in either childhood or adulthood, such as Pompe disease, Krabbe disease, Fabry disease, or cardiac arrhythmias. For this reason, conditions such as these will be placed with childhood-onset conditions.
- Conditions that have an actionability score below the threshold but have onset before age 18 would be included in the childhood-onset non-medically actionable category.
- Conditions that have an actionability score below the threshold and onset after age 18 would be included in the adult-onset non-medically actionable category (and thus not eligible for return of results).

A list of conditions that have been scored by the NC NEXUS team as of the date of submission are provided in NC NEXUS_Actionability Scores (see Appendix 8). The tables in Appendix 8 document the initial work performed in the NC NEXUS project and previous work (Berg et al. 2015) to develop a semi-quantitative metric for assessing clinical actionability of gene-disease pairs. This list of 658 gene-disease pairs is a work in progress and we expect to curate > 200 gene-disease pairs by the end of the NC NEXUS project. Scores, may be update periodically to reflect progress in the evidence base or advances in management of different genetic disorders. These scores will be used, in combination with curated information regarding the age of onset or age at which interventions would occur, to define the four categories of genomic information defined in this protocol. Since the development of final lists conditions in each of these categories

is a primary outcome of the study, we anticipate that the work of binning each Mendelian disorder is expected to continue throughout the study period, and that periodic updates of the lists will occur. The FDA will be provided with a 5-day notice of any updates or changes in the lists that are implemented in the informatics algorithms.

3.2.3.6.6 Genetic Variant Interpretation and Reporting:

A member of the study team, acting as a molecular analyst, will conduct an initial review of the variant data, including review of quality metrics, visual inspection of variants, and review of the literature. The results of the analysis will be presented to the molecular sign-out committee (board-certified clinical geneticists, genetic counselors, clinical molecular geneticists and pathologists) for discussion. Final interpretations will be added as part of that variant's annotation in the database, so that future instances of that variant can be consistently assigned. The research team will review all variants identified as being possibly reportable (see below for detailed procedures). Those judged to be clinically relevant would be confirmed in the CLIA-certified MGL using the duplicate DNA sample.

Indication-based analysis

In the "Diagnosed Cohort," we will perform an "indication-based analysis" that evaluates variants in genes within a specific diagnostic list that is constructed so as to interrogate all known genes that could be related to a patient's phenotype. In the setting of a diagnostic evaluation, we will review all variants in genes that could be related to the phenotype, using a computational classifier to prioritize variants for analysis (Table 1). Since these individuals are already diagnosed with a rare genetic disorder, we will return variants that are deemed to be "pathogenic," "likely pathogenic," or "variant of uncertain significance," according to accepted practice guidelines developed by the ACMG. It should be noted that the computational prioritization is strictly intended as a way to facilitate human review of the data, and will not constitute an automated assessment of variant pathogenicity. For instance, it has been our experience in prior exome-sequencing related studies that many variants that had previously been identified as pathogenic in databases of human mutation have subsequent evidence calling this pathogenicity into question, supporting the need for manual review of variants even in this high-priority class.

Table 1: Computational classification of genomic variants to prioritize for human review

Class	Present in database of human mutations[1]	Variant Type[2]	Minor Allele Frequency (MAF)[6]
A	Yes	Any	<5%
B	N/A	Truncating[3]	<1%
C	N/A	Missense	<1%
D	N/A	Synonymous, Non-canonical splice site[4], and UTR[5]	<1%
E	N/A	Intronic	<1%
F	N/A	Truncating and Missense	1-5%

G	N/A	All other variants	1-5%
H	N/A	Any	>5%

1. Databases to be used in this computational analysis include HGMD and the NCBI ClinVar database. Variants that qualify for category A are those identified as "Disease Mutation" (DM) in the HGMD or variants identified as "Pathogenic" or "Likely Pathogenic" in ClinVar.
2. For the purpose of computational classification, the "variant type" will default to the most damaging effect for the variant among all of the transcripts represented in the RefSeq database. For example, if the variant has a missense effect in one transcript but is intronic or UTR in another transcript, it will be treated as missense for the purposes of computational classification.
3. Truncating variants include: nonsense, frameshifting insertions/deletions, and canonical splice site alterations (the first two and last two nucleotides of the intron).
4. Non-canonical splice site variants include those that occur within 3-10 nucleotides of the intron-exon border.
5. UTR variants are annotated as being located in the 5' untranslated or 3' untranslated regions of the mRNA.
6. MAF data will be derived from frequency data from the 1000 Genomes Project and ExAC; the highest of the minor allele frequencies for a given variant from any ethnic group will be used to evaluate the MAF threshold criteria.

Integral to the efficient diagnostic assessment of an entire genome or exome will be the establishment of *a priori* panels of genes to be assessed under certain clinical situations. One of the major tasks of the clinical and molecular teams will be the development of such lists relevant to the categories of disorders that are present in the Diagnosed Cohort. Once established, these lists will be used to query patients' variant data to identify all variants in genes of possible diagnostic significance in the context of their medical presentation. A molecular analyst will evaluate the prioritized variant list and provide a preliminary interpretation of the case to the molecular sign-out committee.

The committee will make a final pathogenicity determination and decide whether any variants exceed our threshold for reporting (Table 2). Because of the presence of a phenotype in the individual being sequenced, results considered to be clinically relevant would include the "known pathogenic," "likely pathogenic," and "variant of uncertain significance" as determined by the molecular sign-out committee.

Table 2: Categories of findings deemed reportable for an indication-based analysis

Result Category	Variant types	Zygosity	Phenotype[8]	Inheritance
Positive-Definitive	KP[1]	Heterozygous	Concordant	Dominant[9]
	KP	Homozygous or compound heterozygous[6]	Concordant	Recessive[10]
Positive-Probable	LP[2]	Heterozygous	Concordant	Dominant
	KP	Potentially compound heterozygous[7]	Concordant	Recessive
	LP	Homozygous or potentially compound heterozygous	Concordant	Recessive

Uncertain-VUS	VUS[3]	Heterozygous	Concordant	Dominant
	VUS,	Homozygous	Concordant	Recessive
	VUS plus KP/LP[4]	Compound heterozygous or potentially compound heterozygous		
Uncertain-AR het	KP/LP[5]	Heterozygous	Concordant	Recessive
Uncertain-Contributory	Any	Any	Partially matching	Any

1. KP = Known Pathogenic
2. LP = Likely Pathogenic
3. VUS = Variant of Uncertain Clinical Significance
4. In conditions with recessive inheritance, we may identify one KP or LP variant and one VUS. In this situation, we would report the findings as a type of "Uncertain" result due to the presence of one VUS allele. Parental studies would be requested to determine the phase of the variants.
5. In conditions with recessive inheritance in which we only find a single KP or LP variant, but are unable to identify a second candidate variant, we will report this finding as a type of "Uncertain" result due to the possibility of a missed exonic or partial gene deletion on the opposite allele.
6. With NGS technology, it may not be possible to determine the phase of two variants that are identified. When possible, we will use data from the aligned sequence reads to determine phase. If the two variants can be shown to be on opposite strands using NGS data, they will be reported as "Positive-Definitive" with parental studies requested for confirmation of phase.
7. When aligned sequence data are unable to determine the phase of two candidate variants, they will be deemed "potentially compound heterozygous" and reported as a "Positive-Probable" result until parental studies can be performed to determine phase.
8. If the phenotype of the diagnosed individual matches with the condition predicted by the genetic results, this will be considered a "concordant" result. However, if the phenotype of the diagnosed individual only partly matches or is incompletely explained by the genetic results, this will be considered a "partially matching" result. In cases with "partially matching" phenotype, the result will default to an "Uncertain-Contributory" result communicated with appropriate caveats.
9. Dominant inheritance includes both autosomal and X-linked dominant conditions. For X-linked dominant conditions relevant variants would be hemizygous in males and heterozygous in females.
10. Recessive inheritance includes both autosomal and X-linked recessive conditions. For X-linked recessive conditions, relevant variants would be expected to be hemizygous in males and homozygous or compound heterozygous in females.

<u>NGS-NBS and Additional Genomic Findings</u>

In the "Well-Child" cohort there will be no phenotype to inform a diagnostic list. In addition, all conditions unrelated to the phenotype known for patients in the "Diagnosed Cohort" would be considered "incidental" to their primary indication for sequencing. Therefore, the analysis of the NGS-NBS list and additional genomic findings will be more akin to screening than diagnostic testing. In this setting, the prior probability that an individual has a rare Mendelian disorder will be very small (based on the population prevalence of the disorder), and thus the positive predictive value of genomic information will be strongly influenced by the specificity of the results. Both the "Diagnosed Cohort" and "Well-Child Cohort" will receive results from the "NGS-NBS" gene list. In order to minimize false positives, we will use stringent criteria for return of results and will only return variants that are deemed to be "pathogenic" or "likely pathogenic" and consistent

with the expected inheritance pattern of the condition (eg. homozygous or presumed compound heterozygous variants in the case of a recessive condition).

Variants will be computationally selected for their presence in a gene on the NGS-NBS list, their likely pathogenic significance and, in the case of recessive conditions, whether one or two mutations are present (34). Given the large number of possible genomic findings, and the low *a priori* likelihood that individuals in the "Well-Child" cohort would be affected with any given rare genetic disorder, it will be critical to strike a balance between the "sensitivity" and the "specificity" of the analysis so as to correctly identify individuals at high risk for a treatable genetic condition without overwhelming the molecular analysts, MGL, and clinicians with large numbers of variants of uncertain significance. This analysis will utilize the same computational classes as described in Table 1, with additional informatic filtering to determine which variants qualify for human review. We will review variants that satisfy the following conditions:

1) For genes associated with conditions inherited in a dominant fashion, we will review any variants in computational classes A and B;
2) For genes associated with conditions inherited in a recessive fashion, we will review variants in cases where two or more variants from computational classes A, B, or C are present.

Cases fulfilling these criteria will undergo review by a molecular analyst and the molecular sign-out conference, and only variants determined to be "known pathogenic" or "likely pathogenic" and consistent with the expected inheritance pattern would be returned in the context of NGS-NBS. All other cases will be reported as "negative." This approach is an inherently conservative one. We recognize that the strict thresholds outlined above will inherently have imperfect sensitivity for detecting clinically relevant variants, but at the same time this approach will have higher specificity and therefore protect against false positive results. This is also a pragmatic approach, since it will be impossible for a human to comprehensively review each of the many variants that will be identified in every individual. Thus, the balance we are striving to achieve is to maximize clinical sensitivity and specificity, while minimizing the effort required of a human molecular analyst.

3.2.3.6.7 CLIA Confirmation:

All results deemed reportable in the NC NEXUS study will be confirmed by orthogonal methods (Sanger sequencing) in the CLIA-certified UNC Hospitals MGL (Mol Gen Custom DNA Sequencing, see Appendix 7). A clinical report will be generated and approved by a board-certified molecular geneticist or pathologist. This result will be provided to the parents (see Section 3.2.3.6.9) and will be eligible to be included in the electronic health record (NC NEXUS NGS-NBS Electronic Medical Record (EMR): Appendix 20 and NC NEXUS Additional Results Electronic Medical Record (EMR) Consent: Appendix 21).

3.2.3.6.8 Randomization:

After sequencing and analysis of the indication-based analyses and/or NGS-NBS are complete, the couple will be randomized into a "decision group" or a "control group". The couple will be contacted to arrange a return of results visit with a geneticist and genetic counselor.

Randomization will be computer implemented using permutated block randomization with blocks of randomly varying size. Participants will be stratified for block randomization based on three parameters: study cohort ("Diagnosed Cohort" or "Well-child Cohort"), language preference ("English" or "Spanish"), and the relationship status of the parent(s) giving consent ("Single" or "Couple"). In this way, we will achieve optimum randomization within each of these groups.

Those in the "decision group" will be given information about this visit and future study activities. In addition, they will also be given access to a supplement to the electronic decision aid that explains the three categories of additional genomic findings (adult-onset medically actionable, childhood-onset non-medically actionable, and carrier status), and how such information might be of potential benefit or harm**.** They will be able to decide which categories of additional findings to learn (all, none, or any combination of some of the categories). We will encourage parents to use the aid at home prior to the return of result visit. During the visit, they will have the opportunity to discuss the information about the categories from which they can request results and how to communicate their decisions about requesting categories of additional genomic information. They will be asked to sign a form that documents which categories of results they have requested.

The "control group" will not have the option to request the "additional genomic findings" and will not receive access to the supplement to the electronic decision aid. They will only receive information about the upcoming return of result visit.

3.2.3.6.9 Return of Results Visit:

Qualified genetic professionals (physicians and genetic counselors) who are part of the research team will meet with the couples after sequencing is complete to disclose clinically confirmed variants that meet a high bar for evidence of pathogenicity. This form of return of results accompanied by comprehensive genetic counseling is the gold standard in genetic testing, and will be complemented by parental utilization of the decision aid and genetic counseling during the first study visit. Negative results will be accompanied by a discussion of the limitations of NGS-NBS, essentially communicating the caveat that a negative result does not fully rule out the possibility of any health conditions developing in the future, as is the case with any screening test. Clinically confirmed results will be summarized by a laboratory report given to the couple at the visit. They will be asked whether or not they consent to having the results placed into the child's medical record and will sign a form indicating this decision. A clinical follow-up plan for all results will be established with the parents (see section 3.2.3.7.2).

3.2.3.6.10 Subsequent Return of Additional Results (Decision Group only):

Election to receive any additional results will trigger an independent analysis of genomic data and CLIA confirmation. Couples requesting results from the carrier status category will be able to learn these results during a scheduled phone call with a genetic counselor. Requests for the other two categories will trigger a second return of results visit with a geneticist and genetic counselor. Couples in the "decision group" will be asked to complete a brief post-decision questionnaire (the Time 2A questionnaire) after they decide whether or not to request additional genomic findings to assess the consequences of having this option.

3.2.3.6.11 Follow-up Questionnaires:

All participants will complete two follow up questionnaires following the return of result visit; a Time 3 questionnaire completed within 2 weeks of the visit and a Time 4 questionnaire completed 3 months after the visit. Both will assess the short- and longer-term consequences of decision-making and results disclosure.

Couples who do not complete a questionnaire in a timely manner may be offered the option of completing questionnaires in a telephone interview. Questionnaires will be administered in Qualtrics, accessed via a computer or mobile device, with paper and pencil versions of the questionnaires available to those who prefer not to complete them online.

3.2.3.7 Follow-up procedures

3.2.3.7.1 Revised results:

Advancements in medical genetics and our understanding of variant pathogenicity will continuously evolve and thus impact the clinical interpretation of variants identified in the study participants. One scenario that can be anticipated is that the pathogenicity assessment for a particular variant may change over time. This means that the initial classification of a variant may be superseded by a subsequent reclassification. This could mean that a result that was previously "negative" could change to "positive" if a variant initially classified as VUS is reclassified as pathogenic. It is also possible that a variant initially classified as "pathogenic" or "likely pathogenic" would be reclassified to VUS. In this scenario the previous "positive" result could change to "negative." These types of revised result will be communicated to participants without notification of the FDA.

In addition to the expected reclassification of variants, there are other types of revised results that we can anticipate. Over time the association of more genes with diseases and the development of prevention or treatment will result in reassignment of loci and lead to changes in the interpretation of sequencing results. In addition, advancements in sequencing technology, bioinformatics analysis, and variant assessment will inherently necessitate periodic alterations of the established analytic pipelines. Among the major research activities of the NC NEXUS project will be the refinement of the genes/loci that

are assigned to each category of genomic information (as defined above), evaluation of new bioinformatics algorithms, and the criteria used to assess pathogenicity of variants.

- Advancements in the science of medicine will continuously add to our knowledge of the genetic underpinnings of disease. Although most of the inborn errors of metabolism that will be present in the "diagnosed cohort" have definitively established genetic etiologies (eg. PKU, MCADD), other conditions represented in this cohort (eg. hearing loss) are still subject to active investigation. Therefore, the diagnostic lists utilized for the "indication-based analysis" will be updated periodically to include newly discovered genes, when the research team deems those discoveries to have sufficient clinical validity. Similarly, new treatments and management strategies will be defined for many genetic conditions, thus changing their potential clinical actionability. Therefore, the list of genes that constitutes the "NGS-NBS" may be updated to account for such advancements. Finally, newly discovered genetic conditions will be reviewed and assigned to other categories of genomic information (adult-onset medically actionable, childhood-onset non-medically actionable, and carrier status) as appropriate.

- Advancements in computational processing and analysis of NGS data will continuously improve the analytic validity of variant calling pipelines, improving the sensitivity and specificity of the variants that are identified. Therefore, we will utilize the raw sequence data generated through the NC NEXUS project (and other ongoing projects at UNC) to evaluate new informatics pipelines. These analyses will be performed in parallel with the established procedures described in section 3.2.3.6.3 above, and only when the research team identifies substantially improved performance will the pipelines described in section 3.2.3.6.3 be updated.

- Optimizing the criteria for analysis and reporting of genomic variants in the context of NGS-NBS is a core research question for the NC NEXUS project, and thus defining the most effective informatics algorithms is expected to be an ongoing task. For example, one goal of the research project is to investigate informatics algorithms that can be used to select variants for human review, optimizing the clinical sensitivity and clinical specificity while minimizing human workload. Therefore, we will utilize the variant data generated through the NC NEXUS project (and other ongoing projects at UNC) to evaluate these algorithms, in parallel with the procedures described in section 3.2.3.6.6 above. One example will be the development of algorithms that can evaluate variant annotations in order to satisfy specific criteria for pathogenicity assessment, in order to provide a more accurate preliminary classification than the computational classifier described in Table 1. The algorithms will be updated only when

the research team is satisfied that an updated informatics algorithm is superior.

If, as a result of any of the advancements described in the bullets above, the research team determines the need to update gene lists, bioinformatics pipelines, variant analysis algorithms the FDA will be provided a 5-day notice. All participants analyzed using the previous version of the protocol will have their data reanalyzed, and in the event that any new findings qualify for return of results, they will be confirmed in the MGL. Parents will be re-contacted that the results they have received during the study have changed as a result of reanalysis.

Thus, by design there will be developmental changes in the protocol, accompanied by periodic reanalysis of the NGS data, with the possibility that the results of the analysis may change over time and some participants will be re-contacted for updated results. This situation will be clearly described in the informed consent.

3.2.3.7.2 Longitudinal follow-up of results:

The downstream implications of positive results are of great interest for the NC NEXUS project. We will therefore plan to engage in longitudinal follow-up for the duration of the study (as long as funding allows). This follow-up will include both clinical follow-up and psychosocial evaluations of parents.

Clinical follow-up

The clinical follow-up of participants in the NC NEXUS study will depend on the cohort to which they belong and the type of result they receive.

- ***Previously known diagnoses:*** Participants from the "Diagnosed cohort" will already have established standard-of-care follow-up through the specialty clinics from which they are recruited, and their participation in the study will have no impact on this ongoing clinical care. Results will be provided to the parents by a certified genetic counselor and MD medical geneticist, and communicated to their clinical providers via secure messaging. Any variants that are confirmed in the CLIA lab will be eligible for placement in the EHR. The findings may be utilized to guide care, but this would be entirely at the discretion of the established clinical providers. In this case, clinicians associated with the NC NEXUS study will serve as consultative resources for clinical providers but will not direct the care of the patients. Study personnel will actively monitor the EHR as part of an observational study to track how genomic findings are utilized.

- ***Previously unknown diagnoses:*** Participants in both the "Well-child cohort" and "Diagnosed cohort" will have the potential to receive positive findings from NGS-NBS or other additional categories of genomic information that will represent new information (i.e. unrelated to a diagnostic indication). These results will be provided to parents by a certified genetic counselor and MD medical

geneticist, and a standard-of-care clinical follow-up plan will be established that is appropriate for the finding. This customized plan may involve long-term monitoring by a pediatrician, diagnostic imaging, other screening tests, and referral to specialists. This follow-up plan will be part of the patient's clinical care. Clinicians associated with the NC NEXUS study will serve as consultative resources for clinical providers but will not be directly responsible for the care of the patients. Study personnel will actively monitor the EHR as part of an observational study to track how genomic findings are utilized. In order to mitigate medical risks in this population, study providers will determine specific benchmarks (depending on the individual finding) that will be evaluated to ensure that appropriate follow-up is being given. Study personnel will track these benchmarks and, if a benchmark is not met, we will communicate with the parent to determine why and to develop an alternative clinical follow-up plan depending on the situation.

- ***Negative results:*** Most participants in the "Well-child cohort" and some participants in the "Diagnosed cohort" will have negative findings. Results will be provided to parents by a certified genetic counselor and MD medical geneticist, parents will be counseled regarding the small chance of a false negative result, and the patients will undergo routine clinical follow-up with their providers. Study personnel will passively monitor for rare cases of false negative genomic results by inviting parents to contact the study in the rare event that symptoms of a genetic disorder develop in their child.

Outcomes will be tracked in every participant by way of a chart review performed at the end of the study period. This review will include developmental outcomes, clinical events specific to any diagnoses, and results of any screening or diagnostic tests.

3.2.3.7.3 Indication-based reanalysis:

All parents in the study will be given an opportunity to request an "indication-based analysis" should symptoms of a genetic disorder arise in their child. We anticipate that such requests will be rare. If a request is made, an appropriate diagnostic list will be developed (depending on the child's symptoms) and molecular analysis will be performed in accordance with section 3.2.3.6.6 above. Results will be confirmed in the CLIA lab and provided in accordance with section 3.2.3.6.7 above.

3.2.3.8 Schedule of activities

The schedule of activities is shown in section 3.2.2.2.

The enrollment telephone call will take an estimated 15 minutes to review the parents' participation consent form and schedule Visit 1.

- Visit 1 will take an estimated 30 minutes in order to allow parents to have any questions answered and make a final decision about whether or not to accept

NGS-NBS for their child. We expect that this encounter will be facilitated by the parents having access to the electronic decision aid prior to the visit.

- Visit 2 will occur approximately 3-4 months after Visit 1. For most participants in the "Diagnosed Cohort" the return of results is likely to take 30 minutes to review the indication-based analysis. For >95% of participants in the "Well-Child Cohort" the return of results is likely to take 15 minutes or less for negative results, whereas for the small number that do have a positive finding the return of results could take 30-45 minutes to provide contextualized information and recommend a follow-up plan. For the two-thirds of participants randomized to the "decision group" we estimate that 30 minutes will be needed to review any questions the parents have about the additional categories of genomic information that they may decide whether or not to learn.

- Visit 3 will occur approximately 1-2 months after Visit 2. The length of the visit will depend on how many categories of additional information are requested. We predict that the most typical result will be positive carrier status for 1-4 conditions and negative findings for the other two categories (adult-onset medically actionable, childhood-onset non-medically actionable). For this type of visit we estimate 30 minutes for return of results. In the rare event of a positive finding in the adult-onset medically actionable or childhood-onset non-medically actionable categories we would expect the visit to last 60 minutes or more if needed.

3.2.4 Study outcome evaluations

3.2.4.1 Study endpoints

Primary Objective 1: Scientific endpoints for this objective will be a.) generation of whole exome sequence data and variant call files, b.) analysis of variants to determine whether any disease-causing variants exist, c.) confirmation of any suspected variants in the CLIA laboratory as a measure of analytic validity, and d.) comparison with clinical data to evaluate the sensitivity and specificity of sequencing.

Primary Objective 2: Scientific endpoints for this objective will be a.) curation of gene-disease pairs to define clinical actionability, b.) determination of an actionability threshold for inclusion in the NGS-NBS gene list and definition of the categories of additional genomic information, c.) return of results and observational study of patient outcomes and integration of genetic findings into clinical care.

Primary Objective 3: Scientific endpoints will occur when parents consent to: a.) participate in the NC NEXUS study b.) receive genetic sequencing results (NGS-NBS) for their child c.) if randomized to the "decision group", decide whether to receive results in addition to NGS-NBS. An additional endpoint will occur if clinically significant variants are returned to parents. The final endpoint will take place when parents complete a series of quantitative measures to assess a range of factors related to

participation in the study. Please refer to the list of study measure in NC NEXUS Project 3 Longitudinal Study Measures: see Appendix 19).

3.2.4.2 Sample size determination

This project is one of a consortium of NICHD/NHGRI –funded "NSIGHT" projects. Given that this is an exploratory study, a formal power calculation cannot be performed. We expect to be able to perform joint analyses of data across the consortium to address certain questions that may require larger sample size.

In addition, the study was designed to address the ELSI (Ethical, Legal, and Social Implications) research questions related to the impact of NGS on patients and their families. Using PASS software, we estimated the statistical power for a multiple regression model of mothers' mean scores predicting the decisional conflict scale with two predictor variables (study group [well- and diagnosed-child groups] and race/ethnicity [Black, White, and Hispanic]) and 10 control variables (e.g., demographics, health literacy, trust in medical community) that account for 20% of the variance in scores, assuming a p-value of 0.05. We assumed the sample would be split equally across racial/ethnic groups, consistent with the distribution in our study population. If study group and race/ethnicity account for 2% or more of the variance after controlling for the other variables, we will have statistical power of at least 82%, indicating acceptable power for comparisons by study group and race/ethnicity. We also examined power for detecting differences in decision to screen across study groups. We estimated power for a logistic regression comparing decision to screen between the two study groups, assuming the well-child group has approximately 70% probability of accepting NGS-NBS (based on our Fragile X NBS study) and using a p-value of 0.05. On the basis of these assumptions, we would have 83% power or higher to detect at least a 12% difference in probability of agreeing to screening between the two study groups, which corresponds to an odds ratio of 2.0.

3.2.4.3 Outcome data and data analysis

Primary Objective 1:

A. Generation of whole exome sequence data: Datasets will include raw FASTQ short read files, aligned BAM files, and VCF variant call files. Variants will be annotated and deposited in a local database as described in 3.2.3.6.3.
B. Analysis of variants: Curated clinical significance of individual variants will be stored in the annotated database. Final case-level results (according to Table 2) for each patient will be recorded. Types of mutations that are detected will be characterized in aggregate.
C. CLIA confirmation: Results from NGS will be compared with Sanger results in the CLIA lab to assess the false positive rate (analytic specificity) of NGS.
D. Clinical sensitivity and clinical specificity: Calculations of test sensitivity and specificity will be performed based upon the diagnostic result and the clinical follow-up.

Primary Objective 2:

A. Clinical actionability curation: Curated literature review data for each gene-disease pair will be stored in a REDCap database. Final scores determined by the binning committee will be recorded and analyzed.
B. Definition of categories of genomic information: Based on curation of age of onset and actionability, the binning committee will determine thresholds that define the four categories of genomic information as described in 3.2.3.6.4.
C. Observational study of outcomes and integration of findings into clinical care: Longitudinal outcomes data will be collected in a REDCap database and analyzed.

Primary Objective 3:
Consistent with the primary research questions for this objective, which focus on ethical use of NGS-NBS, we specify the following primary independent variables: Race/ethnicity (non-Hispanic White, Black, Hispanic), health literacy, and child status (diagnosed vs. well). We specify the following primary dependent variables: sequencing-related distress, knowledge about NGS-NBS and (for the "decision group" only) about additional genomic results (continuous variables), decision to accept or decline NGS-NBS and (for the "decision group" only) additional genomic results (dichotomous variables), and decisional conflict (a continuous variable). Primary analyses will focus on these outcomes in mothers. These analyses involve a between-within design and will be analyzed with mixed linear modeling to accommodate nesting (i.e., assessments nested within participants). Models with continuous outcomes will be implemented with SAS PROC MIXED because (a) it can handle nested data; (b) it handles missing data more appropriately than repeated-measures analysis of variance, which uses listwise deletion of missing data; and (c) it provides a wider range of options for modeling the error covariance structure than general linear model procedures, which assume an error structure that is often unrealistic. Models with dichotomous outcomes will be implemented using mixed-effects logistic regression within SAS PROC GENMOD, which shares strengths similar to those offered by PROC MIXED.

3.3 Risk Analysis

The NC NEXUS research study was launched in response to a request from NIH (NICHD and NHGRI), to perform research studies to investigate next-generation sequencing studies in newborns. Although some ethical guidelines in the past have raised concerns about testing children for adult onset conditions, there are few, if any, studies looking at the outcomes of such testing. In order to satisfy the directives for obtaining additional information about this, we have proposed the NC NEXUS study to provide research data to begin to answer some of these questions.

Parents enrolled in the study will not be exposed to any significant physical or social harms. It is possible that they could experience psychological distress due to making decisions about having sequencing for their child, or deciding about whether to learn certain categories of information. One of the objectives of the NC NEXUS project is to study precisely these types of impacts that might accompany genomic sequencing of newborns. We estimate that 80% of the family units will be couples and 20% will be

single parents, so with enrollment of 400 children in the study we expect to enroll 720 parents (400 female, 320 male). The majority will be between 20-40 years of age.

Children and newborns in the study will be exposed to theoretical physical, social, and psychological harms that have been discussed and debated in the medical literature. An additional source of risk relates to positive results (both true positive and false positive) and false negative results. Again, assessment of the magnitude of these potential risks is a major overarching goal of the NC NEXUS project. We estimate that the distribution of 400 children and newborns ages 0-5 enrolled in the study will be roughly 50% female and 50% male.

3.3.1 Anticipated Risks

Potential risks to which the subjects (parents and their children and newborns) will be exposed as a result of their participation in the clinical study can be divided between generic risks that are inherent to human subjects genetic research and risks that are specific and unique to the NC NEXUS project.

The investigators are well aware of guidelines, opinions, and arguments regarding genetic testing in children for adult-onset conditions (2, 6 and 38). Although concern has been raised regarding potential harms (vulnerable child syndrome, genetic discrimination, parental bonding, among others) there has been a dearth of studies that have tracked whether these actually occur. Use of next generation sequencing raises this to a higher level of importance. Additional research in this area has been recommended by stakeholders (48). This is one of the Primary Objectives of this research study that the National Institutes of Health has deemed important to fund.

3.3.1.1 Physical Risks

Discomfort and distress

Risk: Saliva samples will be obtained from infants and children by use of a sponge that will be swabbed along the cheeks and gums. The degree of discomfort is expected to be minimal but, as in any newborn who is disturbed, could cause crying. This sampling procedure was chosen for this study to minimize infant discomfort (compared to venipuncture or heel-sticks).

Mitigation: We will obtain the specimens as quickly as possible (estimate 5-10 minutes) by a nurse with extensive experience handling newborn infants. If the newborn cries excessively during the process of sample collection the collection will be stopped. If insufficient sample (e.g. only one) has been obtained then samples from this infant will not be included in the study.

Effectiveness: Highly effective

Complications of medical management

Risk: Infants and children in the study may receive "positive" genetic findings that indicate a need for medical intervention (longitudinal care, screening tests, procedures). In rare cases, such follow-up may lead to unnecessary interventions in the case of false positives, or complications of interventions in both false positives and true positives. Participants in the "Diagnosed" cohort will all be receiving ongoing standard clinical care in their respective clinics, and the genetic results are not expected to create any additional risk. We expect that <3% of participants will have previously unknown findings from the NGS-NBS screen or the optional additional categories of genomic information (Amendola et al. 2015).

Mitigation: Any children in the study found to have additional findings will be referred for standard of care clinical management.

- Adherence to strict and conservative definitions of "pathogenic" and "likely pathogenic" variant classifications, and overall rules for reporting "positive" findings (as described in 3.2.3.6.6 and Table 2) will maximize specificity and reduce the chance of false positive results.
- False positives will also be minimized in some conditions in which confirmatory clinical testing is available (eg. biochemical assays or enzyme testing).
- All participants with positive NGS-NBS findings will have a standard-of-care clinical follow-up plan established and will be referred to the appropriate specialists for surveillance or treatment.
- Drs. C. Powell and B. Powell are both pediatricians as well as medical geneticists and have experience in appropriate medical follow-up and referral to specialists for children with genetic disorders.

Effectiveness: Moderately effective. Once a participant has embarked on standard-of-care medical follow-up, we cannot further mitigate the risk of complications that may occur.

Failure to diagnose

Risk: Because next-generation sequencing will not achieve 100% sensitivity, there may be participants in the study with false negative results. In addition, the selection of conditions for NGS-NBS will only include a small subset of all genetic disorders. Thus, some participants may not be diagnosed with a condition that is present or will manifest in the future. This is an extremely unlikely outcome for participants in the "Diagnosed Cohort" who have known diagnoses. In addition, due to the very low prevalence of other genetic conditions, it is highly unlikely that any participants in the "Well-Child cohort" will have such a condition. This risk is therefore extremely low.

Mitigation: Participating in the NC NEXUS study does not create any additional risk of a missed diagnosis than any other child in the general population, since they will have equivalent routine standard of care as would any other child in the general population. In addition, elements of the study design will mitigate against this risk:

- Continual improvement of bioinformatics pipelines and variant interpretation procedures, with periodic reanalysis, will reduce false negatives by enhancing the sensitivity of the NGS-NBS.
- Parents will be offered "indication-based reanalysis" if symptoms develop, thus potentially allowing them to arrive at a diagnosis faster than if they were not participating in the study.

Effectiveness: Highly effective.

3.3.1.2 Social risks

Confidentiality

Risk: Loss of confidentiality (personal health and genetic information) due to inadvertent disclosure of genetic findings could lead to adverse personal psychological (moderate, rare) or financial impact (e.g. inability to obtain health or life insurance (moderate, rare), or social harm (moderate, rare).

Mitigation: We will apply all reasonable measures to ensure confidentiality for research subjects.

- Signed consent forms will be stored in a locked office.
- Samples (saliva, isolated DNA, sequencing library samples) and *in silico* data (raw sequencing data, alignment files, called variants) used in this study will be identified using a unique study ID number.
- A password-protected secure REDCap database managed by NC TraCS will contain the link between the patient identity and the study ID number, but the research laboratory will not have access to patient identifiers. Subject identifying information will be accessed only by study personnel with a "need to know" identifying information for the purpose of implementing the study.
- In the clinical laboratory, identifying information will be protected in the same manner as all other clinical samples maintained there. Final genetic test reports (which do include the participant's identifying information) are handled via the UNC Hospitals Molecular Diagnostic laboratory according to CLIA standards. Digital copies of the final reports will be password protected and paper copies will be stored in locked cabinets in a secure office space.
- Any genetic test results that are entered into the electronic medical record after parental consent will have the same HIPAA protections as any other medical information.
- Participants' responses to questionnaires will be entered into our secure database and identified only by their ID number.

Effectiveness: Highly effective

Financial

Risk: There is always a risk that genetic findings could result in financial risk such as loss of insurance or employment, however that risk is very low, both in general and in the current study. Some participants will receive genetic test results that may diagnose a particular condition or indicate that the participant is at-risk to develop a condition in the future.

- For participants in the "Diagnosed cohort," the risks are not greater than they would be if the participant were having clinical testing. The risk is somewhat greater in sum because more genes are being analyzed and because of the small chance of an additional genomic finding with significant clinical implications.
- For participants in the "Well-Child" cohort there is a very small chance (<3%) of a finding in the category of "NGS-NBS" results. However, since these findings will be clearly actionable from a medical standpoint, there would be a significant medical benefit to the subject any time such a finding is revealed.
- For participants randomized to the "decision group" of the study, the risks of disclosing information about additional genomic findings are not known.

Mitigation: Federal legislation called the Genetic Information Nondiscrimination Act (GINA) prohibits the use of genetic information to discriminate against individuals in employment and health insurance settings. The informed consent process, as well as, the consent forms (see NC NEXUS_consent_phase II_diagnosed cohort: Appendix 16 and NC NEXUS_consent_phase II_Well-Child cohort: Appendix 17) will include a discussion of the benefits and the limitations of GINA. There are also North Carolina state laws to protect against genetic discrimination. A major provision of The Affordable Care Act (ACA) of 2010 prohibits issuers of health insurance from discriminating against patients with genetic diseases by refusing coverage because of 'pre-existing conditions'. Parents will be informed that current laws that protect against genetic discrimination do not apply to life insurance, disability insurance or long-term care insurance (NC NEXUS_consent_phase II_diagnosed cohort: Appendix 16 and NC NEXUS_consent_phase II_Well-Child cohort: Appendix 17). Extensive genetic counseling will be provided to parents regarding their significance of any findings and recommended clinical follow-up. Clinically serious adult-onset genomic findings for which there are no available treatments or preventive strategies (for example, early onset dementia) will not be returned to parents.

Effectiveness: Uncertain. GINA does not protect some "optional" forms of insurance, such as disability, life or long-term care insurance, so there is the potential that participation in this study could affect participants' future insurability for these insurance types. In addition, the law does not apply to the U.S. military. Like GINA, the ACA does not apply to non-health insurance types.

Group Harm

Risk: Because we are including ethnic minorities there is a chance that some genetic findings might be reported as linked to a particular racial or ethnic group, producing what has been described as "group harm." The likelihood of this is estimated to be rare.

Mitigation: We, as clinicians and researchers, are sensitive to this issue and will endeavor to avoid such issues when reporting genetic findings of the study.

Effectiveness: Highly effective

Family Dynamics
Risk: As with any genetic information, there is a possible risk that family members may respond negatively to genetic information that was learned during the course of the research project. This is true of any genetic testing (both clinical and research).

Mitigation: Professional genetic counseling will be provided as part of the informed consent and return of results.

Effectiveness: Moderately effective

3.3.1.3 Psychological risks

Parental emotional stress due to study participation
Risk: There is a slight risk of parents experiencing uncomfortable emotional states by completing the psychosocial assessments that ask some personal questions about quality of life and experiences of receiving diagnostic results or incidental findings. The degree of this risk is estimated to be rare to infrequent.

Mitigation: Because study interviews and questionnaires were chosen to reflect what are likely to be preexisting concerns, the study assessments are not expected to markedly increase participants' psychological distress. The project team also has had extensive experience in conducting assessments with individuals and families who receive genetic testing and findings from those tests. Before beginning the assessments and interviews, subjects will be reminded that they can stop the interview at any time, or choose not to answer specific questions.

Effectiveness: Highly effective

Parental distress or anxiety regarding positive genomic findings
Risk: The chief risk to parents participating in this study is anxiety or distress from having learned of genomic information about their child that predicts disease risk or reveals a predisposition to a disorder for which there is no currently effective intervention. For parents of participants in the "Diagnosed cohort," the risk of any additional distress or anxiety as a result of study participation is minimal. Parental distress or anxiety is more likely when unexpected genomic findings are returned.

- Diagnostic Findings: The return of results related to a known diagnosis is straightforward and non-controversial. Emotional distress is possible any time that parents learn their child has a genetic condition. However, parents

participating in the "Diagnosed cohort" already know that their child has a disorder with a likely genetic etiology, and research indicates that risk is minimal when genetic information is relayed by a genetic counseling team in an appropriate setting.

- NGS-NBS Findings: For parents of participants with positive NGS-NBS findings (not related to a known diagnosis), there is risk for an adverse psychological impact. We expect that the degree of anxiety and distress would be equivalent to that of parents whose child receives a positive standard newborn screen result. This psychological reaction is likely to be tempered by the medically actionable nature of the findings.

- Additional genomic findings: For parents randomized to the "decision group" who choose to learn about carrier status in their child, we expect that the psychological impact will be minimal, and similar to that of parents who learn that their child is a carrier of Cystic Fibrosis or Sickle Cell anemia through the standard newborn screening program. For those who choose to learn additional diagnostic information about their child and subsequently receive unexpected information indicating their child has a genetic health risk, the magnitude of psychological distress is unpredictable and depends on the parent and the findings. However, the chance of such findings is very small.

Mitigation: The risks associated with parental responses to genetic information about their child are complex and form the basis of the need for this research project. We will provide genetic counseling and referral to specialists as needed for any positive results.

- All study team members who have contact with study participants are already trained (physicians and genetic counselors) to recognize and probe indicators of possible distress (e.g., participants' description or display of distress-related symptoms).
- We will collect data on distress (depressive symptoms, anxiety) before return of results (to establish baseline levels) and after return of results, allowing us to examine changes in distress over time.
- The decision aid is being designed to help families understand the risks and benefits of study participation and make an informed choice based on their values and preferences.
- Parents are also able to change their minds about any choices made as part of the study before information is returned to them.

Effectiveness: Moderately effective

Parental decision regret

Risk: Parents in the study may experience regret about the decision they make with regard to having their child sequenced, or their choices to receive or not to receive certain categories of additional genomic information. Our experience in the clinical setting indicates that emotional distress requiring referrals is rare or infrequent. Our experience

in the clinical setting indicates that emotional distress requiring referrals is rare or infrequent. In an earlier study (18) there were no significant differences between 18 mothers of screen-positive infants with Fragile X premutation and 18 comparison mothers on measures of anxiety, depression, stress, or quality of life. A subset of mothers experienced clinically significant anxiety and decision regret, but factors associated with these outcomes could not be identified. Greater spousal support was generally associated with more positive outcomes.

Mitigation: Decision regret will be measured after return of results. Scores will be analyzed to detect clinically meaningful increases (0.5 standard deviations or more, according to research on clinically meaningful changes and changes that are noticeable to research participants).

- The decision aid should minimize regret by enabling informed choices based on the parents' values and preferences.
- Any participants flagged based on these monitoring methods will be discussed with the study team, which includes clinical geneticists, certified genetic counselors, and psychological researchers with expertise in psychological distress.
- For any parents who do experience distress, research suggests it would likely involve anxiety and decision regret rather than depressive symptoms or poor quality of life, suggesting that additional counseling would be a first-line response to resolve the distress, psychological counseling or similar referrals may be indicated.

Effectiveness: Highly effective

Psychological impact on child/infant participants

Risk: Return of unexpected results in the context of testing a minor raises new and challenging issues regarding the protection of human subjects. These are mostly theoretical risks, without a great deal of empiric data to indicate the magnitude or likelihood of these risks. As such, one of the key goals of the NC NEXUS project is to begin to provide evidence on the psychological impact of genetic testing in children. The most common type of "unexpected" result (unrelated to a participant's diagnosis) in this study will be carrier status for a recessive disorder, which we expect to have a very low chance of having a detrimental psychological impact. Other potentially more concerning findings that would indicate the likely future onset of disease will be much less likely, estimated at <3% of the cohort. Possible psychological risks include:

- Vulnerable child syndrome in which genetic findings exacerbate childhood developmental or adjustment problems
- Abandonment or neglect of the child as a result of genetic findings
- Abrogation of the future "right not to know" genetic information

Mitigation: The NC NEXUS study follows the model of informed, shared decision making by providing educational resources (understandable decision aids paired with counseling) to help ensure, using rigorous practices, that parents are adequately prepared

for this information. If such issues arise as a result of learning an unexpected genomic finding, we have extensive local experience and resources to help in such situations.

- Dr. C. Powell runs a pre-symptomatic Huntington disease testing program and has experience in such situations. Dr. Berg, Dr. B. Powell and Ms. Roche have had extensive experience returning diagnostic, medically actionable and additional findings in both a clinical and research setting.
- Families experiencing significant distress as a result of unexpected findings will be referred to appropriate mental health specialists as needed. Since clinical geneticists and certified genetic counselors will be on the team conveying these results, our experience in the clinical setting indicates that such emotional distress requiring referrals is rare.
- Parents will not be able to request results that would indicate a risk of an adult-onset condition for which there is no current treatment, such as ALS.

Effectiveness: Uncertain. Some potential harms, such as the long-term implications of learning about genomic information in a healthy newborn, are unknown and somewhat unpredictable, and may manifest long after completion of the study. Although we plan to follow participants longitudinally as long as possible, we cannot guarantee that funding will exist for long-term uninterrupted monitoring of outcomes decades from now.

3.3.2 Adverse Event Recording/Reporting

The study team has considerable expertise in conducting assessments with individuals and families who receive genetic testing and findings from those tests. Key personnel in this grant include certified genetic counselors, clinical geneticists, medical biochemical geneticists and a neurologist, all of whom have extensive experience with medical management of rare genetic conditions and dealing with patient responses to genetic information including newborn screening results in a clinical setting.

3.3.2.1 Adverse Event Definitions

The NC NEXUS study itself does not raise substantial risks for any of the following adverse events:

- Serious injury or illness
- Hospitalization
- Disability
- Life-threatening adverse effect

3.3.2.1.1 Medical management of known diagnoses:

Participants in the “Diagnosed Cohort” will have ongoing medical care for their known conditions, some of which include the potential for serious illnesses, hospitalizations, or even death. However, enrollment in the NC NEXUS research project will not constitute any increased risk for such complications, whether or not a molecular diagnosis is obtained. Participants in the “Well-Child Cohort” will likewise have the potential to

develop any typical injury or pediatric illness. Again, enrollment in the NC NEXUS research project will not constitute any increased risk for these common conditions. Therefore, we will ***not*** consider intercurrent illnesses, sporadic injuries, or the worsening of existing conditions as adverse events associated with the study.

3.3.2.1.2 Medical management of newly identified diagnoses:

Positive findings from the NC NEXUS study may lead to clinical follow-up and possibly medical interventions as part of the clinical management of a newly diagnosed genetic condition. These outcomes are expected and will be documented as part of the longitudinal follow-up of participants with positive findings. Therefore, we will ***not*** consider the existence of additional treatment or further diagnostic tests as an adverse event. However, there may be instances in which physicians take actions due to a genetic finding, but these actions are not considered to be standard of care. In addition, there may be rare instances in which the standard of care management of a diagnosed genetic condition leads to complications that would negatively impact the clinical utility of the genetic result. Therefore, we will monitor for any such adverse events, defined as:

- **Adverse effects associated with the investigational device due to medical interventions undertaken as a result of positive findings:**
    1. There is a reasonable possibility that erroneous (non-standard of care) medical actions may have occurred as a consequence of positive findings.
    2. There is a reasonable possibility that serious injury, hospitalization, or death may have occurred as a complication of standard of care medical follow-up of positive findings.

3.3.2.1.3 Psychosocial complications of newly identified diagnoses*:*

Positive findings from the NC NEXUS study may lead to low-level parental anxiety and/or distress. This is an expected reaction to a medical diagnosis and should be short-lived and relatively minor. Therefore, we will ***not*** consider the existence of mild or time-limited psychological complications as an adverse event. However there may be rare instances in which study members deem the level of psychological distress to require referral to a specialist or other intervention. Therefore, we will monitor for any such adverse events, defined as:

- **Adverse psychosocial effects associated with the investigational device due to revelation of positive findings:** There is a reasonable possibility that serious psychosocial harms may have occurred as a consequence of reporting positive genomic findings to the parents.

3.3.2.1.4 False negative results:

The NC NEXUS study is evaluating a screening test using a technology that is certain to have imperfect sensitivity for most genetic conditions. Therefore, there is a chance that

some individuals in the study will develop a genetic condition that was not detected by the sequencing test (false negative results). This is a predictable event, and thus will ***not*** be considered an adverse effect, since these individuals would have the same outcome as if they had not enrolled in the study. With longitudinal follow-up over the course of the study it is possible that we will identify a small number of false negative results, but based on the sample size this is an extremely unlikely occurrence. Furthermore, all participants will already be receiving standard of care newborn screening and pediatric care and thus will not be relying on NC NEXUS for the detection of conditions that are currently deemed to be part of the recommended uniform screening panel.

3.3.2.1.5 False positive results:

NGS technology (like any test) is known to have technical false positives, essentially variant calls that are due to errors in mapping, other variant calling artifacts, or inherent limitations in the current state of knowledge about the human genome (eg. unmapped pseudogenes). All results will be confirmed by Sanger sequencing in the CLIA-certified MGL, so it is very unlikely that any technical false positives will be inadvertently returned to participants. On the other hand, the process of variant analysis and interpretation is part of the practice of medicine and is subject to variability between laboratories. A board-certified molecular geneticist or pathologist will review and sign-out all results that are deemed returnable in the NC NEXUS study in order to ensure the highest threshold of quality. However, there is a possibility that some of the positive results could be reinterpreted in the future (and thus become false positive results). In this case, it is possible that actions may be taken due to the finding, which are later determined to have been unnecessary. Therefore, we will monitor for such adverse events, defined as:

- **Unnecessary medical care associated with the investigational device due to false positive findings:** There is a reasonable possibility that medical actions taken by the patient's physician were related to a genetic finding that was later deemed to be a false positive result.

3.3.2.1.6 Other unexpected adverse effects:

The NC NEXUS study may also involve risk for unexpected adverse effects, which we define as any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc.)
- Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research)
- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

3.3.2.2 Recording and Assessment of Adverse Effects

All observed or volunteered adverse effects, regardless of cohort or randomization group, that have a reasonable possibility of a causal relationship to the investigational device will be recorded in the REDCap database entry for the participant. For all adverse effects, sufficient information will be pursued and/or obtained so as to permit 1) an adequate determination of the outcome of the effect (i.e., whether the effect should be classified as a serious adverse effect) and; 2) an assessment of the causal relationship between the adverse effect and the investigational device or, if applicable, the other subsequent treatment or diagnostic procedure.

The minimum initial information to be captured in the subject's REDCap form concerning the adverse device effect includes:

- A narrative description of the event
- Classification of the event's severity and rationale for classification
- Investigator assessment of the association between the event and study treatment
- Current status

3.3.2.2.1 Reporting adverse effects to FDA

Adverse Device Effects

The NC NEXUS study itself does not raise substantial risks for any of the following adverse device events:

- Results in death
- Is life-threatening
- Results in permanent impairment of a body function or permanent damage to body structure
- Necessitates medical or surgical intervention to preclude permanent impairment of a body function or permanent damage to a body structure

  -or-
- A previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting noted above (reporting such events within 10 working days from when event was deemed reportable).

  Such reports will be submitted within ***10 working days***

Unanticipated Adverse Device Effects (UADEs)

The NC NEXUS does not contemplate having UADEs associated with this study.

Withdrawal of IRB approval

The Sponsor shall notify the FDA, all participating IRBs and participating investigators of any withdrawal of approval of the study by a reviewing IRB ***within 5 working days*** after receipt of the withdrawal of approval.

FDA Reporting Process

Medical Device Reports, whether for anticipated or unanticipated device-related effects, are to be submitted on FDA Form 3500A. The contact information for submitting MDR reports is noted below:

Food and Drug Administration
Center for Devices and Radiological Health
Medical Device Reporting
PO Box 3002
Rockville, MD 20847-3003

3.3.2.2.2 Reporting adverse effects to the responsible IRB

Federal regulations require timely reporting by investigators to their local IRB of unanticipated problems posing risks to subjects or others. The following describes the UNC-CH IRB reporting requirements, though Investigators at participating sites are responsible for meeting the specific requirements of their IRB of record.

Report Promptly, but no later than 5 working days:
Researchers are required to submit reports of the following problems promptly but no later than10 working days from the time the investigator becomes aware of the event:

- ***Unanticipated problems including adverse events*** **that are unexpected and related**
  - *Unexpected: An event is "unexpected" when its specificity and severity are not accurately reflected in the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document and other relevant sources of information, such as product labeling and package inserts.*
  - *Related to the research procedures: An event is related to the research procedures if in the opinion of the principal investigator or sponsor, the event was more likely than not to be caused by the research procedures.*
  - *Harmful: either caused harm to subjects or others, or placed them at increased risk*

- ***Unanticipated adverse device effect***: Any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application, or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

Other Reportable events:
The following events also require prompt reporting to the IRB, though no later than 10 working days:

- ***Complaint of a research subject*** when the complaint indicates unexpected risks, or the complaint cannot be resolved by the research team.
- ***Protocol deviations or violations*** (includes intentional and accidental/unintentional deviations from the IRB approved protocol) for any of the following situations:
  - *one or more participants were placed at increased risk of harm*
  - *the event has the potential to occur again*
  - *the deviation was necessary to protect a subject from immediate harm*
- ***Breach of confidentiality***

Reporting Process
The reportable events noted above will be reported to the IRB using the form: "Reportable Event Form" or as a written report of the event (including a description of the event with information regarding its fulfillment of the above criteria, follow-up/resolution and need for revision to consent form and/or other study documentation).

Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's study file.

3.3.3 Withdrawal of subjects from the study

The informed consent materials will clearly state that parents can withdraw from the study at any time, that this will not impact the child's medical care in any way. Parents who refuse to use the decision aids or fail to comply with the questionnaires will be considered withdrawn and their child's sample will not undergo further sequencing or analysis. They will not participate in the randomization. Specimens from parents who desire to terminate participation will be destroyed and no further analysis of data in such individuals will be pursued.

3.4 Description of Investigational Device

On August 27, 2014, the FDA provided a response to a pre-submission inquiry and determined that the proposed NC NEXUS study would be required to submit an Investigational Device Exemption (IDE) due to the significant risk associated with the potential long-term consequences to both the parent(s) (i.e., psychological impact) and the child (i.e., denial of life and/or long-disability insurance and having medical records containing WES information of which he/she did not consent to) after learning of the WES finding. The FDA has determined that the decision aid that will be developed and the psychosocial research that will be conducted are also part of the "device" that is being evaluated. In that case, the "device" would be best described as:

> "Informed parental decision-making aided by an electronic decision aid regarding their acceptance of next-generation sequencing newborn screening for their child; whole exome sequencing with targeted analysis and Sanger confirmation of

positive findings; return of results through standard-of-care genetic counseling; and follow-up questionnaires regarding the psychosocial impact of the screening."

This device encompasses all aspects of our study, including informed consent forms, shared-decision making tools (i.e., electronic decision aid), sample collection, next-generation sequencing, bioinformatics pipelines including variant calling and selection algorithms, confirmation of variants with Sanger sequencing, procedures for returning different categories of genomic results to parents, and follow-up procedures with parents before and after learning of research results (questionnaires).

Each of the steps involved in the NCNEXUS project can be envisioned as an element of an instructional manual containing (but not limited to) the following instructions of use: script used for the initial recruitment, study brochure, decision aids, questionnaires, consent forms, sample collection, laboratory methods (DNA extraction, exome library preparation, and massively parallel sequencing), bioinformatics pipeline (initial informatics analysis and variant annotation), clinical interpretation of exome sequence variants (screening and indication-based analysis), variant confirmation by Sanger sequencing (in the CLIA-certified Molecular Genetics Laboratory), randomization, return of results, and other detailed procedures describing precautions and safeguards that will be utilized before and after the parents have been informed of the investigational results. Thus, the "device" is all aspects of the study as described above.

Possible modifications to the device that we can anticipate occurring throughout the study are discussed in previous sections and briefly summarized here. The clinical actionability of all Mendelian disorders will continue to evolve over the foreseeable future. The development of the list of conditions that have been scored by the NC NEXUS Actionability team (Appendix 8) as described in Section 3.2.3.6.5, Defining Clinical Actionability is expected to continue throughout the study period and after, and therefore, periodic updates of the lists will occur. Slight changes or adjustments based on feedback from user testing may lead to minor changes in the Longitudinal Study Measures (Appendix 19) described in Section 3.2.2.1.3, Study Narrative. Among the major research activities of the NC NEXUS project will be the evaluation of new bioinformatics algorithms; in addition, we anticipate improvement in the evidence available to assess pathogenicity of variants (as defined in Section 3.2.3.7.1, Revised Results in the Follow-up Procedures, Section 3.2.3.7). Thus, by design there will be developmental changes in the protocol, accompanied by periodic reanalysis of the NGS data, with the possibility that the results of the analysis may change over time and some participants will be re-contacted for updated results. This situation will be clearly described in the informed consent.

The FDA will be provided with a 5-day notice of any updates or changes in the measures, lists that are implemented in the informatics algorithms, or types of revised results that are returned to patients.

3.5 Monitoring Plan/Procedures

The Sponsor-investigator (Principal Investigator) and key study personnel will meet on a quarterly basis to discuss aspects of the study, review unanticipated problems and adverse events, evaluate results, scrutinize data and anticipate problems relevant to subject safety. An independent Study Data Monitor and Medical Safety Monitor will be established prior to enrollment of participants. These individuals will meet with the Principal Investigator and other key study personnel as described in sections 3.5.1 and 3.5.2 below.

As our study involves a unique aspect of genomic sequencing in the pediatric population not typically considered under most "medical monitoring" plans, including the option for some parents to receive results on variants in genes that are associated with carrier status and adult-onset conditions in their infant, we held a 2-day conference at the beginning of our project to solicit opinions about our proposed study protocol including return of results from an external group of consultants including experts in biomedical ethics, genetic counseling, newborn screening and clinical genetics. We received support for our plan as outlined above. These experts have agreed to continue to serve as external consultants throughout our study period and include Dr. Eric Juengst, Director of the UNC Center for Bioethics, as well as others from outside our institution. They will be available as needed to review any ethical concerns or questions that arise.

3.5.1 Study Data Monitor

The data in this study will be reviewed on a regular basis by an independent data monitor. The role of the data monitor will be to review study documentation, regulatory files, and informed consent to ensure the quality and integrity of the data collected and adherence to good clinical practices. An important focus of the data monitoring will be to ensure appropriate informed consent has been obtained from the research participants.

3.5.2 Medical Safety Monitoring

The Principal Investigator will oversee the safety of the study at her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above. Medical monitoring will include a regular assessment of the number and type of adverse events as defined in section 3.3.2.1. Any safety concerns or unanticipated problems will be relayed to an independent medical safety monitor for immediate review. In addition, there will be a quarterly project meeting at which concerns can be raised for discussion by the entire project team. Such opportunities will allow us to monitor for the expected psychosocial impact of genetic testing as well as being alert to otherwise unexpected participant safety issues. The medical safety monitor will have expertise in pediatrics and research ethics. The role of the medical officer will be to advise the Principal Investigator and project team and make recommendations about continuing the study.

3.5.3 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the EC/IRB, the sponsor, government regulatory bodies, and University compliance and

quality assurance groups of all study-related documents and study-related files. The investigator will help coordinate inspections of applicable study-related facilities. Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

3.6 Additional records and reports

Reports from the study data monitor describing records reviewed, any concerns noted, and recommendations made to correct deficiencies; as well as correspondence from the medical safety monitor following any necessary reviews will be provided to the IRB, FDA, and NHGRI/NICHD. Reporting will also include annual progress reports to NHGRI/NICHD.

3.6.1 Data Handling and Record Keeping

Most of the data collected in the NC NEXUS study will be stored electronically as follows (and detailed in 3.6.1.2 below):

- A REDCap database managed by the North Carolina Translational and Clinical Sciences (NC TraCS) Institute will be used to store demographic information and baseline clinical data for each participant (parents and children) enrolled in the clinical study. This database is within the UNC Hospitals firewall and will serve as the database of record linking personally identifiable information with the unique study identifier. Entry and maintenance of the study records will be a shared responsibility of study investigators.
- Project-related tasks and laboratory data will be recorded in a custom workflow management system managed by RENCI that has access restricted to specific roles. No personally identifiable health information is included.
- Data from questionnaires will be stored on a secure drive at UNC with restricted access to only those personnel who are involved in data analysis.
- Data from the decision aid will be stored on a secure drive at RTI with restricted access to only those personnel who are involved in data analysis.
- Sequence data and called variants will be stored in the UNC Research Computing system. Raw sequence data will be stored for the duration of the study on tape backup through UNC Research Computing. A reduced representation of the participant's variant calls (currently in the form of a VCF file, although standard formats may change over time) and a file that comprises the clinically relevant variants to be reviewed and confirmed will be stored in a data repository managed by RENCI and will have access restricted to a subset of study personnel who are involved in data analysis.

Results that are confirmed in the MGL will be reported as clinical genetic test results and parents will be provided with a paper copy of the report for their personal records. These official reports will have participant names and medical record numbers and, if consent is given, will become part of the permanent medical record, subject to the protections afforded by the HIPAA regulations. Otherwise, subject names or other directly

identifiable information will not appear on any reports, publications, or other disclosures of clinical study outcomes.

3.6.1.1 Record Maintenance and Retention

The investigator-sponsor will maintain records in accordance with Good Clinical Practice guidelines, to include:

- FDA correspondence related to the IDE application and Investigational Plan
- IRB correspondence related to the clinical protocol, current and past versions of the IRB-approved clinical protocol and corresponding IRB-approved consent forms
- Signed Investigator's Agreements and Certifications of Financial Interests of Clinical Investigators
- Certificates of required training (e.g., human subject protections, Good Clinical Practice, etc.) for investigator-sponsor and listed sub-investigators
- Signed informed consent forms
- Copies of adverse event reports and annual or interim reports
- Monitoring visit reports
- Final clinical study report.

3.6.1.2 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

All clinical information will be kept confidential, and will only be accessed by those directly involved in the research. The FDA may request and be granted access to the records. The digital file containing the linked participant names, UNC medical record numbers and unique study identifiers will be stored in a password-protected REDCap database managed by NC TraCS. Paper copies of consent forms and any personal health information will be stored in a locked filing cabinet in a locked office. All identifiers (name, date of birth, etc.) will be removed from the saliva samples before they are sent to

the BSP or MGL and all samples used in this study will be labeled only with the participant's unique study identifier.

Genetic variant data: Each participant's genetic variant data will be stored using a unique participant ID number and stored for the duration of the study on tape backup through UNC Research Computing. A file that comprises the clinically relevant variants to be reviewed and confirmed will be stored in a data repository managed by RENCI, which will have access restricted to a subset of study personnel. A reduced representation of the participant's variant calls will be stored with the unique participant ID number to allow for re-analysis.

Questionnaire Responses: Participants' responses to study questionnaires (research data) will be identified only by their unique participant ID number, whether collected in an online questionnaire format implemented in Qualtrics or in a paper-and-pencil questionnaire or interview by a trained staff member (e.g., for participants who cannot or prefer not to complete the online questionnaire). The questionnaires will not collect information that could be used to identify participants.

Decision Aid Usage Data: Data gathered through participant interaction with the electronic decision aid (e.g., app performance metrics, usage metrics, and participant inputs) will be associated with unique participant ID numbers. The decision aid will not collect information that could be used to identify participants. All usage data will be stored in a secure password protected database at RTI secured by industry standard firewalls and a stringent IT security policy framework. Data and query tools published via web interfaces will be encrypted.

4. MANUFACTURING INFORMATION

Due to the nature of the NC NEXUS study, this section is not applicable per guidance received during the Pre-IDE Submission process in Q140207 12 2014 UNC email – FINAL. See Cover Letter for more details.

5. INVESTIGATOR INFORMATION

5.1 Investigator Agreement

INVESTIGATOR AGREEMENT FOR THE CLINICAL INVESTIGATION OF NC NEXUS (North Carolina Newborn Exome Sequencing for Universal Screening). All investigators have signed the agreement and any additional investigators will not be added until the agreement is signed

5.2 Investigator certification

PHYSICIAN CO-INVESTIGATORS (i.e., physicians participating as co- or sub-investigators on this clinical investigation under supervision of the Principal Investigator.

5.2 Investigator CVs

Investigators CVs. See Appendix 22

5.3 Investigator Certifications of Financial Interest

Not applicable

6. INSTITUTIONAL REVIEW BOARDS

6.1 The reviewing institutional review board (IRB) for clinical investigation(s) of the NC NEXUS conducted at the University of North Carolina at Chapel Hill is the:

Institutional Review Board
CB # 7097, Medical School Building 52
105 Mason Farm Road
Chapel Hill, NC 27599-7097
Chairperson: Herbert Patterson, Ph.D.

The UNC-Chapel Hill Institutional Review Board (IRB) will not grant IRB approval of the clinical investigation(s) of NC NEXUS under this IDE application until such time that the FDA has accepted the IDE application. A copy of the written notification of approval of the UNC-Chapel Hill IRB for the conduct of the clinical investigation of NC NEXUS under this IDE application will be submitted to the FDA upon issuance. Clinical investigation(s) of the NC NEXUS will not commence at the University until such time that the UNC-Chapel Hill IRB has granted approval of such.

7. OTHER INVOLVED INSTITUTIONS

All participating institutions have been previously identified in Section 6, Institutional Review Boards.

8. FINANCIAL CLAIMS

There is no intent to sell any component of the investigational device in this study.

9. ENVIRONMENTAL ASSESSMENT

An environmental assessment as required under 21 CFR 25.40 or a claim for categorical exclusion under 21 CFR 25.30 or 25.34 is no longer required.

10. LABELING

Due to the nature of the NC NEXUS study, this section is not applicable per guidance received during the Pre-IDE Submission process in Q140207 12 2014 UNC email – FINAL. However, we will ensure that the limitations of the NGS-NBS technology, as well as its potential risks and benefits, are adequately addressed in the informed consent documents and electronic decision aid content.

11. INFORMED CONSENT

The informed consent to enroll in the study will be obtained by a trained recruiter using the study pamphlet and a recruitment script. This consent will be for participation in the study which includes learning more about NGS-NBS, collection of basic demographic information, scheduling an in person visit for considering consent for NGS-NBS, and access to the NGS-NBS decision aid. The consent will be obtained either in person or by phone.

The informed consent process for deciding about accepting NGS-NBS will be done at an in person study visit and facilitated by one of the clinical geneticists and/or genetic counselors on the research team. Topics will include genetic testing and the types of results that could be learned, the assessments in Project 3, and storage and use of genetic data and biospecimens as well as the risks and benefits of genomic sequencing.

All couples will sign a written informed consent form to obtain NGS-NBS for their child.

All children will be younger than 5 years old so will not be asked to give assent.

Consent forms will be translated to Spanish for non-English, Spanish, speaking participants. Additionally, a Spanish speaking interpreter or a Spanish speaking genetic counselor will be present during the informed consent process to communicate the information in the consent documents to the participants and will recruit the Spanish speaking participants. Our Spanish language specialists have extensive experience in medical translation and will strive to maintain the simplest translation possible from the English text.

Please see Appendix 11: NC NEXUS Information Sheet Phase I Diagnosed Cohort
Please see Appendix 12: NC NEXUS Information Sheet Phase I Well-Child Cohort
Please see Appendix 16: NC NEXUS Consent Phase II Diagnosed Cohort
Please see Appendix 17: NC NEXUS Consent Phase II Well-Child Cohort
Please see Appendix 20: NC NEXUS NGS-NBS Electronic Medical Record (EMR)
Please see Appendix 21: NC NEXUS Additional Results Electronic Medical Record (EMR) Consent

12. ADDITIONAL INFORMATION

All meeting dates regarding the pre-submission IDE discussions (e.g., meetings and communications) are provided below. The cover letter contains identical information
Date: May 02, 2014

Purpose: NC NEXUS teleconference with the FDA for clarification/feedback of study protocol

Pre-submission number: Q140207 *Meeting minutes are provided on the next page..*

NC NEXUS Answer: A.) Parents of all participants will receive standard of care genetic counseling by board certified (American Board of Medical Genetics (ABMG) or American Board of Genetic Counseling (ABGC)) genetic counselors and medical geneticists during decision-making and return of results. One of the PI's (Dr. Powell) in addition to being a board-certified clinical geneticist is also a board certified genetic counselor. Additional genetic counselors are part of our study team and will participate in return of results sessions with study participants. This discussion will include the risks, benefits, and limitations of exome sequencing with focused informatics analysis. For example, parents will be told about the Genetic Information Nondiscrimination Act (GINA) and the types of insurance (eg. life and long-term care) that are not covered. Parents will be given information about the likelihood of any given type of results and the follow-up plan that would be put in place if such a result was present. Finally, parents will be educated about the limitations of the process, including the nuances of what a "negative" result would mean.

B.) A "decision aid" will be provided to parents that clearly outlines the pros and cons of choosing to learn different types of results, and there will be ample opportunities for questions. A similar decision aid was developed and used previously by Dr. Powell in the context of a Fragile X study. The NC NEXUS decision aid will be shown to focus groups to confirm clarity and ease of understanding before being given to the parents of NC NEXUS participants. The decision aid and counseling sessions will be divided up by the categories of choices that may be available. First, the choice to participate in the NC NEXUS study and receive the NGS-NBS panel, and second, the additional three categories of information that parents may choose to learn if they are randomized into the experimental arm of the study (as described above). Information will be provided in the decision aid that describes the legal consequences of placing results in the electronic medical record. Since all results will be subject to confirmation, clinical interpretation, and reporting by ABMG-certified molecular geneticists in the hospital's CLIA lab, such results could be made part of the official medical record. We do not yet have a determination from the Institutional Review Board as to whether parents will be required to sign an additional consent in order to place positive results in the medical record, or whether the consent to participate in the study will be sufficient.

- **FDA Question: Will parents of participants who choose only to get pathogenic results be able to get more information (e.g. a file of the full dataset of variants)?**

  NC NEXUS Answer: No. We do not have any plans to release complete variant datasets to parents. If parents disagree with the method of analysis they can elect not to participate in the study.

- **FDA Question: Will people understand that they may be getting back unanticipated results?**

NC NEXUS Answer: Yes, this will be clearly stated in the consent documents that parents of subjects will be given prior to enrollment in any part of the study. If they do not wish to receive any unanticipated results they will have the option of not participating in the study. In the group randomized to decide whether or not to learn additional results (beyond those related to their child's condition and/or those that are included in the NGS-NBS panel results) the decision aid tool that is being developed as part of the research study will enable them to learn more about these options and decide what, if any, additional results they wish to learn.

- **FDA Question: How will negative results be reported?**

  NC NEXUS Answer: We propose to return negative results to parents in the form of a "research report" that will summarize the test process, including coverage metrics, genes tested, aggregate information about the number of variants, and a disclaimer about the limitations of whole exome sequencing. The research report would not be placed in the medical record.

- **FDA Question: Is Cohort 2 already diagnosed with a medical condition?**

  NC NEXUS Answer: Yes, Cohort 2 will have been clinically diagnosed with a genetic disorder (eg. via biochemical assay or other clinical work-up). Parents will already be aware that their child is affected with a medical condition, even though the genetic etiology (specific gene mutations) may not be known. One important aspect of the NC NEXUS project is evaluating the performance of NGS-NBS in predicting and diagnosing the causal genetic factor underlying rare disorders as well as disorders with known genetic and environmentally-induced components (e.g. hearing loss).

- **FDA Question: How does Cohort 3 factor into the ELSI research about decision-making by parents?**

  NC NEXUS Answer: The healthy newborn cohort provides information about parental decision-making in a "real world" setting, as would be expected for pregnant couples who are at no increased risk for genetic disorders. The ELSI component of the NC NEXUS project is comprised of a series of questionnaires and surveys as part of a longitudinal study to look at hopes, expectations, and anxieties associated with making choices about the return of NGS-NBS results.

- **FDA Question: How are threshold cut-offs and actionability scores determined?**

  NC Nexus Answer: The actionability score will be based on an algorithm, which incorporates factors such as severity and knowledge of the disease and intervention. The actionability score along with age of onset will help to determine which bin the disease will be categorized in to. Along with informatics, an expert panel will also help set the threshold and decision making process. For example, Duchene's Muscular Dystrophy would be

categorized in the childhood onset non-medically actionable bin, however treatment can delay the onset of disease, thus this may be categorized in the NGS-NBS bin.

- Summary of FDA's feedback and action items

NC NEXUS provided an informative ~~satisfactory level of information to~~ presentation to help clarify some of the issues in the "Q140207 Memo to Sponsor – FINAL" that arose from the presubmission questions below. Further internal discussions within the FDA will be required before answers can be provided. NC NEXUS will send draft meeting minutes within two weeks of the teleconference. The FDA will request any additional information that is necessary, and conduct internal meetings to provide updated ~~answers~~ responses to the NC NEXUS presubmission questions. The FDA will respond in the context of the presentation and make specific suggestions about what, if any, aspect(s) of the proposed study might trigger a determination of significant risk. The FDA will also provide specific feedback about any changes, if necessary, that would ameliorate the need for an IDE and provide helpful information for future studies. NC NEXUS and the FDA will maintain open communication via email and teleconference(s), as needed, in a timely fashion. ~~The FDA expects to be able to get back to NC NEXUS fairly soon.~~ Some additional discussion points regarding the study and associated risk are noted below:

1. **What level of risk is involved in the proposed study?**
   - More specific details on the information/reports that will be returned to parents and placed in the medical file.
   - Examples of diagnostic methods besides Sanger sequencing that may be needed to confirm certain types of mutations, such as large deletions, that may be detected by WES were provided to the FDA. NC Nexus stated that the~~These~~ confirmatory tests will also be done in a CLIA-approved laboratory.

2. **Will our proposed study require an IDE?**
   a. Which results will be returned to parents?
   b. Which results will be placed in patient's electronic file and associated ethical implications regarding the children?
   - Other confirmatory methods besides Sanger
   - Examples of diseases in the distinct binning categories ~~(~~FDA requested a list of all diseases, if available, that will be included in the study).

3. **What modifications of the protocol are recommended by the FDA?**
   - More information on the proposed study, including ethical implications regarding how data generated in the study will be used is needed for the FDA to understand the risks of the study and the mitigations that could be put in place to address these risks. The addition of mitigations is an example of protocol modifications. ~~Modifications based on feedback about reporting negative results~~

4. **During the course of the study, what changes to the protocol or IRB would require additional review by the FDA?**

Date: July 02, 2014

Purpose: Pre-submission IDE review request

Pre-submission number: Q140207/S001

FDA contact person: Kellie B. Kelm, PhD

Date: July 14, 2014

Purpose: Addendum to the pre-submission IDE (Q140207/S001)

Pre-submission number: Q140207/S002

FDA contact person: Sunita J. Shukla, MPH, PhD

Date: August 27, 2014

Purpose: FDA responses following review by the Office of In-Vitro Diagnostics and Radiological Health

Pre-submission number(s): Q140207/S001 and Q140207/S002

FDA Lead Reviewer: Sunita J. Shukla, MPH, Ph.D.

FDA responses are provided below.

DEPARTMENT OF HEALTH & HUMAN SERVICES — Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Building 66

TO: Laura V. Milko, PhD
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Cynthia M. Powell, MD
Professor of Pediatrics and Genetics
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FROM: Sunita Shukla, PhD
Lead Reviewer
FDA/CDRH/OIR/DCTD
Sunita.Shukla@fda.hhs.gov
301-796-6406

RE: Q140207/S001 and Q140207/S002
DEVICE: NC NEXUS
DATED: Q140207/S001: 7/02/14
Q140207/S002: 7/14/14
RECEIVED: Q140207/S001: 7/02/14
Q140207/S002: 7/14/14

DATE: August 27, 2014

Dear Dr. Milko,

Thank you for submitting the follow-up requested information for our review. The pre-submissions noted above seek FDA input regarding your clinical protocol.

This is an informal communication that represents the best judgment of the Office of In Vitro Diagnostics and Radiological Health staff and consultants who reviewed the protocols. It does

not constitute an advisory opinion and does not bind or otherwise obligate or commit the agency to the views expressed, as per 21 CFR 10.85(k). We have provided an evaluation of your proposed studies below.

Proposed Intended Use/Indications for Use (excerpted from the submission):

The NC NEXUS study will evaluate the use of exome sequencing as a potential means to augment newborn screening (NBS). The main technical outcome will be to examine the sensitivity and specificity of this technology in detecting conditions that are currently screened for in newborns. Another technical outcome will be to examine the capacity of exome sequencing to detect other conditions that would be beneficial to identify at an early age in children but for which there is currently no available diagnostic method.

Aim of Study (excerpted from the submission):

Carry out Whole Exome Sequencing (WES) from various target populations (see below) using DNA collected from buccal swabs (using Oragen Discover (OGR-250) sample collection kits. Key aim of study is how to divide the broad range of genomic variants into categories that will allow parents to make well-informed decisions about a) whether or not to pursue exome sequencing for their newborn; and b) what types of genomic information they are interested in learning. The study proposes a "binning" method of the results based on clinical validity and clinical actionability (see below) and the development of a standardized procedure for categorizing genomic loci into the binned categories (below). To assess the impact of non-medically actionable WES findings, parents will be randomized into 2 groups: control group (will receive all medically actionable results) and experimental group (will be asked to decide what, if any, of the non-medically actionable information they choose to learn about their child. A decision aid (see below) will be used to help parents make an informed decision about study participation and parental preference for return of results.

Binning Categories (excerpted from the submission):

- **Bin 1**: Findings that provide medically actionable incidental results, including conditions screened for in the current NBS context as well as other medical conditions that are not currently included in current NBS protocols. This category will represent the core results from NGS-NBS.
- **Bin 2**: Findings related to a childhood health condition with no specific medical intervention (non- medically actionable). (These findings, along with Bin R, will be returned to parents randomized to have the opportunity to learn them, if they request them after making an informed decision to do so)
  - Bin 2a: Findings selected by as likely to cause people very little distress
  - Bin 2b: Findings selected as likely to cause some people moderate distress
  - Bin 2c: Findings selected as likely to cause most people a considerable amount of stress
- **Bin R**: Findings about reproductive risks, likely to cause little to moderate stress
- **Bin X**: Findings related to untreatable adult-onset health conditions (not to be returned to parents)
- **Bin 3**: Findings that have no clear association with any genetic disorder (not to be returned to parents)

Decision Aid: You state that your study team has extensive experience in health communication, consent, health literacy, NBS and informed consent. Drawing on this experience the study team will develop and test an electronic Decision Aid tool that will explain the complexities of WES to parents and their options for return of results. The Decision Aid tool will be utilized by parents during the consent process and by those participating in a longitudinal study to investigate the acceptability of Next-Generation Sequencing (NGS)/WES for their children. The control group will be given access to a version of the online decision aid that provides information about sequencing and the NGS-NBS panel, and helps them decide whether they want to agree to sequencing. The experimental group will be given access to a version of the online Decision Aid that provides information about sequencing, the NGS-NBS panel, and the categories of additional information they can request to learn. Parents can opt to learn some, all, or none of the additional categories of information. More information regarding the target populations is shown in the table below:

Target Populations (excerpted from submission):

NC NEXUS Study Population and Recruitment Estimates				
	Cohorts	Estimated numbers of subjects available for recruitment		
		Current Patients (Age 0-5 years)	New cases* or births/yr	Total
Disorders currently detected through NBS	PKU	33	5-7	60
	MCADD	28	5-7	60
	CF confirmed	65	12-22	155
	CF with false positive NBS	N/A	130	500
	CRMS	10	1-3	20
	Hearing Loss	1800	200	2600
Disorders that currently cannot be detected by NBS	Other patients in Genetics & Metabolism Clinic, Neurology Clinic	20	5	50
	PCD	20	5	45
	Well Child	N/A	3500	5080

Disease cohorts are ascertained using current standard newborn screening methods

* New cases are identified in the newborn period and enrolled by 6 months of age

Confirmation of Results (excerpted from the submission):

Many of the variants, including rare variants, will be confirmed using Sanger sequencing. However, it is possible that WES may identify mutations for which clinical testing is currently available but for which Sanger sequencing is not ideal. If Sanger is not optimal, gold standard molecular diagnostics tests will be performed (for example, the Qiagen Pyromark MD (pyrosequencing) and Affymetrix GeneChip system (expression, copy number variation, etc) will be available). Clinical reports regarding any positive findings will be generated by the CLIA-based lab after confirmation through Sanger sequencing, which will then be provided to parents and placed in the electronic medical record. Research reports, describing the aggregate exome

sequencing results such as total number of variants identified in different categories (but no specific variant details), will be provided to all parents, but will NOT be placed in the electronic medical record.

Bioinformatics: You propose to develop and evaluate various bioinformatics approaches for the utilization in NGS-NBS. You also state that you will determine the types of variants that can reliably be detected using your current pipeline, and you will explore novel methods that promise to detect types of variants not readily detectable by current approaches to WES. Specifically, you propose to explore thresholds for selecting variants to be further analyzed in an effort to optimize the performance characteristics. In order to enhance the sensitivity of your approach, you will compare methods for calling single nucleotide variants and explore methods of detecting certain types of variants to determine those types that can or cannot be reliably detected. In order to enhance the specificity of your approach, you will investigate the application of a gene-specific mutational burden metric to help adjudicate and re-classify genetic variants (which may result in re-classification of bins for various incidental findings).

Revised Results: You state that over the course of time, association of more genes with diseases and the development of prevention or treatment will result in reassignment of loci and lead to changes in the interpretation of WES findings. When such reassignment occurs, parents will be re-contacted if the results they have received change during the period of the Project.

Specific questions for FDA:

UNC requests FDA feedback on the following questions:

1. *What level of risk is involved in the proposed study?*

- **FDA Response:** Based on the information provided, FDA has determined that your proposed clinical investigation is a Significant Risk device study and you will need to submit an IDE application for this investigation**.** This risk assessment is based on the following rationale:

  1) In your proposal (Q140207/S001), you have stated the following, "*The risk of parental anxiety due to return of unexpected incidental findings raises new and challenging human subjects issues. Further complicating the return of incidental findings is their heterogeneity with potential psychological and clinical impact on patients ranging from trivial to profound……How to handle the return of incidental findings is a central challenge to genomic medicine and will be particularly important in the use of WES and other forms of whole genome sequencing in children*." Thus, your study proposes to evaluate the risk associated with the return of incidental (investigational) findings (of varying degrees) to parents and the psychological impact this will have upon the parents and children over a given period of time. FDA agrees that this is an important study objective. In addition to the potential psychological risks, we also point out that there may also be physical and social risks to the children depending on what parents choose to do as a result of the research. The probability and magnitude of these risks cannot be quantified, especially in the cohort where children are not currently experiencing symptoms. Therefore, we cannot determine that these risks are non-significant.

As a mitigation for the risk, on page 155 you have stated that the binning strategy will "*allow for a systematic approach to parent education and informed consent as it relates to newborn screening.....Finally, the manner in which incidental findings are delivered (if parents so choose) will also be category-driven and risk-calibrated to protect them and their offspring from harm.*" Although the decision aid tools will take the nature of the incidental findings into account, given the aim of your study, the risks of sharing all types of incidental findings cannot be fully anticipated. For example, parents may view children as "sick" or especially vulnerable as a result of the research findings, even if the incidental results have no known medical significance. Such unforeseen or unpredictable consequences for patients may warrant an ongoing relationship between the parents, researchers, and child advocates.

2) You state that: *Many of the variants, including rare variants, will be confirmed using Sanger sequencing. However, it is possible that WES may identify mutations for which clinical testing is currently available but for which Sanger sequencing is not ideal. If Sanger is not optimal, gold standard molecular diagnostics tests will be performed (for example, the Qiagen Pyromark MD (pyrosequencing) and Affymetrix GeneChip system (expression, copy number variation, etc) will be available).* We acknowledge that you state that investigational test results will be confirmed; however you also state that test results will be revised over time based on evolving bioinformatics approaches that you will develop. In such cases, this re-categorization of information will result in changes in the interpretation of WES findings, and will lead to re-contacting of parents to notify them of these changes. Since the bioinformatics approaches involved in potentially revising investigational results will be developed, modified, and evaluated throughout the course of the study, the probability and magnitude of the risk of re-analysis of results cannot be defined. Therefore, we cannot determine that such risk is non-significant.

3) We also point out that, as a result of this research, detailed information will be available in the child's medical record that may have long-term effects that are difficult to predict. The Agency is aware of circumstances where genetic information obtained in research has affected insurability, has been discoverable in legal proceedings or has otherwise been used against the research participant or a member of his/her family. We are particularly concerned because this information will be obtained about children who cannot consent or refuse for themselves. This risk is significant, and may not be mitigable.

2. *Will our proposed study require an IDE?*

- **FDA Response:** As outlined in our response to #1 above, the proposed study will require an IDE.

3. *What modifications of the protocol are recommended by the FDA?*

- **FDA Response:** We would like to emphasize that we believe you have proposed a study that may answer some important questions in the evolving field of NBS. FDA herein offers to work with you as your study evolves in order to suggest ways of mitigating potential risks and to expedite the IDE process. For example, review of informed consent forms is a part of the IDE process. Thus, it may be helpful to provide this information (as a supplement to this pre-submission) to us in advance of your IDE submission so that we can provide any suggested modifications at that time.

The following modifications are also recommended at this time:

1. Please provide details in your IDE submission or as a supplement to your pre-submission that outline the duration of follow-up of parents and children after parents are informed of research results. In addition, please provide information about who will be providing this follow-up, how often, and how this follow-up may help to mitigate any medical or social risks that may occur as a result of the research. Information on what actions will be taken to maintain contact with parents, and procedures for parents who wish to drop out of the study, should also be provided. In particular, it may be helpful to have the binning committee (or another body of experts) suggest additional precautions or safeguards for oversight before and after parents have been informed of the investigational findings.

*Please note that additional mitigations and safeguards may be recommended by FDA during the IDE process and/or as we continue to discuss your study proposal with you in any subsequent pre-submissions.

4. During the course of the study, what changes to the protocol or IRB would require additional review by the FDA?

- **FDA Response:** Due to the evolving nature of the study components (such as binning categories, informatics changes, etc), FDA will provide further guidance regarding which modifications would result in a need for an IDE supplement prior to proceeding. We can discuss this in more detail in our upcoming teleconference on 8/28/14 from 1-2 PM ET.

Note that any revisions that you would like to submit in response to this letter (after the meeting) or new protocols for FDA feedback (called a pre-submission supplement) should be submitted as an eCopy[1] to the address below and should reference the pre-submission number above (Q140207) in the cover letter to facilitate processing.

U.S. Food and Drug Administration
Center for Devices and Radiological Health
Document Control Center – WO66-G609
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002

If you have any questions or comments regarding this review, please contact Sunita Shukla, at (301) 796-6406 or at sunita.shukla@fda.hhs.gov

Sunita J. Shukla -S
2014.08.26 16:05:46
-04'00'

Branch Concurrence:
Toxicology Branch Chief Denise Johnson-lyles -S

Date: September 22, 2014

Purpose: Email from Jonathan Berg to Sunita Shukla with questions about the IDE and requesting guidance.

Pre-submission number: Q140207/S002

FDA contact person: Sunita J. Shukla, MPH, PhD

Date: December 2, 2014

Purpose: Email from Jonathan Berg to Sunita Shukla with questions about the IDE and requesting guidance.

Pre-submission number: Q140207/S002

FDA contact person: Sunita J. Shukla, MPH, PhD

Date: December 10, 2014

Purpose: FDA responses (in red) to the questions that UNC emailed on 12/2/14.

Pre-submission number: Q140207/S002

FDA contact person: Sunita J. Shukla, MPH, PhD

FDA responses are provided below.

Dear Dr. Berg,

Please find attached FDA responses (in **bold** red) to the questions that you emailed on 12/2/14. Please let me know if you have any further questions. Thank you, Sunita

Sunita Shukla, MPH, Ph.D.
Scientific Reviewer
Division of Chemistry and Toxicology Devices
Office of In Vitro Diagnostics and Radiological Health (OIR)
Food and Drug Administration
10903 New Hampshire Avenue
WO66, Room 5647
Silver Spring, MD 20993-0002
Tel. (301) 796-6406

****

From: Shukla, Sunita
Sent: Tuesday, December 02, 2014 3:52 PM
To: 'Berg, Jonathan'
Cc: Milko, Laura V.; Powell, Cynthia M.; Bailey, Don
Subject: RE: IDE questions

Dear Dr. Berg,

Thank you for your email and questions. I am going to review your questions and go over these with our review team. I will email you our feedback prior to 12/11/14. Thank you, Sunita

Sunita Shukla, MPH, Ph.D.
Scientific Reviewer
Division of Chemistry and Toxicology Devices
Office of In Vitro Diagnostics and Radiological Health (OIR)
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10903 New Hampshire Avenue
WO66, Room 5647
Silver Spring, MD 20993-0002
Tel. (301) 796-6406

****

From: Berg, Jonathan [mailto:jonathan_berg@med.unc.edu]
Sent: Tuesday, December 02, 2014 10:59 AM
To: Shukla, Sunita
Cc: Milko, Laura V.; Powell, Cynthia M.; Bailey, Don
Subject: IDE questions

Hi Sunita,

We have made some progress here at UNC in preparing our IDE, but we have lots of questions! We've had meetings with both the IRB and the regulatory specialist within the CTSA here at UNC, and really no one has much experience with this type of submission. We are planning a meeting with the regulatory specialists at RTI to see if they can provide any additional assistance.

For now, could you please have a look at the following questions and provide guidance?

1. Report of prior investigations:
What constitutes "prior investigations" if this is a new device specific to this research project?
- We have experience with preparation of the exome sequencing libraries through other ongoing projects but these projects are not directly related to NC NEXUS. ***How much detail do we need to provide about the library production process?***
- The location where sequencing will occur is a core facility at UNC and is not under our direct control. The core facility has generated large amounts of sequence data for other projects, including for example the TCGA. ***What level of detail do we need to provide about the core facility's capabilities and previous sequence output?***
- Our group has broad experience with psychosocial research in children and adults, as well as the development of educational materials, both of which will be part of the ELSI aspect of this project, but these prior projects do not directly relate to NC NEXUS. ***How much detail do we need to provide about previous psychosocial research?***

2. Labeling:
How shall we "label" our device, if the "device" includes the technical and psychosocial aspects of the project?
- From a technical standpoint we would describe the "next-generation sequencing newborn screening" platform as "exome sequencing with focused informatics analysis and Sanger confirmation of positive results." The FDA has determined that the decision aid that will be developed and the psychosocial research that will be conducted are also part of the "device" that is being evaluated. In that case, the "device" would be described as "informed parental decision-making aided by an electronic decision aid regarding their acceptance of next-generation sequencing newborn screening for their child, with follow-up surveys and questionnaires regarding the psychosocial impact of the screening." ***Can you provide us with guidance about how we would label this device, and what that "labeling" would entail? How do we provide "copies of all labeling for the device"?***

3. Manufacturing information:
What constitutes the "manufacture" of our "device"?

- As described above, our "device" does not naturally adhere to what is described in the regulatory guidance: "A description of the methods, facilities, and controls used for the manufacture, processing, storage, and, where appropriate, installation of the device, in sufficient details so that a person generally familiar with good manufacturing practice can make a knowledgeable judgment about the quality control used in the manufacture of the device." ***If we are not manufacturing a product for any kind of distribution, how do we respond to this section of the IDE application?***

4. Investigational plan/protocol:
Is there a standard format for the protocol?
- The three examples that you sent us are very different in terms of structure and level of detail. Since we are currently working on the IRB submission for this project, we would like to avoid duplicating effort. ***Would it be reasonable to submit the IRB protocol, assuming that it covered the study design, patient selection, procedures, safety monitoring, and analysis plan? If so, can you please send us a list of subcategories from the main template that need to be covered for a "device" such as the NC NEXUS project?***

Thank you for your answers. We have a meeting next Thursday morning (12/11/14) to discuss the IDE application and would appreciate your responses by then.

Sincerely,
Jonathan

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FDA responses (in bold) to the questions that UNC emailed on 12/2/14

1. Report of prior investigations:
What constitutes "prior investigations" if this is a new device specific to this research project?
If there have not been any prior investigations using your device (which would include laboratory/animal studies and reports of prior publications), you should state this in your IDE application.
- We have experience with preparation of the exome sequencing libraries through other ongoing projects but these projects are not directly related to NC NEXUS. ***How much detail do we need to provide about the library production process?*** **Although your experience with the preparation of the exome sequencing libraries are not related directly to the NC NEXUS project, please provide relevant information regarding the preparation of the library that will be used for the current study. Relevant information would include: an SOP/written protocol describing the preparation of the exome sequencing library and its components and properties (such as reagents, stability, etc), instrumentation to be used, enrichment of exon targets, verification of library quality and other quality control steps that are performed during library preparation.**
- The location where sequencing will occur is a core facility at UNC and is not under our direct control. The core facility has generated large amounts of sequence data for other projects, including for example the TCGA. ***What level of detail do we need to provide about the core facility's capabilities and previous sequence output?*** **Please provide a description of the core facility where the sequencing will occur and its role. For example, please indicate the facility's role in the preparation of the sequencing libraries, evaluation of the quality of the libraries, sample extraction/storage/handling, sequence output, etc. Please note that if the**

sample extraction and library preparation are occurring outside of the core facility, please indicate where these will take place. You may include a brief description of the core facility's relevant prior experience with sequencing and other aspects that are similar to your study.

- Our group has broad experience with psychosocial research in children and adults, as well as the development of educational materials, both of which will be part of the ELSI aspect of this project, but these prior projects do not directly relate to NC NEXUS. ***How much detail do we need to provide about previous psychosocial research?*** **Since the ELSI component of your study represents a unique aspect of the IDE application with regard to study risk, please include a brief description of the past relevant experience that will be applicable to the development of the ELSI component described in the proposed IDE study. Relevant information should also include past experience regarding follow-up, mitigation of risks and other safeguards related to the investigational findings.**

2. Labeling:

<u>How shall we "label" our device, if the "device" includes the technical and psychosocial aspects of the project?</u>

- From a technical standpoint we would describe the "next-generation sequencing newborn screening" platform as "exome sequencing with focused informatics analysis and Sanger confirmation of positive results." The FDA has determined that the decision aid that will be developed and the psychosocial research that will be conducted are also part of the "device" that is being evaluated. In that case, the "device" would be described as "informed parental decision-making aided by an electronic decision aid regarding their acceptance of next-generation sequencing newborn screening for their child, with follow-up surveys and questionnaires regarding the psychosocial impact of the screening." ***Can you provide us with guidance about how we would label this device, and what that "labeling" would entail? How do we provide "copies of all labeling for the device"?*** **Please note that for the purposes of the current IDE study, device labeling is not applicable. However, as provided in prior FDA feedback, please ensure that the relevant information regarding the device/study and ELSI components, for example, are provided as part of the IDE (which will also include the study protocol and informed consent documents).**

3. Manufacturing information:

<u>What constitutes the "manufacture" of our "device"?</u>

- As described above, our "device" does not naturally adhere to what is described in the regulatory guidance: "A description of the methods, facilities, and controls used for the manufacture, processing, storage, and, where appropriate, installation of the device, in sufficient details so that a person generally familiar with good manufacturing practice can make a knowledgeable judgment about the quality control used in the manufacture of the device." ***If we are not manufacturing a product for any kind of distribution, how do we respond to this section of the IDE application?*** **Due to the nature of your device, this section will not be applicable to your IDE application.**

4. Investigational plan/protocol:

<u>Is there a standard format for the protocol?</u>

- The three examples that you sent us are very different in terms of structure and level of detail. Since we are currently working on the IRB submission for this project, we would like to avoid duplicating effort. ***Would it be reasonable to submit the IRB protocol, assuming that it covered the study design, patient selection, procedures, safety monitoring, and analysis plan? If so, can you please send us a list of subcategories from the main template that needs to***

be covered for a "device" such as the NC NEXUS project? **The information contained within the IRB protocol may be appropriate for the IDE application. Based on the above noted feedback and the example IDE content that was emailed to you on 9/22/14, it is acceptable to state "Not applicable" for sections of the IDE application that do not apply to your device. The example below illustrates a few potential subsections regarding your device (please note that the subsections below are examples and may not be inclusive of other information you will include in your IDE, such as safety monitoring and ELSI). Please note that any additional information may be requested interactively during the review of your IDE.**

3.2 Internal Validation of Performance
3.2.1 Accuracy
3.2.5 Potential Interfering Substances
3.2.6 Reagents and Stability.
3.2.8 Sample to Sample Carry-over
4.4 Description of Device
4.4.2 Instrument
4.4.3b Software
4.4.4 Data Analysis
4.4.5 Anticipated Changes
4.5 Monitoring Procedures .(QC)

Date: May 21, 2015

Purpose: FDA draft document review for NC NEXUS.

Pre-submission number: Q140207/S003

FDA contact person: Sunita J. Shukla, MPH, PhD

Communications are provide below. Please note: Documents/files provided to the FDA on this date are list below and are located in Section 14.

APPENDIX 23: 21MAY2015_NCNEXUS_information_sheet_Phase_I_Diagnosed cohort

APPENDIX 24: 21MAY2015_NCNEXUS_information_sheet_phase I WC cohort

APPENDIX 25. 21MAY2015_NC NEXUS_consent_phase II_diagnosed cohort

APPENDIX 26. 21MAY2015_NC NEXUS_consent_phase II_WC cohort

APPENDIX 27. 21MAY2015_NC NEXUS_Recruitment Decision Aid

APPENDIX 28. 21MAY2015_NEXUS_Online DA_Decision 1_Shooting script_05 19 2015

APPENDIX 29. 21MAY2015_NEXUS_Online DA_Decision 2_Shooting script_05 19 2015

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From: Berg, Jonathan [mailto:jonathan_berg@med.unc.edu]
Sent: Wednesday, May 27, 2015 6:21 PM
To: Shukla, Sunita <Sunita.Shukla@fda.hhs.gov>; Milko, Laura V. <laura_milko@med.unc.edu>
Cc: Powell, Cynthia M. <powellcm@med.unc.edu>; Bailey, Don <dbailey@rti.org>
Subject: Re: FDA draft document review for NC NEXUS

Sunita,

Thank you for your input. We have been working with our regulatory groups on the IDE submission but do not have a target submission date yet. We will update you with our expected timeframe when it seems clearer.

-Jonathan

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From: <Shukla>, Sunita <Sunita.Shukla@fda.hhs.gov>
Date: Wednesday, May 27, 2015 at 4:46 PM
To: "Berg, Jonathan" <jonathan_berg@med.unc.edu>, "Milko, Laura V." <laura_milko@med.unc.edu>
Cc: "Powell, Cynthia M." <powellcm@med.unc.edu>, "Bailey, Don" <dbailey@rti.org>
Subject: RE: FDA draft document review for NC NEXUS

Dear Dr. Berg,

Thank you for submitting the documents containing the decision aid information. Based on a cursory review of the documents, we have the following general questions/suggestions for your IDE:

1. Please provide a timeframe for when you will be submitting your IDE. This will allow for us to coordinate our efforts, workload, timelines and set up any necessary meetings with you prior to the receipt of the IDE (especially since we will only have a 30 day review clock for the IDE). This will also allow for us to provide any appropriate background for internal review team members prior to the receipt of the IDE.
2. Although we may not have specific comments on the documents you provided on 5/21/15, there may be additional comments once we have received the full IDE package. Please note that we will work interactively with you throughout the review of the IDE to work through any issues.
3. Although you have provided the online decision aid content in the 5/21/15 email, please note that it will be useful for you to provide the associated screenshots that will be

viewed by the study participants. This will help us evaluate the content, presentation of material, and other aspects that will be seen by the study participants.

4. As with the feedback that was provided to you during the review of Q140207 (and related Supplements), please ensure that you provide your plan/SOP for how you will address any triggers/changes to your analytical processes (such as those that would affect binning of results, etc) or ELSI components. Please also describe what risks such changes will be associated with and how these changes/risks will be conveyed to study participants.

We look forward to hearing back from you regarding your timeframe of your IDE submission. Please let me know if you have other questions. Thank you, Sunita

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From: Berg, Jonathan [mailto:jonathan_berg@med.unc.edu]
Sent: Tuesday, May 26, 2015 10:53 PM
To: Shukla, Sunita; Milko, Laura V.
Cc: Powell, Cynthia M.; Bailey, Don
Subject: Re: FDA draft document review for NC NEXUS

Hi Sunita,

The documents we sent are only for a preliminary review. The complete IDE will contain much more detail about our project.

We were under the impression that it would be helpful for you to see the consent forms and the decision aid content so that you could provide feedback before the full IDE is submitted. Was that incorrect?

Thanks,

-Jonathan

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From: <Shukla>, Sunita <Sunita.Shukla@fda.hhs.gov>
Date: Tuesday, May 26, 2015 at 6:01 PM
To: "Milko, Laura V." <laura_milko@med.unc.edu>
Cc: "Powell, Cynthia M." <powellcm@med.unc.edu>, "Berg, Jonathan"

<jonathan_berg@med.unc.edu>, "Bailey, Don" <dbailey@rti.org>
Subject: RE: FDA draft document review for NC NEXUS

Dear Ms. Milko,

We will be discussing your documents internally, however I wanted to check if the documents you emailed on 5/21/15 are part of the IDE submission (and there will be other documents describing the test, etc) or are the documents you emailed going to be the entirety of the IDE submission you plan on submitting? Thank you, Sunita

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From: Milko, Laura V. [mailto:laura_milko@med.unc.edu]
Sent: Thursday, May 21, 2015 7:32 AM
To: Shukla, Sunita
Cc: Powell, Cynthia M.; Berg, Jonathan; Bailey, Don
Subject: FDA draft document review for NC NEXUS

Dear Dr. Shukla,

Prior to finalizing the IDE application for the NC NEXUS study, we have several draft documents that we'd like to provide for informal review and feedback. These documents, once finalized, will be important for establishing the timeline, goals, and endpoints for the study, and we'd appreciate your suggestions for ways to mitigate potential risks and expedite the IDE process. Please find the following attached:

- Study flows for the 'well-child and 'diagnosed' cohorts. Abbreviations: T1 Q, T2 Q, and T3 Q refer to questionnaires that will be given at three different time points. NGS-NBS refers to the select group of conditions that we determine to be similar to current RUSP conditions; these are childhood onset medically actionable conditions, and positive findings will be confirmed and returned to all participants. RoR is a return of results encounter. AI refers to "additional information" that parents randomized to the decision arm will be asked to decide about whether to learn after viewing the second part of the online decision aid; categories include adult onset medically actionable, childhood onset non-medically actionable, and carrier status.

- NC NEXUS Recruitment Decision Aid – This will be in the form of a brochure that is given to prospective parent participants prior to enrollment in the study.

- Decision Aid "shooting script" files - These documents show our working shooting scripts for the online decision aid, part 1 (whether to have their child undergo NGS-NBS) and part 2 (whether to learn additional information). It shows the content for each group (single parent versus couple) or for parents with a newborn versus child with a diagnosed condition. In addition the columns show the narration, the text that would appear on screen, any animation or data capture. There may be small changes to wording during the development process to accommodate suggested by the team programming the decision aid or by the NEXUS steering committee as the decision aid takes shape.

- Well-Child cohort information sheet to get verbal consent for parent(s) to participate in the study (Phase I)
- Diagnosed cohort information sheet to get verbal consent for parent(s) to participate in the study (Phase I)
- Well-Child cohort parental consent for their child to have genomic sequencing for Phase II
- Diagnosed cohort parental consent for their child to have genomic sequencing for Phase II

We look forward to hearing from you. Please contact us if you have any questions.

Best,
Laura

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13. REFERENCE LIST

(References in Bold Type are provided and have been selected as being the most relevant to this IDE application)

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14. APPENDIX

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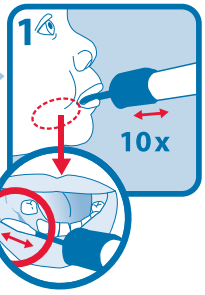
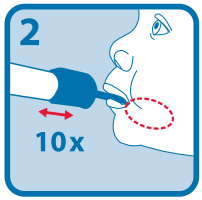
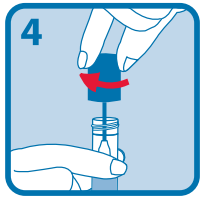
ORAcollect for pediatrics

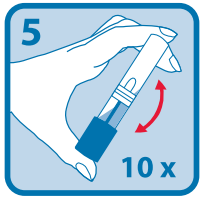
REF OC-175

For Research Use Only
Not for use in diagnostic procedures









OPEN HERE

DNA genotek
www.dnagenotek.com

Made in Canada
DNA Genotek Inc.
Ottawa, ON, Canada K2K 1L1
Subsidiary of OraSure Technologies, Inc.

Contents: Contains 1 collection kit.
Intended for the collection and stabilization from pediatric oral samples.

Warning and precautions: Ensure the sponge tip does NOT come into contact with any surface prior to collection. Choking hazard. Do not leave kit unattended in presence of a child or infant. Wash with water if the liquid comes in contact with eyes or skin. **Do NOT ingest**. See MSDS at www.dnagenotek.com

Storage: 15°C – 25°C
Patent (www.dnagenotek.com/legalnotices)
PROTOTYPE (P-152) PRO-LB-00198 Issue 1/2015-08

VOL 2 000001

Label legend:
- Manufacturer
- 15°C – 25°C Storage instructions
- REF Catalog number
- Use by

UNC BIOSPECIMEN PROCESSING FACILITY

BSP Facility MSMI Automated DNA extraction from OC-175 collection systems SOP protocol ver. 03_09_15

I Purpose: To insure high molecular weight DNA that is free from contaminants, suitable for sequencing and genotyping. This extraction protocol is based on the extraction of nucleic acids through their capture on highly specific binding M-PVA Magnetic Beads that are thereafter attracted to metal rods, magnetized by an electromagnet. While these magnetized rods transfer the DNA bound to the particles through the different process, wash, and elution solutions, the rod rotation, switched on after deactivation of the electromagnet, leads to efficient and homogeneous re-suspension of the particles during the preparation steps. This results in high yield purity DNA/RNA and success in the following downstream applications.

II Materials:

- Field sample delivered either by study staff or overnight courier, fresh or frozen
- Gloves
- 4 ml MSMI elution tubes
- 24 well deep-well blocks
- 24 rod sheaths
- 5ml syringe
- Sterile gauze
- Proteinase K 20ng/ul
- MSMI and all reagents (binding buffer 2, wash buffers 3-7, and beads from either kits 1074 or 1081). Do not use any solutions marked for RNA or circulating nucleic acids.
- Screw top 1.5-2.0ml microcentrifuge tubes
- -80º C freezer

III Procedure:

Collection

- Universal precautions for working with human specimens should be followed during all stages of specimen handling. Universal (or Standard) precautions such as hand washing, contaminated needle and sharps disposal, use of personal protective equipment, decontamination of equipment and work surfaces, and labeling of specimen containers are described in the UNC Exposure Control Plan (located on lab bookshelf).
- Receive Sample into the BSP facility by courier or overnight carrier and log into current BSP facility's Laboratory Information Management System (LIMS).
- **Immediately prior to extraction on the MSMI, add 10ul prot K. (20ng/ul stock) to the OC-175 tube, vortex, and incubate at 55º C for at least 2 hours.**
- **After incubation, combine liquid and collection swab in the barrel of a 5ml syringe placed in a 15ml conical tube, and spin for 2 minutes at 7000xg. (Put a small piece of**

sterile gauze in the bottom of the barrel first, to keep the swab from spinning through.)

- Bring samples up to 2mls with Tissue lysis buffer (Perkin Elmer Art No 805).
- Duplicate samples should be processed in separate runs to avoid loss through machine failure.

MSMI Run

- Prime lines 2-6
- Choose the "8 saliva 2ml autofill h24 4ml.che" protocol.
- Place disposable rod sheaths in position 1
- Transfer saliva into the deep well block in position 2
- Pipet 240ul beads in the deep well block in position 3
- Place empty deep well racks in position 4, 5 And 6
- Place elution tubes in position 7.
- Fill elution tubes with 170ul elution buffer
- Scan input and output tubes
- Ensure all buffer lines are connected, and carboys contain sufficient buffer
- Start run

Post MSMI Run

- After run has finished remove elution tubes, and transfer to 1.5ml snapcap tube.
- Spin the tubes for 5 minutes at 14000xg.
- Transfer solution to a 1.5ml screwcap tube, taking care not to transfer any of the pellet.
- Place DNA at 4°C prior to quantitation and gel analysis (if requested by project) for short term storage. For long-term storage, store at -80°C.

DNA Quantitation

- DNA is quantitated using UV absorbance on either the Nanodrop instrument or the Dropsense, as well as via picogreen assay. See specific SOPs for the use of these pieces of equipment.
- Optional quantitation using a human-specific qPCR may be performed based on the requirements imposed by the study or the BSP.

Change log

- 03_01_15 is Initial version.
- More extensive introduction added
- Additional information added about solutions to be used.
- Additional supplies added
- Comment about optional quantitations added.

REFERENCES:

1. BioRobot EZ1 User Manual, Version 1.1, July 2005

2. EZ1 DNA Handbook, 3rd Edition, February 2008
   (EN-EZ1-DNA-Blood-Handbook, April 2010)

PRINCIPLE:

Magnetic particle technology combines the speed and efficiency of silica-based DNA purification with the convenient handling of magnetic particles. DNA is isolated from lysates in one step through its binding to the silica surface of the particles in the presence of a chaotrophic salt. The particles are separated from the lysates using a magnet. The DNA is then efficiently washed and eluted in elution buffer.

SPECIMENS:

DNA is extracted from newborn saliva sample collected from the OC-175 ORAcollect for Pediatrics (DNA Genotek, Inc. Ottawa, ON, Canada) with swab and preservation buffer in collection tube. After collection, ensure the collected sample is in an upright position to keep swab in the preservation solution and store at 15ºC to 25ºC.
Extracted DNA not used for requested testing is stored at 4°C short term and then at –20°C for a minimum of two years.

APPARATUS/REAGENTS:

1. BIOROBOT® EZ1 – Qiagen
2. EZ1 DNA Blood 350 µl Kits – Qiagen (cat# 951054)

PROCEDURE:

Proteinase K pretreatment:
Add 10 µl of 20 mg/mL Proteinase K solution to 350 µl of saliva sample. Mix the sample by inverting the sample tube 5 times and incubate at 56^{O}C hybridization oven

for overnight. Proteinase K-treated samples may be stored at room temperature until ready for purification.

EZ1 purification:

1. Insert the appropriate EZ1 Card (EZ1 DNA blood card) completely into the EZ1 Card slot.
2. Switch on the BioRobot EZ1.
3. Press “Start” to display the “Protocols” menu.
4. For worktable setup, press “**2**” to start for the **350** µl **sample protocol**. Select“**2**” to **elute in 100 µl** elution volume. **Pure ethanol wash, select** “**2**” **Yes**.
5. Press any key to proceed through the text displayed in the LCD.
6. Open the workstation door.
7. Invert 1-6 reagent cartridges twice to mix the magnetic particles. Then tap the cartridges to deposit the reagents at the bottom of their wells.
8. Load the reagent cartridges into the cartridge rack.

   Note: After sliding a reagent cartridge into the cartridge rack, ensure that you press down on the cartridge until it clicks into place.

9. Load 1-6 opened elution tubes in to the first row.
10. Load 1-6 tip holders containing filter-tips into the second row.
11. Load 1-6 each of 2 mL tube with 1800 µl of **80% Ethanol** (1440 µl of 100% Ethanol and 360 µl of molecular grade water) into the 3rd row.
12. Load 1-6 opened sample tubes containing 200 µl l saliva samples into the fourth row.
13. Close the workstation door.
14. Press “Start” to start the protocol.
15. When the protocol ends (~20 min), the LCD displays “Protocol Finished”. Open the workstation door.
16. Remove the elution tubes containing the purified DNA. Discard the sample preparation waste.
17. To run another protocol, press “ESC”, prepare samples as described in step 1, and follow the procedure from step 5 onward. Otherwise, press “Stop” twice to return to the first screen of the LCD, close the workstation door, and switch off the BioRobot EZ1.
18. Clean the BioRobot EZ1with Sani wipes.

Berg Lab Exome Library Production companion to the SureSelect (SSEL) Automated Library Prep and Enrichment System

Step I. Shear DNA

In order to successfully extract the exonic region of DNA, it is first necessary to shear the DNA into smaller manageable sizes (generally 150-200 base pairs in size).

Prior to shearing, samples are quantified using the Qubit dsDNA Assay. The Qubit assay uses fluorometry to verify the quality and concentration of the gDNA sample.

Qubit Fluorometer Protocol

Reagent/Sample Prep:

1.) Remove Qubit 0/100 ng/mL standard reagents from the 4° fridge. Allow to equilibrate to room temperature for 30 minutes. Pull Qubit reagent kit containing buffer + dye from the drawer but keep in a dark place until ready for use (dye is light sensitive).

2.) Prepare samples and reagents as follows:

For x number of samples:

a) For x number of samples, add 2. This accounts for each standard (i.e.: 8 samples + 2 standards = 10 total samples).
b) Take total sample number + standard number and place a '.2' decimal behind it (i.e.: 10.2) to account for an average.
c) To make the Qubit working stock mix, perform protocol as follows:
    i. X total sample * 199 = _____ buffer.
    ii. X total sample * 1 = _____ dye.
    iii. Add these two numbers together to equal total working stock.
d) Calculate amount of mix and sample to equal 200 µL for X number of samples and 2 standards.
    i. X µL mix + 2 µL sample = 200 µL.
    ii. X µL mix + 10 µL sample = 200 µL.

Qubit Results:

3.) Vortex mixture of sample and working stock for 2-3 seconds, then incubate at room temperature for 2 minutes.

4.) Take samples and standards to Qubit machine and select 'DNA Concentration – Broad Range.'

5.) Select "New Calibration."

6.) Run 0 ng/mL standard to equilibrate machine, then run 100 ng/mL to finish equilibration.

7.) Run each sample in order, recording each reading.

8.) Run 100 ng/mL standard.

9.) Run 0 ng/mL followed by 100 ng/mL and record results.

10.) Unplug Qubit machine.

Once the gDNA is verified to be non-degraded (A_{260}/A_{280} is 1.8 to 2.0), 3 µg aliquots of each sample are diluted with 1X Low TE Buffer to a final volume of 130 µl for shearing utilizing the Covaris E220 platform.

Covaris E220 DNA Shearing and PostShear SPRI Cleanup

1) After completion of Preshear Qubit, enter concentration result into PreCap page of excel spreadsheet titled 'Library Production.'

2) Spreadsheet will calculate the Vsx (or the volume of DNA that will be aliquotted into Covaris tube for shearing).

3) Spreadsheet will next calculate the Vte (or the volume of TE buffer to add to the aliquotted DNA to bring the total volume in the Covaris tube to 130 µL.

4) Once all calculations are complete, aliquot correct amounts of DNA and TE buffer into individually labeled Covaris Crimp tubes for a total of 130 µL. (Practice slowly inserting the pipette tip into the Crimp tube, slowly ejecting contents, and slowly pulling tip back out to ensure that no sample is lost.

5) Store tubes in 4° fridge until ready for use.

6) When ready to shear, grab tubes and any other pertinent information/lab supplies and head over to the High Throughput Sequence Facility (HTSF).

7) Once in the HTSF, proceed to set-up Covaris.

8) To set-up/start Covaris, ensure that the transducer/arm apparatus are in an elevated position (out of the water basin). Remove water basin from base of machine and fill to the 12----6 line. Place basin back into machine.

9) Take both hoses and connect the main machine to the water pump (longer hose connects to the left port of the water basin while the shorter hose connects to the right port. (Both ports are located on the front of the basin).

10) Open the computer program titled 'SonoLab.'

11) Once basin is filled and hoses are connected, turn on the pump, machine, and chiller (located under the lab bench).

12) The chiller must be between 7 and 8°.

13) Select 'DeGas On' and allow water to DeGas for 30-45 min.

14) Select the correct wells for samples on the 96 tube diagram on screen.

15) Ensure that all SonoLab settings match the Covaris DNA Shearing Quick guide.

16) To start program, ensure that 96 well tube holder plate is secured in the transducer arm. Close the machine door.

17) Click 'Start.'

18) After starting the program, machine will run on each sample for ~7 min.

19) Remove samples after shearing is complete, using nutcracker, and transfer into WHITE store plate for post-shear SPRI cleanup.

20) Begin post-shear SPRI cleanup by opening Maestro on the SciClone computer.

21) Go to WorkBooks-New Shortcut and select "SureSelectXT Workbook Illumina."

22) Ensure that the correct number of samples (highlighted in yellow) is chosen, and be sure to SAVE document once editing.

23) In Maestro, select open application and start the SureSelect workbook.

24) The program will do a run through to assist in setting up the robot deck with all necessary supplies.

25) Ensure that you are sitting with the machine during active moments so that pipetting errors may be addressed when necessary.

Step 2: Assess Sample Quality

Following shearing, samples are applied to a mixture of Solid Phase Reversible Immobilization (SPRI) beads (ultra magnetic beads that selectively bind to DNA fragments and impurities potentially introduced during the shearing process) by MagBio Genomics (Gaithersburg, MD), and are washed from the samples using ethanol. The purified sample is eluted in nuclease-free water and loaded onto the Agilent 2200 TapeStation for a QC step to quantify the concentration and verify that the size range (in base pairs) of the sheared samples is between 75 and 500 base pairs.

Agilent 2200 TapeStation Protocol:

1) Click the 2200 TapeStation controller icon on laptop.

2) Insert tube strip (or plate) sample block into TapeStation.

3) Place loading tips into loading tip holder and insert into TapeStation.

4) Remove ScreenTape from foil packet.

5) Hold tape with ScreenTape label facing you and gently flick the top of the tape.

6) Insert the ScreenTape into the TapeStation, with the label towards the front of the instrument and the barcode facing right.

7) Prepare samples according to the Quick Guide on the lab bench, and place samples into the sample block inside the TapeStation.

8) Ensure that lids have been removed from samples.

9) Select the tubes/wells you wish to run by clicking and dragging the mouse over the sample locations.

10) Once samples are setup and entered in properly, click the start button. This will produce a SAVE AS window. Save file to computer under TapeStation folder.

11) Perform a final check to ensure tips and samples are loaded, and click OK to start.

12) When TapeStation finishes, remove the tip cartridge and tape.

13) Empty the tip bucket before the next experimental run.

Once all samples have been verified to fall within 150 and 200 base pairs in size, precapture library preparation follows the SureSelectXT Automated Target Enrichments for Illumina Paired-End Multiplexed Sequencing; Automated using Agilent NGS Workstation Option B (Version G. 2, April 2015)

The next steps of the pipeline describe the manipulation of each individual gDNA sample to create a prepped library that can then be selected for specific regions of the genome. The gDNA will go through an End-Repair, A-Tail, Adaptor Ligation, and final PCR steps to yield a prepared library. A final quantitation/quality check will be performed at the very end of these four steps to ensure that the gDNA libraries are within the size and concentration range necessary to move forward with the protocol. (An ideal size for the libraries during the PreCap quantitation step is 225-275 base pairs in length. These will be eventually submitted for sequencing and further downstream analysis. The following document describes in detail the steps performed by the Bravo Automated Liquid Handling Platform

Step 3: Modify DNA ends for target enrichment

First, sheared gDNA samples must be repaired. The ends of the DNA need to be polished so that an A-tail facilitating downstream ligation step can be added at a later point. In order to repair the ends, a mixture of 10X End-Repair buffer, dNTP mix, T4 DNA Polymerase, Klenow DNA Polymerase, and T4 Polynucleotide Kinase enzymes is added to each sample. After mixture is added to each sample, incubation using an Eppendorf Polymerase Chain Reaction (PCR) Thermocycler allows for the reactions to catalyze to completion. Following End-Repair incubation, samples are applied to MagBio beads, and impurities are washed from the samples using ethanol. The purified sample is eluted in nuclease-free water.

Below is an explanation of each of the components in the End-Repair mixture:

End-Repair Mixture

-Water/10X End Repair Buffer – provides stability and volume to mixture

-dNTP mix – provides the necessary nucleotide bases to be added to the existing DNA strand

-T4 DNA Polymerase – catalyzes synthesis of DNA in the 5' → 3' direction

-Klenow DNA Polymerase – forms blunt ends by removing 3' overhangs and adding 5' overhangs

-T4 Polynucleotide Kinase – adds 5'-phosphates to allow subsequent ligation in downstream pipeline

Next, end-repaired gDNA must receive an addition of adenosine bases to the ends of the sequence. The A-Tailing process is an enzymatic method for adding a non-template nucleotide to the 3' blunt end of a double stranded DNA molecule. This method allows for subsequent ligation of adaptors to the individual gDNA samples and for preparation of PCR to exponentially increase the amount of prepped library that is created. In order to add a series of adenosine bases to the gDNA samples, a mixture of 10X Klenow Polymerase buffer, dATP bases, and Exo Minus Klenow Polymerase enzyme is added to each sample. After mixture addition, incubation using an Eppendorf PCR Thermocycler allows for the reactions to catalyze to completion. Following A-Tailing incubation, samples are applied to MagBio beads, and impurities are washed from the samples using ethanol. The purified sample is eluted in nuclease-free water.

Below is an explanation of each of the components in the A-Tail mixture:

A-Tail Mixture

-Water/10X Klenow Polymerase buffer – provides stability and volume to mixture

-dATP – provides the adenosine bases to be added to each gDNA end-repaired sample

-Exo Minus Klenow Polymerase enzyme – catalyzes the addition of nucleotide bases to the ends of gDNA

Third, A-Tailed gDNA must be ligated to the fragmented DNA of interest that will allow for the selection of specific regions of the library during the PostCapture pipeline downstream. In order to ligate adaptors to the ends of the gDNA strands, a mixture of 5x T4 DNA Ligase Buffer, SureSelect Adaptor Oligo Mix, and T4 DNA Ligase enzyme is added to each sample. After mixture addition, incubation using an Eppendorf PCR Thermocycler allows for the reactions to catalyze to completion. Following Adaptor Ligation incubation, samples are applied to MagBio beads, and impurities are washed from the samples using ethanol. The purified sample is eluted in nuclease-free water. At this point in the pipeline, half of the eluted material is stored at -20 degrees Celsius and half is amplified via PCR as described in the last step below. The ability to store half of the material up to this point allows for a safety net in the instance that PCR does not work properly to yield enough amplified library to proceed.

Below is an explanation of each of the components in the Adaptor Ligation mixture:

Adaptor Ligation Mixture

-Water/5X T4 DNA Ligase Buffer – provides stability and volume to mixture

-SureSelect Adaptor Oligo Mix – provides the DNA fragments of interest to which will be selected

-T4 DNA Ligase – catalyzes the ligation of the gDNA sample to the adaptors

<u>Step 4: Amplify adaptor-ligated libraries:</u>

Adaptor Ligated gDNA must be amplified to yield enough prepared library to enter the PostCapture pipeline to enrich for the DNA regions of interest. This amplification utilizes Polymerase Chain Reaction to exponentially increase the amount of prepared gDNA up to this point. An Eppendorf ThermoCycler is used to perform three steps through a number of set cycles (denaturation of the double-stranded DNA, annealing of primers to the open reading frame of the DNA, extension of the DNA by the addition of nucleotide bases). To perform the PCR reaction with the adaptor ligated gDNA, a mixture of SureSelect Primer, SureSelect ILM Indexing PreCapture PCR Reverse Primer, 5x Herculase II Reaction Buffer, 100mM dNTP Mix, and Herculase II Fusion DNA Polymerase enzyme is added to each individual sample. After mixture addition, incubation using an Eppendorf PCR Thermocycler allows for the reactions to catalyze to completion.

Below is an explanation of each of the components in the PreCapture PCR mixture:

PreCapture PCR Mixture

-Water – provides stability and volume to mixture

-SureSelect Primer/SureSelect ILM Indexing Pre-Capture PCR Reverse Primer – provide the starting point for the addition of nucleotide bases to the opened reading frames during extension of gDNA

-5X Herculase II Reaction Buffer – provides stability and volume to reaction mixture

-100mM dNTP mix – provides the necessary nucleotide bases to be added to the growing DNA strand

-Herculase II Fusion DNA Polymerase enzyme – catalyzes synthesis of DNA in the 5’ → 3’ direction.

Following PreCapture PCR, samples are applied to MagBio beads, and impurities are washed from the samples using ethanol. The purified sample is eluted in nuclease-free water.

In order to move the amplified gDNA samples into the next phase of the exome sequencing library production pipeline, each must be verified as pure and within a particular concentration

and base-pair size range (to confirm efficacy of the library preparation process thus far). The Agilent 2200 TapeStation platform is used to verify this important quantitation step. This platform utilizes credit card-sized Agilent ScreenTapes designed for DNA use that each contain 16 lanes for 15 individual gDNA samples plus 1 reference DNA ladder to check size and separation during DNA electrophoresis. A very small amount of gDNA amplified library is added to a running buffer and automatically loaded into the ScreenTape via a small internal pipetting liquid handling system within the platform. The platform then draws the samples through the individual lanes of the ScreenTape via DNA electrophoresis, and a virtual gel image is provided with reference measures of size and concentration. This method provides a robust way to determine size and concentration of each individual library. Once all samples have been verified to a specific size and concentration, they are now moved into the next step of the pipeline.

III. Hybridization

Now that the gDNA libraries contain specific adaptors for which they will be enriched, they must hybridize to an RNA probe. The probe is a complementary sequence to which the adaptor is permitted to bind and thus isolates specific regions of interest.

The prepared library must be dried down to a volume that contains a high enough concentration (1 ug is preferable) to enter the hybridization phase. A ThermoFisher Savant ISS110 SpeedVac apparatus is used to lyophilize each individual gDNA library by concentrating the sample. After lyophilization, a mixture of hybridization buffer is mixed and heated at 65 degrees Celsius for 5 minutes using a ThermoFisher heat block meticulously controlled with metal beads to prevent temperature fluctuation. Biotinylated RNA baits are mixed with an RNAse block buffer solution to create a Capture Library mix. Finally, a SureSelect Block Mix is created using three different index block buffers. The Block Mix is added to the adapter ligated gDNA library to ensure that library does not hybridize to library, but rather that library hybridizes to the RNA bait assisting in the enrichment process. A short incubation using an Eppendorf Thermocycler ensures successful hybridization of the block mix to the ends of the libraries.

Once the index block has been hybridized to the gDNA libraries, the hybridization buffer is added to the mixture, along with the capture library containing the RNA biotinylated baits, and the mixture is sealed in a 96-well Eppendorf PCR plate. This plate is incubated for 24 hours to ensure successful hybridization of RNA baits to gDNA libraries.

IV. PostCapture Library Preparation

Immediately after library hybridization to RNA biotinylated baits, all individual libraries are removed from the thermocycler and are sent through a series of washes using a Dynabead MyOne Streptavidin T1 bead. The Streptavidin bead is a superparamagnetic bead that successfully isolates biotinylated molecules, thus serves to enrich for the hybridized library completed in the previous step.

First, Agilent Binding Buffer is used to clean the Streptavidin beads to ensure a clean, purified capture system to be used for target enrichment. Next, hybridized library is added to an aliquot of Streptavidin bead and sent through two wash buffers to further purify the remaining library. Finally, purified library is eluted in water.

Following purification and elution, a series of SureSelect XT Indexes labeled '1-16' for Illumina are assigned to each individual gDNA library. These indexes are 5 nucleotide base sequences that tag each individual library to allow for proper identification further down the sequencing pipeline.

Finally, Indexed gDNA libraries must be amplified to yield enough prepared library to enter the final pooling pipeline to be sent off for sequencing. This amplification utilizes Polymerase Chain Reaction to exponentially increase the amount of indexed gDNA library. An Eppendorf ThermoCycler is used to perform three steps through a number of set cycles (denaturation of the double-stranded DNA, annealing of primers to the open reading frame of the DNA, extension of the DNA by the addition of nucleotide bases). To perform the PCR reaction with

the adaptor ligated gDNA, a mixture of SureSelect ILM Indexing PostCapture PCR Forward Primer, 5x Herculase II Reaction Buffer, 100mM dNTP Mix, and Herculase II Fusion DNA Polymerase enzyme is added to each individual indexed sample. The index assigned to each library serves as the reverse primer. After mixture addition, incubation using an Eppendorf PCR Thermocycler allows for the reactions to catalyze to completion.
Below is an explanation of each of the components in the PreCapture PCR mixture:

PostCapture PCR Mixture

-Water – provides stability and volume to mixture
-SureSelect ILM Indexing Post-Capture PCR Forward Primer – provide the starting point for the addition of nucleotide bases to the opened reading frames during extension of gDNA
-5X Herculase II Reaction Buffer – provides stability and volume to reaction mixture
-100mM dNTP mix – provides the necessary nucleotide bases to be added to the growing DNA strand
-Herculase II Fusion DNA Polymerase enzyme – catalyzes synthesis of DNA in the 5' → 3' direction.

Following PostCapture PCR, the final libraries are sent through one last clean-up phase where they are applied to a mixture of MagBio ultra magnetic beads that selectively bind to DNA fragments. Once bound to beads, ethanol is applied twice to wash away reagents and impurities introduced during the Post-Capture target enrichment process, and the purified library material is efficiently eluted in RNAse/DNAse free laboratory water.

In order to pool the final libraries to be submitted for sequencing, each must be verified as pure and within a particular concentration and base-pair size range (to confirm efficacy of the library preparation process). The Agilent 2200 TapeStation platform is used to verify this important quantitation step.

V. Library Pooling

In order to successfully sequence individual libraries, they must be pooled in groups of 4 in order to fill one individual lane of the HiSeq2500 sequencing platform that is utilized. The final step in the library process consists of manually assigning individual indexed libraries to "pools" or groups of samples to be submitted for sequencing.

The method for pooling consists of assigning four different indexed libraries to a standardized pool based on size, concentration, and index tag. It is important to standardize the final library concentrations to allow for equal representation of each library in each pool. To standardize, the ThermoFisher Savant ISS110 SpeedVac apparatus is used to lyophilize each final gDNA library by concentrating the sample. Once pooling is complete, all pools are securely transferred to the High Throughput Sequencing Facility at the University of North Carolina at Chapel Hill for further analysis and downstream sequencing.

Sources

Rehm, Heidi et al. "ACMG clinical laboratory standards for next-generation sequencing." *Genetics in Medicine* 15.9 (2013): 733-47. Print.

SureSelect XT Target Enrichment System for Illumina Paired-End Sequencing Library. Version 1.5, November 2012, Agilent Technologies.

SureSelect^{XT} Automated Target Enrichment for Illumina Paired-End Multiplexed Sequencing

Automated using Agilent NGS Workstation Option B

Protocol

Version G.2, April 2015

SureSelect platform manufactured with Agilent SurePrint Technology

Research Use Only. Not for use in Diagnostic Procedures.

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Notices

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Technical Support

For technical product support, contact your local Agilent Support Services representative. For Agilent's worldwide sales and support center telephone numbers, go to www.agilent.com/chem/contactus

or send an email to:
SureSelect.Support@agilent.com

Notice to Purchaser

Research Use Only. Not for use in diagnostic procedures.

SureSelect capture libraries and reagents must be used within one year of receipt.

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CAUTION

A **CAUTION** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a **CAUTION** notice until the indicated conditions are fully understood and met.

WARNING

A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

In this Guide...

This guide describes an optimized protocol for Illumina paired-end multiplexed library preparation using the Agilent SureSelect^{XT} Automated Library Prep and Capture System.

This protocol is specifically developed and optimized to capture the genomic regions of interest using Agilent's SureSelect system to enrich targeted regions of the genome from repetitive sequences and sequences unrelated to the research focus prior to sample sequencing. Sample processing steps are automated using the Agilent NGS Workstation Option B.

1 Before You Begin

This chapter contains information (such as procedural notes, safety information, required reagents and equipment) that you should read and understand before you start an experiment.

2 Using the Agilent NGS Workstation for SureSelect Target Enrichment

This chapter contains an orientation to the Agilent NGS Workstation, an overview of the SureSelect target enrichment protocol, and considerations for designing SureSelect experiments for automated processing using the Agilent NGS Workstation.

3 Sample Preparation (3 µg DNA Samples)

This chapter describes the steps to prepare the DNA samples for target enrichment when starting with 3 µg of gDNA.

4 Sample Preparation (200 ng DNA Samples)

This chapter describes the steps to prepare the DNA samples for target enrichment when starting with 200 ng of gDNA.

5 Hybridization

This chapter describes the steps to hybridize and capture samples.

6 Indexing

This chapter describes the steps to amplify, purify, and assess quality of the sample libraries. Samples are pooled by mass prior to sequencing.

7 Reference

This chapter contains reference information.

What's New in Version G.2

- Support for the OneSeq Capture Libraries (see Table 3 on page 14 and Table 75 on page 141).
- Support for ClearSeq Capture Libraries, including ClearSeq Comprehensive Cancer XT Libraries (see Table 2 on page 13).
- Correction to ordering information for Axygen 96 Deep Well Plates (see Table 4 on page 15).
- Updates to workflow diagram (see Figure 2 on page 29).
- Updates to sequencing data guidelines (see page 133).

What's New in Version G.1

- Support for kits with either 8-bp indexes A01 to H12 (revised index configuration, typically received December 2014 or later) or 6-bp indexes 1 to 16 (original index configuration, typically received before December 2014).

  Kits with 8-bp indexes include 96 indexing primers provided in a blue plate format. For indexing protocol details, see page 134. For kit content details see page 154. For nucleotide sequences of the 8-bp indexes, see Table 86 on page 158. **User guide version G.1 includes updates to the 8-bp index sequences in Table 86** on page 158**.** Do not use version G.0 for 8-bp index sequence information.

  Kits with 6-bp indexes include 16 indexing primers provided in clear-capped tubes. For indexing protocol details, see page 134. For kit content details see page 159. For nucleotide sequences of the 6-bp indexes, see Table 91 on page 162.
- Updates to sequencing sample and run setup guidelines (see page 152).
- Removal of SureSelect Elution Buffer and SureSelect Neutralization Buffer from SureSelect Target Enrichment Box 1 (p/n 5190-8646, provided with kits with revised index configuration; see Table 83 on page 156).

Content

1 Before You Begin

Make sure you read and understand the information in this chapter and have the necessary equipment and reagents listed before you start an experiment.

CAUTION

This Protocol supports the SureSelect Target Enrichment workflow with on-bead post-capture PCR, using version 1.5.1 (**v1.5.1)** VWorks SureSelect automation protocols.

If your VWorks SureSelect setup form displays earlier versions of the automation protocols, please contact service.automation@agilent.com for assistance.

NOTE

This protocol describes automated sample processing using the Agilent NGS Workstation. For non-automated sample processing procedures for Agilent's SureSelect^{XT} Target Enrichment Kit for Illumina Multiplex Sequencing, see publication G7530-90000.

NOTE

This protocol differs from other SureSelect protocols at several steps. Pay close attention to the primers used for each amplification step and the blocking agents used during hybridization.

Procedural Notes

- This User Guide includes protocols for library preparation using either 3 µg DNA samples (see Chapter 3 on page 35) or 200 ng DNA samples (see Chapter 4 on page 71). Make sure that you are following the appropriate protocol for your DNA input amount. After the prepared libraries are amplified, both DNA input options use the same protocol for hybridization and post-capture processing.
- Certain protocol steps require the rapid transfer of sample plates between the Bravo deck and a thermal cycler. Locate your thermal cycler in close proximity to the Agilent NGS Workstation to allow rapid and efficient plate transfer.
- Prepare and load the Agilent NGS Workstation as detailed in each of the protocol steps before initiating each automated protocol run. When loading plates in the workstation's Labware MiniHub, always place plates in the orientation shown in Figure 3 on page 41.
- To prevent contamination of reagents by nucleases, always wear powder-free laboratory gloves and use dedicated solutions and pipettors with nuclease-free aerosol-resistant tips.
- Maintain a clean work area.
- Do not mix stock solutions and reactions containing gDNA on a vortex mixer. Instead, gently tap the tube with your finger to mix the sample.
- Avoid repeated freeze-thaw cycles of stock and diluted gDNA solutions. Possible stopping points, where gDNA samples may be stored overnight at 4°C, are marked in the protocol. When storing samples for >24 hours, store the samples at –20°C, but do not subject the samples to multiple freeze/thaw cycles.
- When preparing frozen reagent stock solutions for use:
  1. Thaw the aliquot as rapidly as possible without heating above room temperature.
  2. Mix briefly on a vortex mixer, then spin in a centrifuge for 5 to 10 seconds to drive the contents off of walls and lid.
  3. Store on ice or in a cold block until use.
- In general, follow Biosafety Level 1 (BL1) safety rules.

Safety Notes

CAUTION

- Wear appropriate personal protective equipment (PPE) when working in the laboratory.

Required Reagents

Table 1 Required Reagents

Description	Vendor and part number
SureSelect, ClearSeq or OneSeq Capture Library*	Select the appropriate library from Table 2 or Table 3
SureSelect^{XT} Automation Reagent Kit*†	
HiSeq platform (HSQ), 96 reactions	Agilent p/n G9641B
HiSeq platform (HSQ), 480 reactions	Agilent p/n G9641C
MiSeq platform (MSQ), 96 reactions	Agilent p/n G9642B
MiSeq platform (MSQ), 480 reactions	Agilent p/n G9642C
Herculase II Fusion DNA Polymerase, 400 reactions (includes dNTP mix and 5x Buffer)	Agilent p/n 600679
QPCR NGS Library Quantification Kit (Illumina GA)	Agilent p/n G4880A
Nuclease-free Water (not DEPC-treated)	Ambion Cat #AM9930
1X Low TE Buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA)	Life Technologies p/n 12090-015, or equivalent
Agencourt AMPure XP Kit	Beckman Coulter Genomics
60 mL	p/n A63881
450 mL	p/n A63882
Quant-iT dsDNA BR Assay Kit, for use with the Qubit fluorometer	Life Technologies
100 assays, 2-1000 ng	Cat #Q32850
500 assays, 2-1000 ng	Cat #Q32853
Dynabeads MyOne Streptavidin T1	Life Technologies
2 mL	Cat #65601
10 mL	Cat #65602
100 mL	Cat #65603
100% Ethanol, molecular biology grade	Sigma-Aldrich p/n E7023

* SureSelect, ClearSeq, and OneSeq reagents must be used within one year of receipt.

† Each 96-reaction kit contains sufficient reagents for 96 reactions used in runs that include at least 3 columns of samples per run.

Table 2 SureSelect^{XT} Automation Capture Libraries

Capture Library	96 Reactions	480 Reactions
SureSelect^{XT} Clinical Research Exome	5190-7344	–
SureSelect^{XT} Focused Exome	5190-7789	–
SureSelect^{XT} Focused Exome Plus 1	5190-7792	–
SureSelect^{XT} Focused Exome Plus 2	5190-7796	–
SureSelect^{XT} Human All Exon v5	5190-6210	–
SureSelect^{XT} Human All Exon v5 + UTRs	5190-6215	–
SureSelect^{XT} Human All Exon v5 + lncRNA	5190-6448	–
SureSelect^{XT} Human All Exon v5 Plus	5190-6224	–
SureSelect^{XT} Human All Exon v4	5190-4633	5190-4635
SureSelect^{XT} Human All Exon v4 + UTRs	5190-4638	5190-4640
SureSelect^{XT} Mouse All Exon	5190-4643	5190-4645
SureSelect^{XT} Custom 1 kb up to 499 kb (reorder)	5190-4808 (5190-4813)	5190-4810 (5190-4815)
SureSelect^{XT} Custom 0.5 Mb up to 2.9 Mb (reorder)	5190-4818 (5190-4823)	5190-4820 (5190-4825)
SureSelect^{XT} Custom 3 Mb up to 5.9 Mb (reorder)	5190-4828 (5190-4833)	5190-4830 (5190-4835)
SureSelect^{XT} Custom 6 Mb up to 11.9 Mb (reorder)	5190-4838 (5190-4843)	5190-4840 (5190-4845)
SureSelect^{XT} Custom 12 Mb up to 24 Mb (reorder)	5190-4898 (5190-4903)	5190-4900 (5190-4905)

Table 3 Compatible ClearSeq and OneSeq Automation Capture Libraries

Capture Library	96 Reactions	480 Reactions
ClearSeq Comprehensive Cancer XT	5190-8013	–
ClearSeq Comprehensive Cancer Plus XT	5190-8016	–
ClearSeq Inherited Disease XT	5190-7520	–
ClearSeq Inherited Disease Plus XT	5190-7523	–
ClearSeq DNA Kinome XT	5190-4648	5190-4650
OneSeq Constitutional Research Panel	5190-8704	–
OneSeq Hi Res CNV Backbone + Custom 1–499 kb	5190-8888	–
OneSeq Hi Res CNV Backbone + Custom 0.5 –2.9 Mb	5190-8891	–
OneSeq Hi Res CNV Backbone + Custom 3–5.9 Mb	5190-8894	–
OneSeq Hi Res CNV Backbone + Custom 6–11.9 Mb	5190-8897	–

Required Equipment

Table 4 Required Equipment

Description	Vendor and part number
Agilent NGS Workstation Option B, with VWorks software version 11.3.0.1195	Agilent p/n G5522A Contact Agilent Automation Solutions for more information: Customerservice.automation@agilent.com
Robotic Pipetting Tips (Sterile, Filtered, 250 µL)	Agilent p/n 19477-022
Thermal cycler and accessories	SureCycler 8800 Thermal Cycler (Agilent p/n G8810A), 96 well plate module (Agilent p/n G8810A) and compression mats (Agilent p/n 410187) or equivalent
PCR plates compatible with selected Thermal Cycler, e.g. Agilent semi-skirted PCR plate for the SureCycler 8800 Thermal Cycler See Table 9 on page 33 for a list of supported PCR plates for automation protocols	Agilent p/n 401334
Eppendorf twin.tec full-skirted 96-well PCR plates	Eppendorf p/n 951020401 or 951020619
Thermo Scientific Reservoirs	Thermo Scientific p/n 1064156
Nunc DeepWell Plates, sterile, 1.3-mL well volume	Thermo Scientific p/n 260251
Axygen 96 Deep Well Plate, 2 mL, Square Well (waste reservoirs; working volume 2.2 mL)	Axygen p/n P-2ML-SQ-C E & K Scientific p/n EK-2440
DNA LoBind Tubes, 1.5-mL PCR clean, 250 pieces	Eppendorf p/n 022431021 or equivalent
Qubit Fluorometer	Life Technologies p/n Q32857
Qubit assay tubes	Life Technologies p/n Q32856
Covaris Sample Preparation System, S-series of E-series model	Covaris
Covaris sample holders	
96 microTUBE plate (E-series only)	Covaris p/n 520078
microTUBE for individual sample processing	Covaris p/n 520045

Table 4 Required Equipment (continued)

Description	Vendor and part number
DNA Analysis Platform and Consumables	
Agilent 2100 Bioanalyzer Laptop Bundle	Agilent p/n G2943CA
Agilent 2100 Bioanalyzer Electrophoresis Set	Agilent p/n G2947CA
DNA 1000 Kit	Agilent p/n 5067-1504
High Sensitivity DNA Kit	Agilent p/n 5067-4626
OR	
Agilent 2200 TapeStation	Agilent p/n G2964AA or G2965AA
D1000 ScreenTape	Agilent p/n 5067-5582
D1000 Reagents	Agilent p/n 5067-5583
High Sensitivity D1000 ScreenTape	Agilent p/n 5067-5584
High Sensitivity D1000 Reagents	Agilent p/n 5067-5585
Centrifuge	Eppendorf Centrifuge model 5804 or equivalent
P10, P20, P200 and P1000 pipettes	Pipetman P10, P20, P200, P1000 or equivalent
Vacuum concentrator	Savant SpeedVac, model DNA120, with 96-well plate rotor, model RD2MP, or equivalent
Magnetic separator	DynaMag-50 magnet, Life Technologies p/n 123-02D or equivalent
Mx3005P Real-Time PCR System	Agilent p/n 401449 or equivalent
Mx3000P/Mx3005P 96-well tube plates	Agilent p/n 410088 or equivalent
Mx3000P/Mx3005P optical strip caps	Agilent p/n 401425 or equivalent
NucleoClean Decontamination Wipes	Millipore p/n 3097
Ice bucket	
Powder-free gloves	
Sterile, nuclease-free aerosol barrier pipette tips	
Vortex mixer	
Timer	

2 Using the Agilent NGS Workstation for SureSelect Target Enrichment

This chapter contains an orientation to the Agilent NGS Workstation, an overview of the SureSelect^{XT} target enrichment protocol, and considerations for designing SureSelect experiments for automated processing using the Agilent NGS Workstation.

About the Agilent NGS Workstation

About the Bravo Platform

The Bravo platform is a versatile liquid handler with a nine plate-location platform deck, suitable for handling 96-well, 384-well, and 1536-well plates. The Bravo platform is controlled by the VWorks Automation Control software. Fitted with a choice of seven interchangeable fixed-tip or disposable-tip pipette heads, it accurately dispenses fluids from 0.1 µL to 250 µL.

CAUTION Before you begin, make sure that you have read and understand operating, maintenance and safety instructions for using your Bravo platform. Refer to the *Bravo Platform User Guide* (G5409-90006) and the *VWorks Software User Guide* (G5415-90063).

Bravo Platform Deck

The protocols in the following sections include instructions for placing plates and reagent reservoirs on specific Bravo deck locations. Use Figure 1 to familiarize yourself with the location numbering convention on the Bravo platform deck.

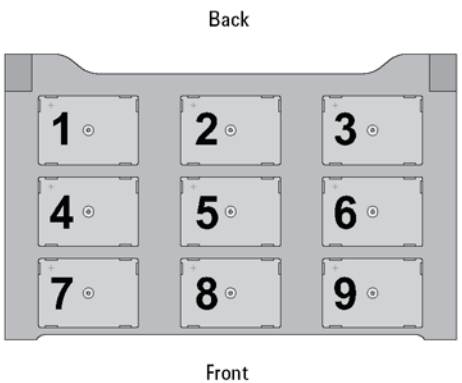


Figure 1 Bravo platform deck

Setting the Temperature of Bravo Deck Heat Blocks

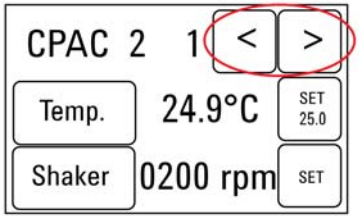
Bravo deck positions 4 and 6 are equipped with Inheco heat blocks, used to incubate sample plates at defined temperatures during the run. Runs that include high- (85°C) or low- (4°C) temperature incubation steps may be expedited by pre-setting the temperature of the affected block before starting the run.

Bravo deck heat block temperatures may be changed using the Inheco Multi TEC Control device touchscreen as described in the steps below. See Table 5 for designations of the heat block-containing Bravo deck positions on the Multi TEC control device.

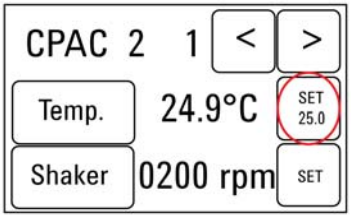
Table 5 Inheco Multi TEC Control touchscreen designations

Bravo Deck Position	Designation on Inheco Multi TEC Control Screen
4	CPAC 2 1
6	CPAC 2 2

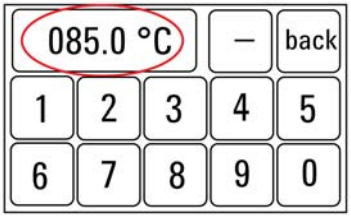
1 Using the arrow buttons, select the appropriate block (CPAC 2 block 1 or CPAC 2 block 2).



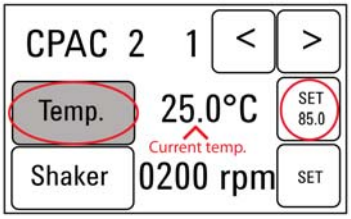
2 To set the temperature of the selected block, press the SET button.



3 Using the numeral pad, enter the desired temperature. The entered temperature appears in the top, left rectangle. Once the correct temperature is displayed, press the rectangle to enter the temperature.



4 Press the Temp button until the new temperature is displayed on the SET button and until the Temp button is darkened, indicating that the selected heat block is heating or cooling to the new temperature setting. The current temperature of the block is indicated in the center of the display.



Setting the Temperature of Bravo Deck Position 9 Using the ThermoCube Device

Bravo deck position 9 is equipped with a ThermoCube thermoelectric temperature control system, used to incubate components at a defined temperature during the run. During protocols that require temperature control at position 9, you will be instructed to start and set the temperature of the ThermoCube device before starting the run.

ThermoCube temperature settings are modified using the control panel (LCD display screen and four input buttons) on the front panel of the device using the following steps.

1. Turn on the ThermoCube and wait for the LCD screen to display **TEMP.**
2. Press the **UP** or **DOWN** button to change **SET TEMP 1** to the required set point.
3. Press the **START** button.

The ThermoCube then initiates temperature control of Bravo deck position 9 at the displayed set point.

VWorks Automation Control Software

VWorks software, included with your Agilent NGS Workstation, allows you to control the robot and integrated devices using a PC. The Agilent NGS Workstation is preloaded with VWorks software containing all of the necessary SureSelect system liquid handling protocols. General instructions for starting up the VWorks software and the included protocols is provided below. Each time a specific VWorks protocol is used in the SureSelect procedure, any settings required for that protocol are included in the relevant section of this manual.

NOTE

The instructions in this manual are compatible with VWorks software version 11.3.0.1195, including SureSelect^{XT} automation protocols version 1.5.1.

If you have questions about VWorks version compatibility, please contact service.automation@agilent.com.

Logging in to the VWorks software

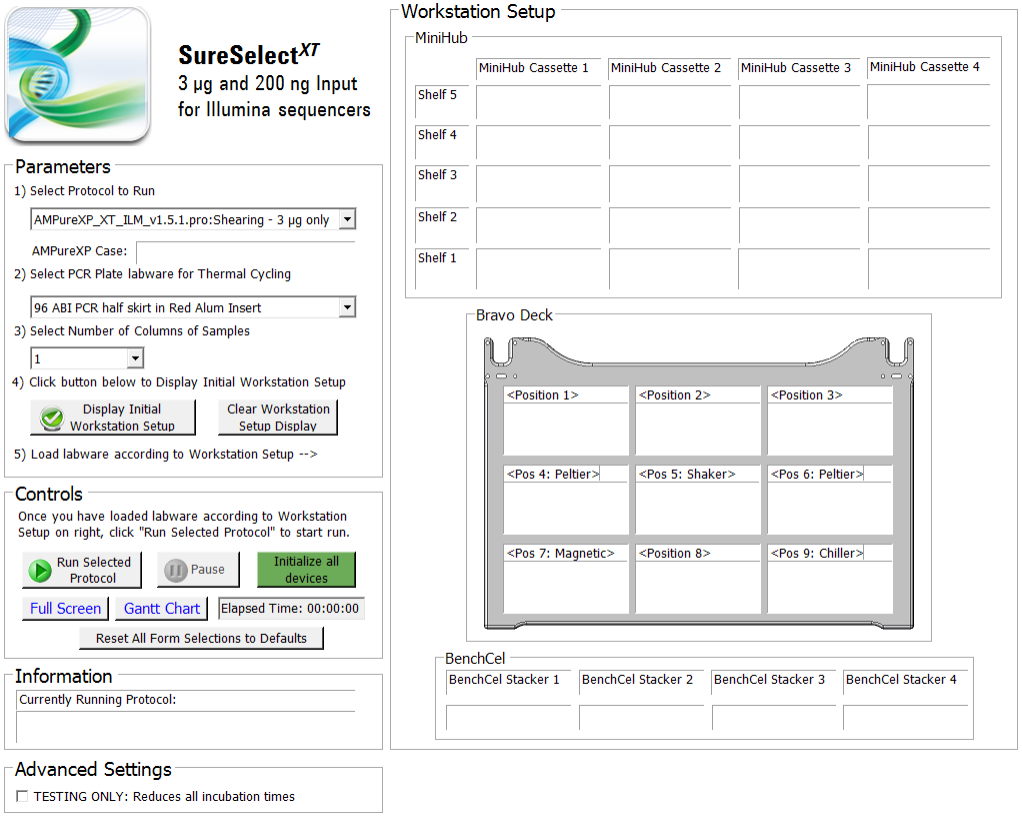
1. Double-click the VWorks icon or the XT_ILM_v1.5.1.VWForm shortcut on the Windows desktop to start the VWorks software.
2. If User Authentication dialog is not visible, click **Log in** on the VWorks window toolbar.
3. In the User Authentication dialog, type your VWorks user name and password, and click **OK**. (If no user account is set up, contact the administrator.)

VWorks protocol and runset files

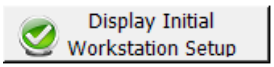
VWorks software uses two file types for automation runs, .pro (protocol) files and .rst (runset) files. Runset files are used for automated procedures in which the workstation uses more than one automation protocol during the run.

Using the SureSelectXT_ILM_v1.5.1.VWForm to setup and start a run

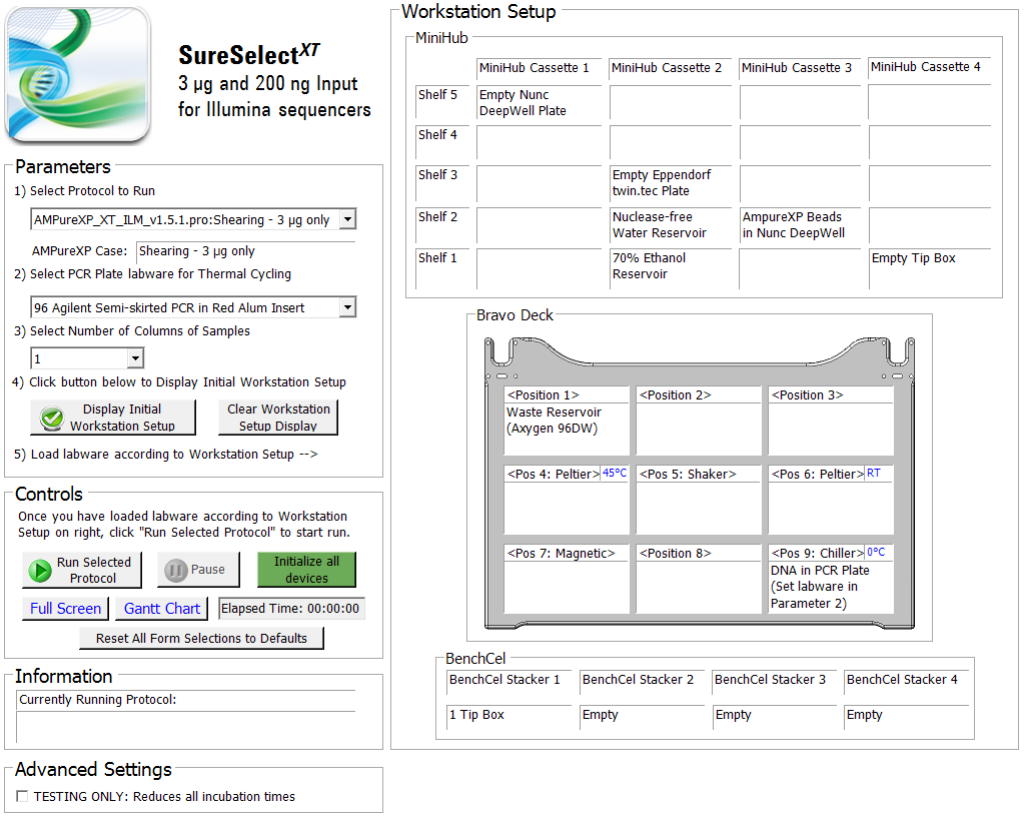
Use the VWorks form SureSelectXT_ILM_v1.5.1.VWForm, shown below, to set up and start each SureSelect automation protocol or runset.



1. Open the form using the XT_ILM_v1.5.1.VWForm shortcut on your desktop.
2. Use the form drop-down menus to select the appropriate SureSelect workflow step and number of columns of samples for the run.
3. Once all run parameters have been specified on the form, click **Display Initial Workstation Setup.**



4 The Workstation Setup region of the form will then display the required placement of reaction components and labware in the NGS Workstation for the specified run parameters.

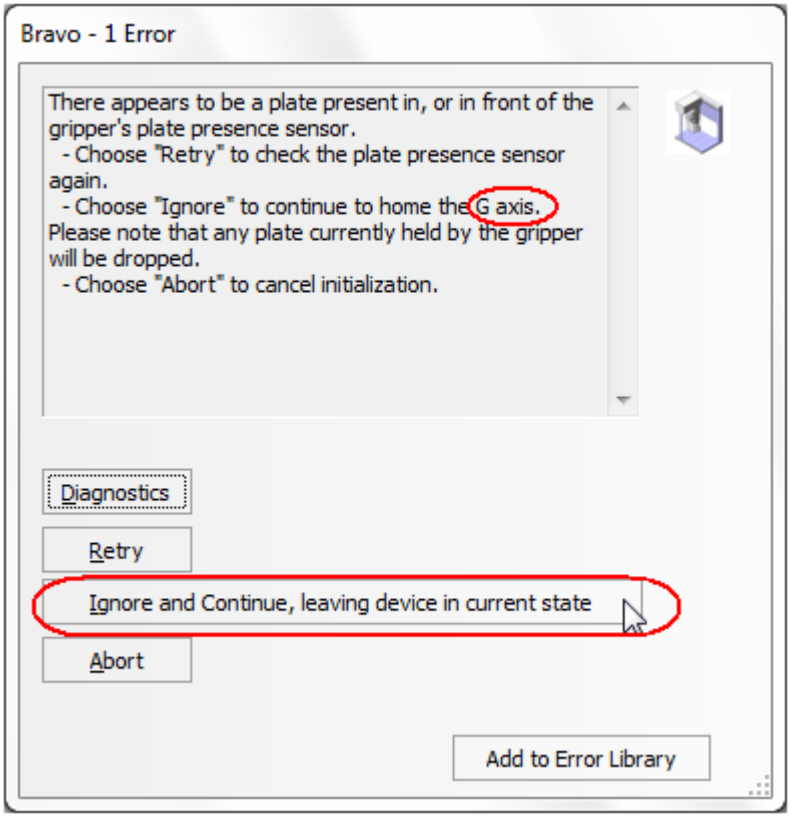


5 After verifying that the NGS Workstation has been set up correctly, click **Run Selected Protocol**.

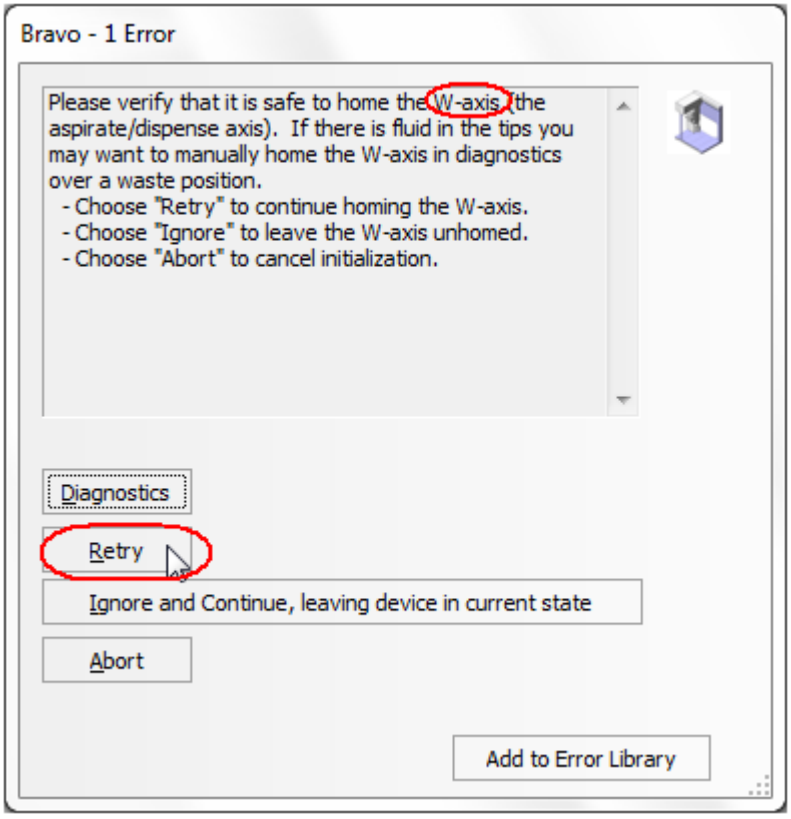
Error messages encountered at start of run

After starting the run, you may see the error messages displayed below. When encountered, make the indicated selections and proceed with the run. Encountering either or both of these error messages is not indicative of a problem with the NGS workstation or your run setup.

1 If you encounter the G-axis error message shown below, select **Ignore and Continue, leaving device in current state**.



2 If you encounter the W-axis error message shown below, select **Retry.**



Verifying the Simulation setting

VWorks software may be run in simulation mode, during which commands entered on screen are not completed by the NGS workstation. If workstation devices do not respond when you start a run, verify the simulation mode status in VWorks using the following steps.

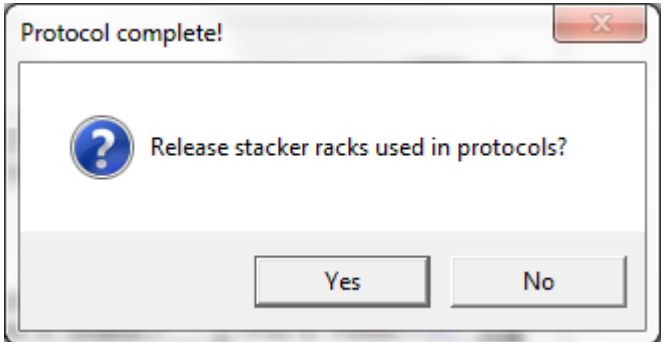
1 Verify that **Simulation is off** is displayed on the status indicator (accessible by clicking **View** > **Control Toolbar**).

2 If the indicator displays **Simulation is on,** click the status indicator button to turn off the simulation mode.

NOTE If you cannot see the toolbar above the SureSelect_XT_Illumina VWorks form, click the **Full Screen** button to exit full screen mode. If the toolbar is still not visible, right-click on the form and then select **Control Toolbar** from the menu.

Finishing a protocol or runset

The window below appears when each run is complete. Click **Yes** to release the BenchCel racks to allow removal of components used in the current run in preparation for the next .pro or .rst run.



Overview of the SureSelect Target Enrichment Procedure

Figure 2 summarizes the SureSelect target enrichment workflow for samples to be sequenced using the Illumina paired-read sequencing platform. For each sample to be sequenced, individual library preparations, hybridizations, and captures are performed. The samples are then tagged by PCR with an index sequence. Depending on the target size of the SureSelect capture, multiple samples can be pooled and sequenced in a single lane using the Illumina-specified multiplex index tags that are provided with SureSelect Library Prep kits.

The SureSelect^{XT} automated target enrichment system is compatible with gDNA samples containing either 3 µg or 200 ng DNA, with minor differences in the VWorks protocols used during the Sample Preparation segment of the workflow for the two DNA input options. Both DNA input options use identical automation protocols for the Hybridization and Indexing segments of the workflow.

When starting with 3 µg gDNA samples, see Table 6 for a summary of the VWorks protocols used during the workflow. Then, see Sample Preparation (3 µg DNA Samples), Hybridization, and Indexing chapters for complete instructions for use of the VWorks protocols for sample processing.

When starting with 200 ng gDNA samples, see Table 7 for a summary of the VWorks protocols used during the workflow. Then, see Sample Preparation (200 ng DNA Samples), Hybridization, and Indexing chapters for complete instructions for use of the VWorks protocols for sample processing.

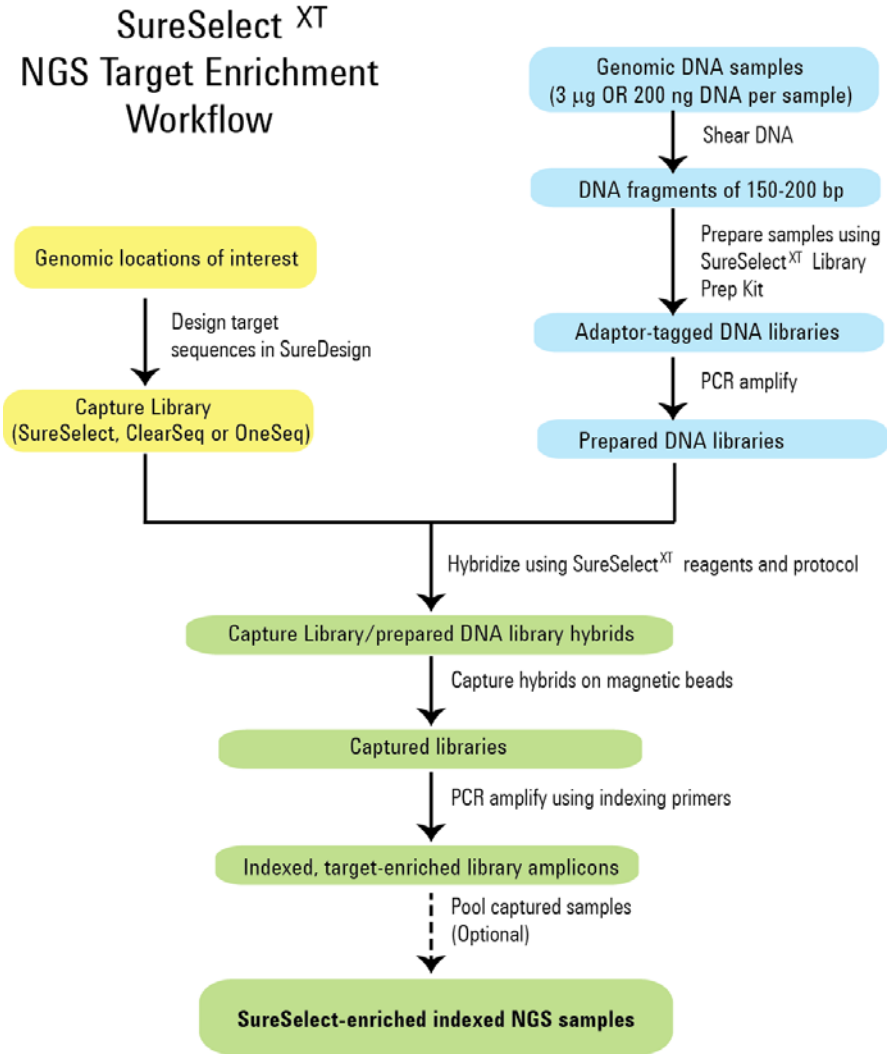


Figure 2 Overall sequencing sample preparation workflow.

Table 6 Overview of VWorks protocols and runsets used for 3 µg gDNA samples

Workflow Step (Protocol Chapter)	Substep	VWorks Protocols Used for Agilent NGS Workstation automation
Sample Preparation	Purify DNA using AMPure XP beads	AMPureXP_XT_ILM_v1.5.1.pro:Shearing-3 µg only
	Prepare adaptor-ligated DNA	LibraryPrep_XT_ILM_v1.5.1.rst
	Amplify adaptor-ligated DNA	Pre-CapturePCR_XT_ILM_3µg_v1.5.1.pro
	Purify DNA using AMPure XP beads	AMPureXP_XT_ILM_v1.5.1.pro:Pre-Capture PCR
Hybridization	Aliquot 750-ng of prepped libraries for hybridization	Aliquot_Libraries_v1.5.1.pro
	Hybridize prepped DNA to Capture Library	Hybridization_v1.5.1.pro
	Capture and wash DNA hybrids	SureSelectCapture&Wash_v1.5.1.rst
Indexing	Add index tags by PCR	Post-CaptureIndexing_XT_ILM_v1.5.1.pro
	Purify DNA using AMPure XP beads	AMPureXP_XT_ILM_v1.5.1.pro:Post-Capture PCR

Table 7 Overview of VWorks protocols and runsets used for 200 ng gDNA samples

Workflow Step (Protocol Chapter)	Substep	VWorks Protocols Used for Agilent NGS Workstation automation
Sample Preparation	Prepare adaptor-ligated DNA	LibraryPrep_XT_ILM_v1.5.1.rst
	Amplify adaptor-ligated DNA	Pre-CapturePCR_XT_ILM_200ng_v1.5.1.pro
	Purify DNA using AMPure XP beads	AMPureXP_XT_ILM_v1.5.1.pro:Pre-Capture PCR
Hybridization	Aliquot 750-ng of prepped libraries for hybridization	Aliquot_Libraries_v1.5.1.pro
	Hybridize prepped DNA to Capture Library	Hybridization_v1.5.1.pro
	Capture and wash DNA hybrids	SureSelectCapture&Wash_v1.5.1.rst
Indexing	Add index tags by PCR	Post-CaptureIndexing_XT_ILM_v1.5.1.pro
	Purify DNA using AMPure XP beads	AMPureXP_XT_ILM_v1.5.1.pro:Post-Capture PCR

Experimental Setup Considerations for Automated Runs

Agilent SureSelect Automated Library Prep and Capture System runs may include 1, 2, 3, 4, 6, or 12 columns (equivalent to 8, 16, 24, 32, 48, or 96 wells) of gDNA samples to be enriched for sequencing on the Illumina platform. Plan your experiments using complete columns of samples.

Table 8 Columns to Samples Equivalency

Number of Columns Processed	Total Number of Samples Processed
1	8
2	16
3	24
4	32
6	48
12	96

The number of columns or samples that may be processed using the supplied reagents (see Table 1) will depend on the experimental design. For greatest efficiency of reagent use, plan experiments using at least 3 columns per run. Each 96-reaction kit contains sufficient reagents for 96 reactions configured as 4 runs of 3 columns of samples per run.

Considerations for Placement of gDNA Samples in 96-well Plates for Automated Processing

- The Agilent NGS Workstation processes samples column-wise beginning at column 1. gDNA samples should be loaded into 96-well plates column-wise, in well order A1 to H1, then A2 to H2, ending with A12 to H12. When processing partial runs with <12 sample columns, do not leave empty columns between sample columns; always load the plate using the left-most column that is available.
- At the hybridization step (see Figure 2), you can add a different Capture Library to each row of the plate. Plan your experiment such that each prepared DNA library corresponds to the appropriate Capture Library row in the sample plate.
- For sample indexing after hybridization to the SureSelect library (see Figure 2), you will need to prepare a separate plate containing the indexing primers. Assign the wells to be indexed with their respective indexing primers during experimental design.
- For post-capture amplification (see Figure 2), different Capture Libraries can require different amplification cycle numbers, based on sizes of the captured targets. It is most efficient to process similar-sized Capture Libraries on the same plate. See Table 75 on page 141 to determine which Capture Libraries may be amplified on the same plate.

Considerations for Equipment Setup

- Some workflow steps require the rapid transfer of sample plates between the Bravo deck and a thermal cycler. Locate your thermal cycler in close proximity to the Agilent NGS Workstation to allow rapid and efficient plate transfer.
- Several workflow steps require that the sample plate be sealed using the PlateLoc thermal microplate sealer included with the Agilent NGS Workstation, and then centrifuged to collect any dispersed liquid. To maximize efficiency, locate the centrifuge in close proximity to the Agilent NGS Workstation.

PCR Plate Type Considerations

Automation protocols include several liquid-handling steps in which reagents are dispensed to PCR plates in preparation for transfer to a thermal cycler. For these steps you must specify the PCR plate type to be used on the SureSelectXT_ILM_v1.5.1.VWForm to allow correct configuration of the liquid handling components for the PCR plate type. Before you begin the automation protocol, make sure that you are using a supported PCR plate type. The PCR plate type to be used in the protocol is specified using the menu below. Vendor and part number information is provided for the supported plate types in Table 9.

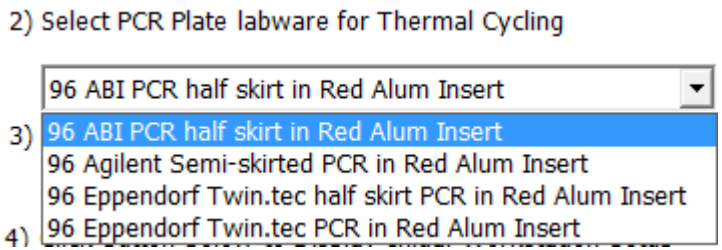


Table 9 Ordering information for supported PCR plates

Description in VWorks menu	Vendor and part number
96 ABI PCR half-skirted plates (MicroAmp Optical plates)	Life Technologies p/n N8010560
96 Agilent semi-skirted PCR plate	Agilent p/n 401334
96 Eppendorf Twin.tec half-skirted PCR plates	Eppendorf p/n 951020303
96 Eppendorf Twin.tec PCR plates (full-skirted)	Eppendorf p/n 951020401

3 Sample Preparation (3 µg DNA Samples)

This section contains instructions for the preparation of gDNA libraries from samples containing 3 µg of DNA. For lower input (200 ng) DNA samples, see the protocol on page 71.

This section contains instructions for gDNA library preparation specific to the Illumina paired-read sequencing platform and to automated processing using the Agilent NGS Workstation. For each sample to be sequenced, individual library preparations, hybridizations, and captures are performed in separate wells of a 96-well plate. The samples are then tagged by PCR with an index sequence. Depending on the target size of the SureSelect capture, multiple samples can be pooled and sequenced in a single lane using the Illumina-specified index tags that are provided with SureSelect^{XT} target enrichment kits.

Step 1. Shear DNA

For each DNA sample to be sequenced, prepare 1 library.

1. Use the Qubit dsDNA BR Assay to determine the concentration of your gDNA sample. Make sure the gDNA is of high quality (non-degraded, A_{260}/A_{280} is 1.8 to 2.0).

   Follow the instructions for the instrument.

2. Dilute 3 µg of high-quality gDNA with 1X Low TE Buffer in a 1.5-mL LoBind tube to a total volume of 130 µL.

3. Set up the Covaris E-Series or S-Series instrument.

   a. Check that the water in the Covaris tank is filled with fresh deionized water to the appropriate fill line level according to the manufacturer's recommendations for the specific instrument model and sample tube or plate in use.

   b. Check that the water covers the visible glass part of the tube.

   c. On the instrument control panel, push the Degas button. Degas the instrument for at least 30 minutes, or according to the manufacturer's recommendations.

   d. Set the chiller temperature to between 2°C to 5°C to ensure that the temperature reading in the water bath displays 5°C.

   e. *Optional.* Supplement the circulated water chiller with ethylene glycol to 20% volume to prevent freezing.

   Refer to the Covaris instrument user guide for more details.

4. Put a Covaris microTube into the loading and unloading station.

   Keep the cap on the tube.

NOTE When using a Covaris E-series instrument to prepare multiple gDNA samples in the same experiment, you can also use the 96 microTube plate (see Table 4 on page 15) for the DNA shearing step.

5. Use a tapered pipette tip to slowly transfer the 130-µL DNA sample through the pre-split septa.

   Be careful not to introduce a bubble into the bottom of the tube.

6. Secure the microTube in the tube holder and shear the DNA with the settings in Table 10 or Table 11, depending on the Covaris instrument SonoLab software version used.

The target peak size is 150 to 200 bp.

Table 10 Shear settings for Covaris instruments using SonoLab software version 7 or newer

Setting	Value
Duty Factor	10%
Peak Incident Power (PIP)	175
Cycles per Burst	200
Treatment Time	360 seconds
Bath Temperature	4° to 8° C

Table 11 Shear settings for Covaris instruments using SonoLab software prior to version 7

Setting	Value
Duty Cycle	10%
Intensity	5
Cycles per Burst	200
Time	6 cycles of 60 seconds each
Set Mode	Frequency sweeping
Temperature	4° to 7° C

7 Put the Covaris microTube back into the loading and unloading station.

8 While keeping the snap-cap on, insert a pipette tip through the pre-split septa, then slowly remove the sheared DNA.

9 Transfer the sheared DNA into the wells of a 96-well Eppendorf plate, column-wise for processing on the Agilent NGS Workstation, in well order A1 to H1, then A2 to H2, ending with A12 to H12.

NOTE

SureSelect Automated Library Prep and Capture System runs may include 1, 2, 3, 4, 6, or 12 columns of the plate. See Using the Agilent NGS Workstation for SureSelect Target Enrichment for additional sample placement considerations.

10 Seal the plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 1.0 sec.

11 Centrifuge the plate for 30 seconds to drive the well contents off the walls and plate seal and to remove air bubbles.

Stopping Point If you do not continue to the next step, store the sample plate at 4°C overnight or at –20°C for prolonged storage.

Step 2. Purify sheared DNA using AMPure XP beads

In this step, the Agilent NGS Workstation transfers AMPure XP beads and gDNA samples to a Nunc DeepWell plate and then collects and washes the bead-bound DNA.

Prepare the workstation and reagents

1. Clear the Labware MiniHub and BenchCel of all plates and tip boxes.
2. Gently wipe down the Labware MiniHub, Bravo deck, and BenchCel with a NucleoClean decontamination wipe.
3. Turn on the ThermoCube, set to 0°C, at position 9 of the Bravo deck. Be sure that the chiller reservoir contains at least 300 mL of 25% ethanol.
4. Let the AMPure XP beads come to room temperature for at least 30 minutes. *Do not freeze the beads at any time*.
5. Mix the bead suspension well so that the reagent appears homogeneous and consistent in color.
6. Prepare a Nunc DeepWell source plate for the beads by adding 185 µL of homogeneous AMPure XP beads per well, for each well to be processed.
7. Prepare a Thermo Scientific reservoir containing 15 mL of nuclease-free water.
8. Prepare a separate Thermo Scientific reservoir containing 45 mL of freshly-prepared 70% ethanol.

9 Load the Labware MiniHub according to Table 12, using the plate orientations shown in Figure 3.

Table 12 Initial MiniHub configuration for AMPureXP_XT_ILM_v1.5.1.pro:Shearing-3 µg only

Vertical Shelf Position	Cassette 1	Cassette 2	Cassette 3	Cassette 4
Shelf 5 (Top)	Empty Nunc DeepWell plate	Empty	Empty	Empty
Shelf 4	Empty	Empty	Empty	Empty
Shelf 3	Empty	Empty Eppendorf Plate	Empty	Empty
Shelf 2	Empty	Nuclease-free water reservoir from step 7	AMPure XP beads in Nunc DeepWell plate from step 6	Empty
Shelf 1 (Bottom)	Empty	70% ethanol reservoir from step 8	Empty	Empty Tip Box

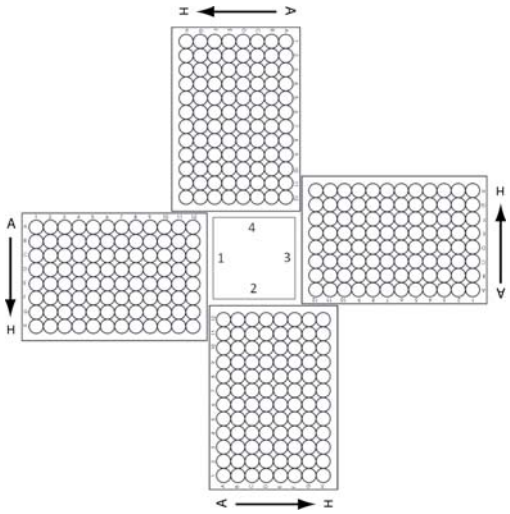


Figure 3 Agilent Labware MiniHub plate orientation. For Thermo Scientific reservoirs, place the notched corner facing the center of the hub.

10 Load the Bravo deck according to Table 13.

Table 13 Initial Bravo deck configuration for AMPureXP_XT_ILM_v1.5.1.pro:Shearing-3 µg only

Location	Content
1	Empty waste reservoir (Axygen 96 Deep Well Plate, square wells)
9	Sheared gDNA samples in unsealed PCR plate seated on red insert (PCR plate type must be specified on setup form under step 2)

11 Load the BenchCel Microplate Handling Workstation according to Table 14.

Table 14 Initial BenchCel configuration for AMPureXP_XT_ILM_v1.5.1.pro:Shearing-3 µg only

No. of Columns Processed	Rack 1	Rack 2	Rack 3	Rack 4
1	1 Tip box	Empty	Empty	Empty
2	1 Tip box	Empty	Empty	Empty
3	2 Tip boxes	Empty	Empty	Empty
4	2 Tip boxes	Empty	Empty	Empty
6	3 Tip boxes	Empty	Empty	Empty
12	6 Tip boxes	Empty	Empty	Empty

Run VWorks protocol *AMPureXP_XT_ILM_v1.5.1.pro:Shearing-3 µg only*

12 Open the SureSelect setup form using the XT_ILM_v1.5.1.VWForm shortcut on your desktop.

13 Log in to the VWorks software.

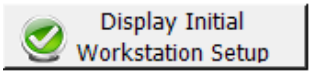
14 On the setup form, under **Select Protocol to Run**, select **AMPureXP_XT_ILM_v1.5.1.pro:Shearing-3 µg only.**

NOTE AMPureXP purification protocols are used during multiple steps of the SureSelect automation workflow. Be sure to select the correct workflow step when initiating the automation protocol.

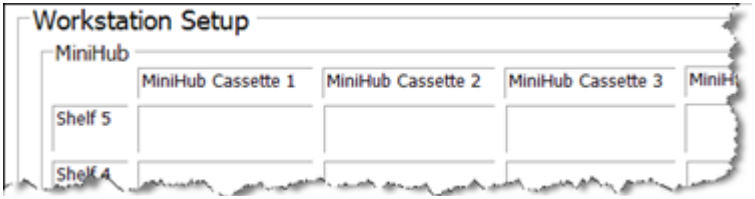
15 Under **Select PCR plate labware for Thermal Cycling**, select the specific type of PCR plate containing the sheared gDNA samples at position 9.

16 Select the number of columns of samples to be processed. Runs must include 1, 2, 3, 4, 6, or 12 columns.

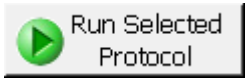
17 Click **Display Initial Workstation Setup**.



18 Verify that the NGS workstation has been set up as displayed in the Workstation Setup region of the form.



19 When verification is complete, click **Run Selected Protocol**.



NOTE If workstation devices do not respond when you start the run, but activity is recorded in the Log, verify that VWorks is not running in Simulation mode. See page 27 for more information.

Running the AMPureXP purification protocol takes approximately 45 minutes. Once complete, the purified DNA samples are located in the Eppendorf plate at position 7 of the Bravo deck.

Step 3. Assess sample quality (optional)

Analysis of the purified sheared DNA samples prior to library preparation is optional. If you elect to include this step, follow the instructions below.

Option 1: Analysis using the 2100 Bioanalyzer and DNA 1000 Assay

Use a Bioanalyzer DNA 1000 chip and reagent kit. For more information, see the *Agilent DNA 1000 Kit Guide* at www.genomics.agilent.com.

1. Set up the 2100 Bioanalyzer as instructed in the reagent kit guide.
2. Seal the sample plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 1.0 sec.
3. Vortex the plate to mix samples in each well, then centrifuge the plate for 30 seconds to drive the well contents off the walls and plate seal.
4. Prepare the chip, samples and ladder as instructed in the reagent kit guide, using 1 µL of each sample for the analysis.
5. Load the prepared chip into the 2100 Bioanalyzer and start the run within five minutes after preparation.
6. Verify that the electropherogram shows the peak of DNA fragment size positioned between 150 to 200 bp. A sample electropherogram is shown in Figure 4.

Stopping Point If you do not continue to the next step, seal the plate and store at 4°C overnight or at –20°C for prolonged storage.

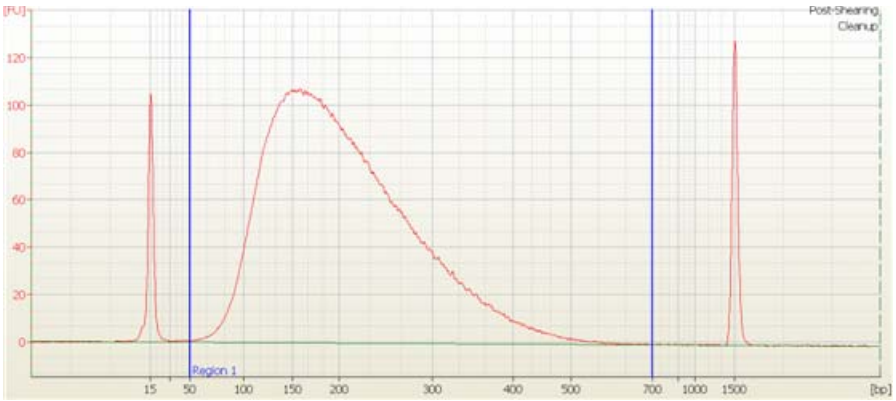


Figure 4 Analysis of sheared DNA using a DNA 1000 Bioanalyzer assay.

Option 2: Analysis using the 2200 TapeStation and D1000 ScreenTape

You can use Agilent's 2200 TapeStation for rapid analysis of multiple samples. Use a D1000 ScreenTape (p/n 5067-5582) and associated reagent kit (p/n 5067-5583). For more information to do this step, see the *Agilent 2200 TapeStation User Manual* at www.genomics.agilent.com.

1 Seal the sheared DNA sample plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 1.0 sec.

2 Vortex the plate to mix samples in each well, then centrifuge the plate for 30 seconds to drive the well contents off the walls and plate seal.

3 Prepare the TapeStation samples as instructed in the *Agilent 2200 TapeStation User Manual*. Use 1 µL of each sheared DNA sample diluted with 3 µL of D1000 sample buffer for the analysis.

CAUTION Make sure that you thoroughly mix the combined DNA and D1000 sample buffer on a vortex mixer for 5 seconds for accurate quantitation.

4 Load the sample plate or tube strips from step 3, the D1000 ScreenTape, and loading tips into the 2200 TapeStation as instructed in the *Agilent 2200 TapeStation User Manual*. Start the run.

5 Verify that the electropherogram shows the peak of DNA fragment size positioned between 150 to 200 bp. A sample electropherogram is shown in Figure 5.

Stopping Point If you do not continue to the next step, seal the sheared DNA sample plate and store at 4°C overnight or at –20°C for prolonged storage.

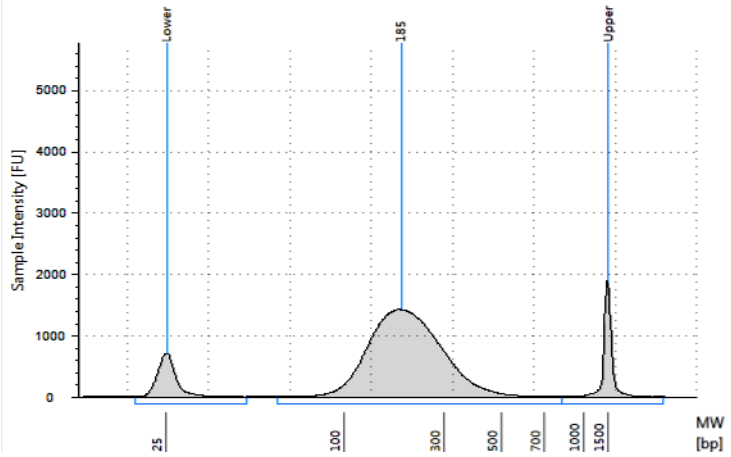


Figure 5 Analysis of sheared DNA using the 2200 TapeStation.

Step 4. Modify DNA ends for target enrichment

In this step, the Agilent NGS Workstation completes the DNA end modification steps required for SureSelect target enrichment, including GA end-repair, A-tailing, and adaptor ligation. After the appropriate modification steps, the Agilent NGS Workstation purifies the prepared DNA using AMPure XP beads.

Before starting the run, you need to prepare master mixes (with overage) for each step, without the DNA sample. Master mixes for runs that include 1, 2, 3, 4, 6, and 12 columns (including overage) are shown in each table.

Prepare each master mix on ice.

Prepare the workstation

1. Clear the Labware MiniHub and BenchCel of all plates and tip boxes.
2. Pre-set the temperature of Bravo deck position 6 to 4°C using the Inheco Multi TEC control touchscreen, as described in Setting the Temperature of Bravo Deck Heat Blocks. Bravo deck position 6 corresponds to CPAC 2, position 2 on the Multi TEC control touchscreen.
3. Turn on the ThermoCube, set to 0°C, at position 9 of the Bravo deck. Be sure that the chiller reservoir contains at least 300 mL of 25% ethanol.

Prepare the SureSelect DNA end-repair master mix

4 Prepare the appropriate volume of end-repair master mix, according to Table 15. Mix well using a vortex mixer and keep on ice.

Table 15 Preparation of End-Repair Master Mix

SureSelect *XT* Reagent	Volume for 1 Library	Volume for 1 Column	Volume for 2 Columns	Volume for 3 Columns	Volume for 4 Columns	Volume for 6 Columns	Volume for 12 Columns
Nuclease-free water	35.2 µL	448.8 µL	748.0 µL	1047.2 µL	1346.4 µL	1944.8 µL	3889.6 µL
10X End-Repair Buffer	10.0 µL	127.5 µL	212.5 µL	297.5 µL	382.5 µL	552.5 µL	1105.0 µL
dNTP mix	1.6 µL	20.4 µL	34.0 µL	47.6 µL	61.2 µL	88.4 µL	176.8 µL
T4 DNA Polymerase	1.0 µL	12.8 µL	21.3 µL	29.8 µL	38.3 µL	55.3 µL	110.5 µL
Klenow DNA Polymerase	2.0 µL	25.5 µL	42.5 µL	59.5 µL	76.5 µL	110.5 µL	221.0 µL
T4 Polynucleotide Kinase	2.2 µL	28.1 µL	46.8 µL	65.5 µL	84.2 µL	121.6 µL	243.1 µL
Total Volume	**52 µL**	**663 µL**	**1105 µL**	**1547 µL**	**1989 µL**	**2873 µL**	**5746 µL**

Prepare the A-tailing master mix

5 Prepare the appropriate volume of A-tailing master mix, according to Table 16. Mix well using a vortex mixer and keep on ice.

Table 16 Preparation of A-Tailing Master Mix

SureSelect^{XT} Reagent	Volume for 1 Library	Volume for 1 Column	Volume for 2 Columns	Volume for 3 Columns	Volume for 4 Columns	Volume for 6 Columns	Volume for 12 Columns
Nuclease-free water	11.0 µL	187.0 µL	280.5 µL	374.0 µL	467.5 µL	654.5 µL	1262.3 µL
10x Klenow Polymerase Buffer	5.0 µL	85.0 µL	127.5 µL	170.0 µL	212.5 µL	297.5 µL	573.8 µL
dATP	1.0 µL	17.0 µL	25.5 µL	34.0 µL	42.5 µL	59.5 µL	114.8 µL
Exo (–) Klenow	3.0 µL	51.0 µL	76.5 µL	102.0 µL	127.5 µL	178.5 µL	344.3 µL
Total Volume	**20 µL**	**340 µL**	**510 µL**	**680 µL**	**850 µL**	**1190 µL**	**2295 µL**

Prepare the adaptor ligation master mix

6 Prepare the appropriate volume of adaptor ligation master mix, according to Table 17. Mix well using a vortex mixer and keep on ice.

Table 17 Preparation of Adaptor Ligation Master Mix **(use only for the 3 µg DNA input workflow)**

SureSelect^{XT} Reagent	Volume for 1 Library	Volume for 1 Column	Volume for 2 Columns	Volume for 3 Columns	Volume for 4 Columns	Volume for 6 Columns	Volume for 12 Columns
Nuclease-free water	15.5 µL	197.6 µL	329.4 µL	461.1 µL	592.9 µL	856.4 µL	1712.8 µL
5X T4 DNA Ligase Buffer	10.0 µL	127.5 µL	212.5 µL	297.5 µL	382.5 µL	552.5 µL	1105.0 µL
SureSelect Adaptor Oligo Mix	10.0 µL	127.5 µL	212.5 µL	297.5 µL	382.5 µL	552.5 µL	1105.0 µL
T4 DNA Ligase	1.5 µL	19.1 µL	31.9 µL	44.6 µL	57.4 µL	82.9 µL	165.8 µL
Total Volume	**37.0 µL**	**471.8 µL**	**786.3 µL**	**1100.8 µL**	**1415.3 µL**	**2044.3 µL**	**4088.5 µL**

Prepare the master mix source plate

7 In a Nunc DeepWell plate, prepare the master mix source plate containing the master mixes prepared in steps 3 to 5. Add the volumes indicated in Table 18 of each master mix to all wells of the indicated column of the Nunc DeepWell plate. Keep the master mixes on ice during the aliquoting steps. The final configuration of the master mix source plate is shown in Figure 6.

Table 18 Preparation of the Master Mix Source Plate for LibraryPrep_XT_ILM_v1.5.1.rst

Master Mix Solution	Position on Source Plate	Volume of Master Mix added per Well of Nunc Deep Well Source Plate					
		1-Column Runs	2-Column Runs	3-Column Runs	4-Column Runs	6-Column Runs	12-Column Runs
End Repair Master Mix	Column 1 (A1-H1)	76.4 µL	131.6 µL	186.9 µL	242.1 µL	352.6 µL	711.8 µL
A-Tailing Master Mix	Column 2 (A2-H2)	40.0 µL	61.3 µL	82.5 µL	103.8 µL	146.3µL	284.4 µL
Adaptor Ligation Master Mix	Column 3 (A3-H3)	54.3 µL	93.7 µL	133.0 µL	172.3 µL	250.9 µL	506.4 µL

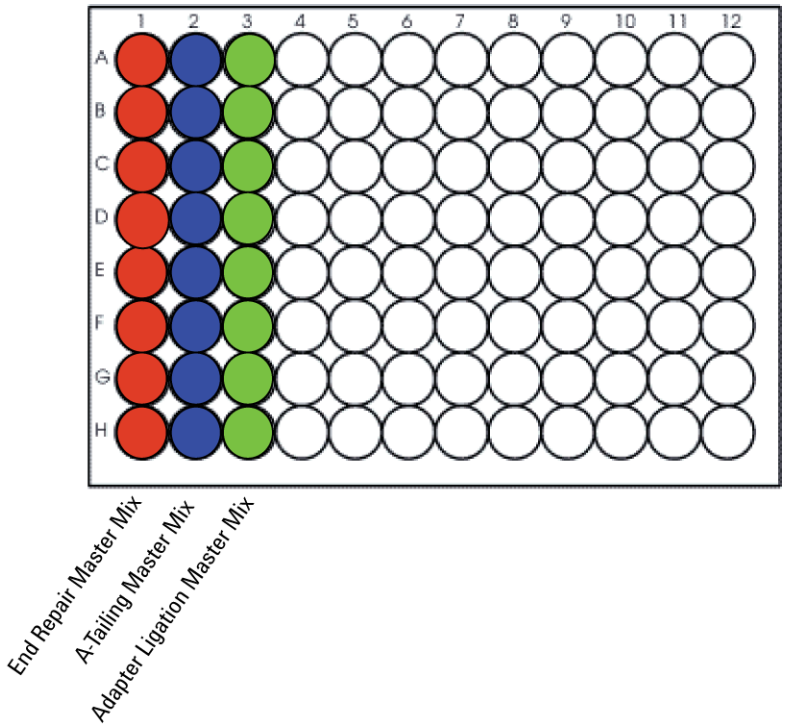


Figure 6 Configuration of the master mix source plate for LibraryPrep_XT_ILM_v1.5.1.rst

8 Seal the master mix source plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 1.0 sec.

9 Centrifuge the plate for 30 seconds to drive the well contents off the walls and plate seal and to eliminate any bubbles. Keep the master mix source plate on ice.

NOTE

The presence of bubbles in source plate solutions may cause inaccurate volume transfer by the Bravo liquid handling platform. Ensure that the source plate is sealed and centrifuged prior to use in a run.

Prepare the purification reagents

10 Verify that the AMPure XP bead suspension is at room temperature. *Do not freeze the beads at any time.*

11 Mix the bead suspension well so that the reagent appears homogeneous and consistent in color.

12 Prepare a separate Nunc DeepWell source plate for the beads by adding 370 µL of homogeneous AMPure XP beads per well, for each well to be processed.

13 Prepare a Thermo Scientific reservoir containing 20 mL of nuclease-free water.

14 Prepare a separate Thermo Scientific reservoir containing 150 mL of freshly-prepared 70% ethanol.

Load the Agilent NGS Workstation

15 Load the Labware MiniHub according to Table 19, using the plate orientations shown in Figure 3.

Table 19 Initial MiniHub configuration for LibraryPrep_XT_ILM_v1.5.1.rst

Vertical Shelf Position	Cassette 1	Cassette 2	Cassette 3	Cassette 4
Shelf 5 (Top)	Empty Nunc DeepWell plate	Empty Nunc DeepWell plate	Empty Nunc DeepWell plate	Empty
Shelf 4	Empty	Empty Eppendorf plate	Empty Eppendorf plate	Empty
Shelf 3	Empty	Empty	Empty	Empty Eppendorf plate
Shelf 2	Empty tip box	Nuclease-free water reservoir from step 13	AMPure XP beads in Nunc DeepWell plate from step 12	Empty
Shelf 1 (Bottom)	New tip box	70% ethanol reservoir from step 14	Empty	Empty tip box

16 Load the Bravo deck according to Table 20.

Table 20 Initial Bravo deck configuration for LibraryPrep_XT_ILM_v1.5.1.rst

Location	Content
1	Empty waste reservoir (Axygen 96 Deep Well Plate, square wells)
6	Empty Eppendorf plate
7	Eppendorf plate containing purified gDNA samples
9	DNA End Modification Master Mix Source Plate, unsealed and seated on silver Nunc DeepWell insert

17 Load the BenchCel Microplate Handling Workstation according to Table 21.

Table 21 Initial BenchCel configuration for LibraryPrep_XT_ILM_v1.5.1.rst

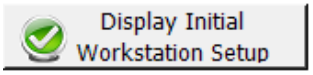
No. of Columns Processed	Rack 1	Rack 2	Rack 3	Rack 4
1	2 Tip boxes	Empty	Empty	Empty
2	4 Tip boxes	Empty	Empty	Empty
3	5 Tip boxes	Empty	Empty	Empty
4	7 Tip boxes	Empty	Empty	Empty
6	10 Tip boxes	Empty	Empty	Empty
12	11 Tip boxes	8 Tip boxes	Empty	Empty

Run VWorks runset LibraryPrep_XT_ILM_v1.5.1.rst

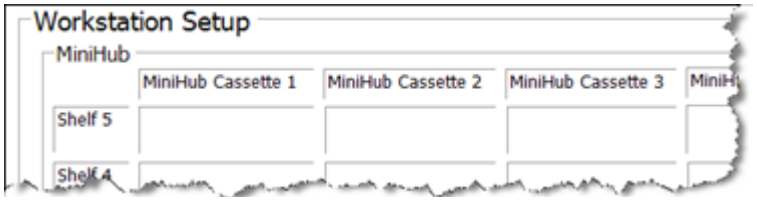
18 On the SureSelect setup form, under **Select Protocol to Run**, select **LibraryPrep_XT_ILM_v1.5.1.rst.**

19 Select the number of columns of samples to be processed. Runs must include 1, 2, 3, 4, 6, or 12 columns.

20 Click **Display Initial Workstation Setup**.

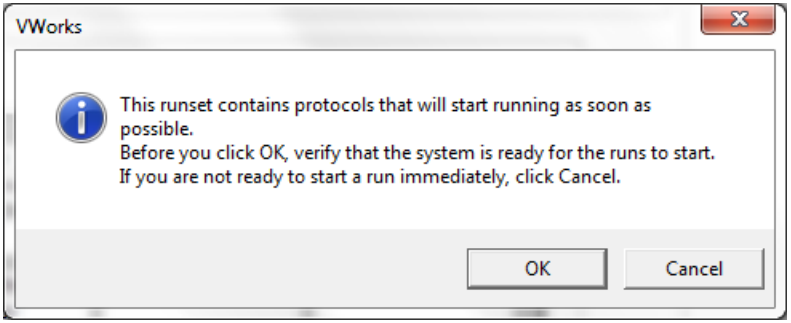


21 Verify that the NGS workstation has been set up as displayed in the Workstation Setup region of the form.



22 When verification is complete, click **Run Selected Protocol**.

23 When ready to begin the run, click **OK** in the following window.



Running the LibraryPrep_XT_ILM_v1.5.1.rst runset takes approximately 3.5 hours. Once complete, the purified, adaptor-ligated DNA samples are located in the Eppendorf plate at position 7 of the Bravo deck.

Stopping Point If you do not continue to the next step, seal the plate and store at 4°C overnight or at –20°C for prolonged storage.

Step 5. Amplify adaptor-ligated libraries

In this step, the Agilent NGS Workstation completes the liquid handling steps for amplification of the adaptor-ligated DNA samples. Afterward, you transfer the PCR plate to a thermal cycler for amplification.

In this protocol, one half of the adaptor-ligated DNA sample is removed from the Eppendorf sample plate for amplification. The remainder can be saved at 4°C for future use or amplification troubleshooting, if needed. Store the samples at –20°C for long-term storage.

CAUTION To avoid cross-contaminating libraries, set up PCR master mixes in a dedicated clean area or PCR hood with UV sterilization and positive air flow.

Prepare the workstation

1. Turn on the ThermoCube, set to 0°C, at position 9 of the Bravo deck. Be sure that the chiller reservoir contains at least 300 mL of 25% ethanol.
2. Leave tip boxes on shelves 1 and 2 in cassette 1 of the Labware MiniHub from the previous LibraryPrep_XT_ILM_v1.5.1.rst run. Otherwise, clear the remaining positions of the MiniHub and BenchCel of plates and tip boxes.
3. Pre-set the temperature of Bravo deck position 6 to 4°C using the Inheco Multi TEC control touchscreen, as described in Setting the Temperature of Bravo Deck Heat Blocks. Bravo deck position 6 corresponds to CPAC 2, position 2 on the Multi TEC control touchscreen.

Prepare the pre-capture PCR master mix and master mix source plate

4 Prepare the appropriate volume of pre-capture PCR Master Mix, according to Table 22. Mix well using a vortex mixer and keep on ice.

Table 22 Preparation of Pre-Capture PCR Master Mix **(use only for the 3 µg DNA input workflow)**

SureSelect^{XT} Reagent	Volume for 1 Library	Volume for 1 Column	Volume for 2 Columns	Volume for 3 Columns	Volume for 4 Columns	Volume for 6 Columns	Volume for 12 Columns
Nuclease-free water	21.0 µL	267.8 µL	446.3 µL	624.8 µL	803.3 µL	1160.3 µL	2320.5 µL
Herculase II 5X Reaction Buffer*	10.0 µL	127.5 µL	212.5 µL	297.5 µL	382.5 µL	552.5 µL	1105 µL
dNTP mix*	0.5 µL	6.4 µL	10.6 µL	14.9 µL	19.1 µL	27.6 µL	55.3 µL
SureSelect Primer† (Forward)	1.25 µL	15.9 µL	26.6 µL	37.2 µL	47.8 µL	69.1 µL	138.1 µL
SureSelect Indexing Pre-Capture PCR (Reverse) Primer‡	1.25 µL	15.9 µL	26.6 µL	37.2 µL	47.8 µL	69.1 µL	138.1 µL
Herculase II Polymerase	1.0 µL	12.8 µL	21.3 µL	29.8 µL	38.3 µL	55.3 µL	110.5 µL
Total Volume	**35 µL**	**446.3 µL**	**743.8 µL**	**1041.3 µL**	**1338.8 µL**	**1933.8 µL**	**3867.5 µL**

* Included with the Herculase II Fusion DNA Polymerase. *Do not use the buffer or dNTP mix from any other kit.*

† Included in SureSelect XT Library Prep Kit ILM.

‡ Included in SureSelect XT Automation ILM Module Box 2. Ensure that the correct primer is selected from Box 2 at this step (do not use the SureSelect Indexing Post-Capture PCR (Forward) Primer).

5 Using the same Nunc DeepWell master mix source plate that was used for the LibraryPrep_XT_ILM_v1.5.1.rst run, add the volume of PCR Master Mix indicated in Table 23 to all wells of column 4 of the master mix source plate. The final configuration of the master mix source plate is shown in Figure 7.

Table 23 Preparation of the Master Mix Source Plate for Pre-CapturePCR_XT_ILM_3µg_v1.5.1.pro

Master Mix Solution	Position on Source Plate	Volume of Master Mix added per Well of Nunc Deep Well Source Plate					
		1-Column Runs	2-Column Runs	3-Column Runs	4-Column Runs	6-Column Runs	12-Column Runs
Pre-Capture PCR Master Mix	Column 4 (A4-H4)	51.4 µL	88.6 µL	125.8 µL	163.0 µL	237.3 µL	479.1 µL

NOTE If you are using a new DeepWell plate for the pre-capture PCR source plate (for example, when amplifying the second half of the adaptor-ligated DNA sample), leave columns 1 to 3 empty and add the PCR Master Mix to column 4 of the new plate.

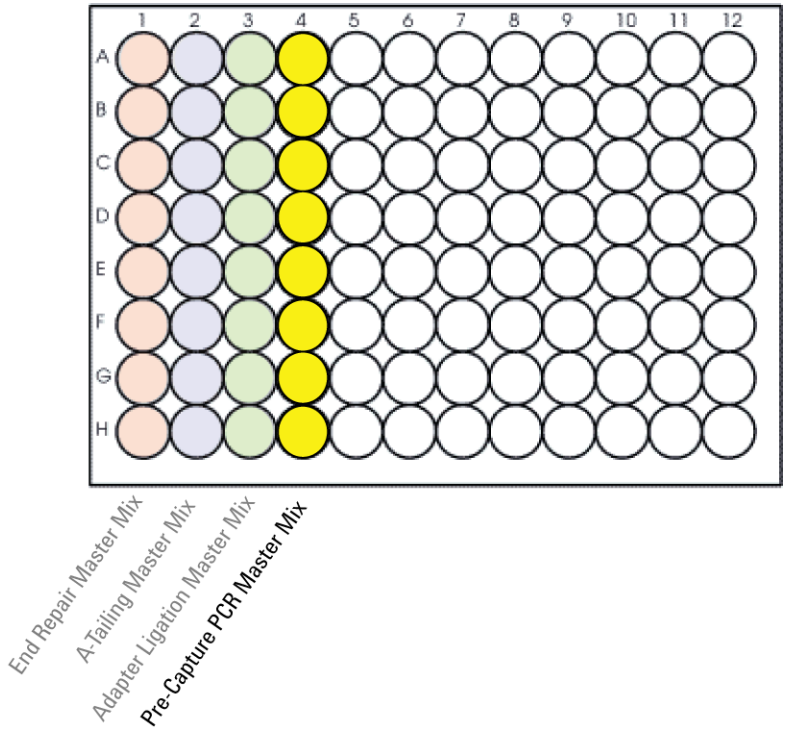


Figure 7 Configuration of the master mix source plate for Pre-CapturePCR_XT_ILM_3µg_v1.5.1.pro. Columns 1-3 were used to dispense master mixes during the previous protocol.

6 Seal the master mix source plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 1.0 sec.

7 Centrifuge the plate for 30 seconds to drive the well contents off the walls and plate seal and to eliminate any bubbles.

NOTE

The presence of bubbles in source plate solutions may cause inaccurate volume transfer by the Bravo liquid handling platform. Ensure that the source plate is sealed and centrifuged prior to use in a run.

Load the Agilent NGS Workstation

8 Load the Labware MiniHub according to Table 24, using the plate orientations shown in Figure 3.

Table 24 Initial MiniHub configuration for Pre-CapturePCR_XT_ILM_3µg_v1.5.1.pro

Vertical Shelf Position	Cassette 1	Cassette 2	Cassette 3	Cassette 4
Shelf 5 (Top)	Empty	Empty	Empty	Empty
Shelf 4	Empty	Empty	Empty	Empty
Shelf 3	Empty	Empty	Empty	Empty
Shelf 2	Waste tip box*	Empty	Empty	Empty
Shelf 1 (Bottom)	Clean tip box*	Empty	Empty	Empty tip box

* The waste tip box (Cassette 1, Shelf 2) and clean tip box (Cassette 1, Shelf 1) are retained from the LibraryPrep_XT_ILM_v1.5.1.rst run and reused here.

NOTE If you are using a new box of tips on shelf 1 of cassette 1 (for example, when amplifying the second half of the adaptor-ligated DNA sample), first remove the tips from columns 1 to 3 of the tip box. Any tips present in columns 1 to 3 of the tip box may be inappropriately loaded onto the Bravo platform pipette heads and may interfere with automated processing steps.

9 Load the Bravo deck according to Table 25.

Table 25 Initial Bravo deck configuration for Pre-CapturePCR_XT_ILM_3µg_v1.5.1.pro

Location	Content
6	Empty PCR plate seated in red insert (PCR plate type must be specified on setup form under step 2)
7	Adaptor-ligated DNA samples in Eppendorf plate
9	Master mix plate containing PCR Master Mix in Column 4 (unsealed)

10 Load the BenchCel Microplate Handling Workstation according to Table 26.

Table 26 Initial BenchCel configuration for Pre-CapturePCR_XT_ILM_3µg_v1.5.1.pro

No. of Columns Processed	Rack 1	Rack 2	Rack 3	Rack 4
1	1 Tip box	Empty	Empty	Empty
2	1 Tip box	Empty	Empty	Empty
3	1 Tip box	Empty	Empty	Empty
4	1 Tip box	Empty	Empty	Empty
6	1 Tip box	Empty	Empty	Empty
12	1 Tip box	Empty	Empty	Empty

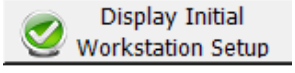
Run VWorks protocol Pre-CapturePCR_XT_ILM_3µg_v1.5.1.pro

11 On the SureSelect setup form, under **Select Protocol to Run**, select **Pre-CapturePCR_XT_ILM_3µg_v1.5.1.pro.**

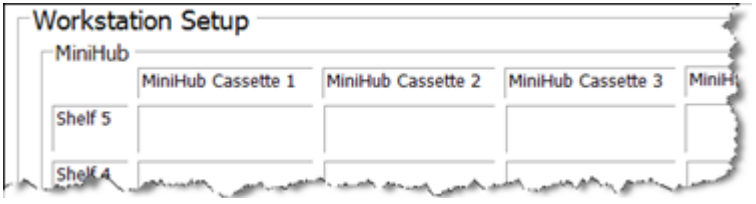
12 Under **Select PCR plate labware for Thermal Cycling**, select the specific type of PCR plate used at position 6 of the Bravo deck.

13 Select the number of columns of samples to be processed. Runs must include 1, 2, 3, 4, 6, or 12 columns.

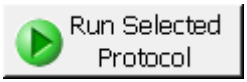
14 Click **Display Initial Workstation Setup**.



15 Verify that the NGS workstation has been set up as displayed in the Workstation Setup region of the form.

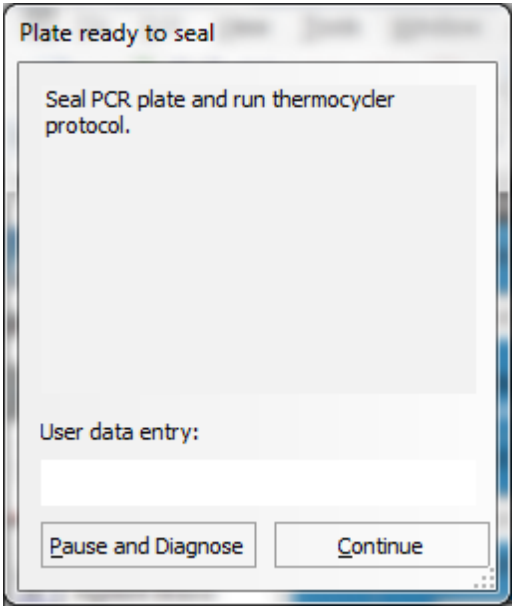


16 When verification is complete, click **Run Selected Protocol**.



Running the Pre-CapturePCR_XT_ILM_3µg_v1.5.1.pro protocol takes approximately 15 minutes. Once complete, the PCR-ready samples, containing prepped DNA and PCR master mix, are located in the PCR plate at position 6 of the Bravo deck. The Eppendorf plate containing the remaining prepped DNA samples, which may be stored for future use at 4°C overnight, or at –20°C for long-term storage, is located at position 7 of the Bravo deck.

17 When you see the following prompt, remove the PCR plate from position 6 of the Bravo deck and seal the plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 3.0 seconds.



18 Centrifuge the plate for 30 seconds to drive the well contents off the walls and plate seal and to eliminate air bubbles.

19 Transfer the PCR plate to a thermal cycler and run the PCR amplification program shown in Table 27.

Table 27 Pre-Capture PCR cycling program **(use only for the 3 µg DNA input workflow)**

Segment	Number of Cycles	Temperature	Time
1	1	98°C	2 minutes
2	4 to 6	98°C	30 seconds
		65°C	30 seconds
		72°C	1 minute
3	1	72°C	10 minutes
4	1	4°C	Hold

NOTE

Different library preparations can produce slightly different results, based on varying DNA quality. In most cases, 5 cycles will produce an adequate yield for subsequent capture without introducing bias or non-specific products. If yield is too low or non-specific high molecular weight products are observed, adjust the number of cycles accordingly with the remaining library template.

Step 6. Purify amplified DNA using AMPure XP beads

In this step, the Agilent NGS Workstation transfers AMPure XP beads and amplified adaptor-ligated DNA to a Nunc DeepWell plate and then collects and washes the bead-bound DNA.

Prepare the workstation and reagents

1 Clear the Labware MiniHub and BenchCel of all plates and tip boxes.

2 Verify that the AMPure XP bead suspension is at room temperature. (If necessary, allow the bead solution to come to room temperature for at least 30 minutes.) *Do not freeze the beads at any time.*

3 Mix the bead suspension well so that the reagent appears homogeneous and consistent in color.

4 Prepare a Nunc DeepWell source plate for the beads by adding 95 µL of homogeneous AMPure XP beads per well, for each well to be processed.

5 Prepare a Thermo Scientific reservoir containing 15 mL of nuclease-free water.

6 Prepare a separate Thermo Scientific reservoir containing 45 mL of freshly-prepared 70% ethanol.

7 Load the Labware MiniHub according to Table 28, using the plate orientations shown in Figure 3.

Table 28 Initial MiniHub configuration for AMPureXP_XT_ILM_v1.5.1.pro:Pre-Capture PCR

Vertical Shelf Position	Cassette 1	Cassette 2	Cassette 3	Cassette 4
Shelf 5 (Top)	Empty Nunc DeepWell plate	Empty	Empty	Empty
Shelf 4	Empty	Empty	Empty	Empty
Shelf 3	Empty	Empty Eppendorf Plate	Empty	Empty
Shelf 2	Empty	Nuclease-free water reservoir from step 5	AMPure XP beads in Nunc DeepWell plate from step 4	Empty
Shelf 1 (Bottom)	Empty	70% ethanol reservoir from step 6	Empty	Empty tip box

8 Load the Bravo deck according to Table 29.

Table 29 Initial Bravo deck configuration for AMPureXP_XT_ILM_v1.5.1.pro:Pre-Capture PCR

Location	Content
1	Empty waste reservoir (Axygen 96 Deep Well Plate, square wells)
9	Amplified DNA libraries in unsealed PCR plate seated in red insert (PCR plate type must be specified on setup form under step 2)

9 Load the BenchCel Microplate Handling Workstation according to Table 30.

Table 30 Initial BenchCel configuration for AMPureXP_XT_ILM_v1.5.1.pro:Pre-Capture PCR

No. of Columns Processed	Rack 1	Rack 2	Rack 3	Rack 4
1	1 Tip box	Empty	Empty	Empty
2	1 Tip box	Empty	Empty	Empty
3	2 Tip boxes	Empty	Empty	Empty
4	2 Tip boxes	Empty	Empty	Empty
6	3 Tip boxes	Empty	Empty	Empty
12	6 Tip boxes	Empty	Empty	Empty

Run VWorks protocol *AMPureXP_XT_ILM_v1.5.1.pro:Pre-Capture PCR*

10 On the SureSelect setup form, under **Select Protocol to Run**, select **AMPureXP_XT_ILM_v1.5.1.pro:Pre-Capture PCR**.

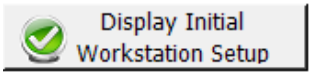
NOTE

AMPureXP purification protocols are used during multiple steps of the SureSelect automation workflow. Be sure to select the correct workflow step when initiating the automation protocol.

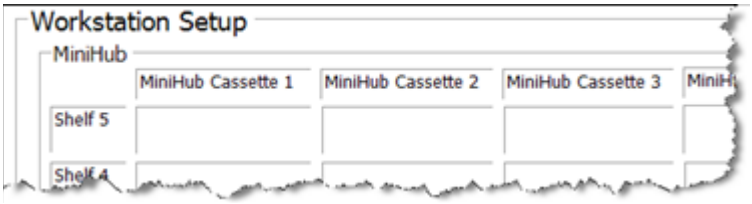
11 Under **Select PCR plate labware for Thermal Cycling**, select the specific type of PCR plate containing the amplified libraries at position 9.

12 Select the number of columns of samples to be processed. Runs must include 1, 2, 3, 4, 6, or 12 columns.

13 Click **Display Initial Workstation Setup**.



14 Verify that the NGS workstation has been set up as displayed in the Workstation Setup region of the form.



15 When verification is complete, click **Run Selected Protocol**.

The purification protocol takes approximately 45 minutes. When complete, the purified DNA samples are in the Eppendorf plate located on Bravo deck position 7.

Step 7. Assess Library DNA quantity and quality

The hybridization protocol in the following section requires 750 ng of each amplified DNA library. Measure the concentration of each library using one of the methods detailed below. Once DNA concentration for each sample is determined, calculate the volume of the library to be used for hybridization using the following formula:

Volume (µL) = 750 ng/concentration (ng/µL)

Option 1: Analysis using the Agilent 2100 Bioanalyzer and DNA 1000 Assay

Use a Bioanalyzer DNA 1000 chip and reagent kit to analyze the amplified libraries. For more information to do this step, see the *Agilent DNA 1000 Kit Guide* at www.genomics.agilent.com.

1. Set up the 2100 Bioanalyzer as instructed in the reagent kit guide.
2. Seal the sample plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 1.0 sec.
3. Vortex the plate to mix samples in each well, then centrifuge the plate for 30 seconds to drive the well contents off the walls and plate seal.
4. Prepare the chip, samples and ladder as instructed in the reagent kit guide, using 1 µL of each sample for the analysis.
5. Load the prepared chip into the 2100 Bioanalyzer and start the run within five minutes after preparation.
6. Verify that the electropherogram shows the peak of DNA fragment size positioned between 225 to 275 bp. A sample electropherogram is shown in Figure 8.
7. Determine the concentration of the library (ng/µL) by integrating under the peak.

Stopping Point If you do not continue to the next step, seal the plate and store at 4°C overnight or at –20°C for prolonged storage.

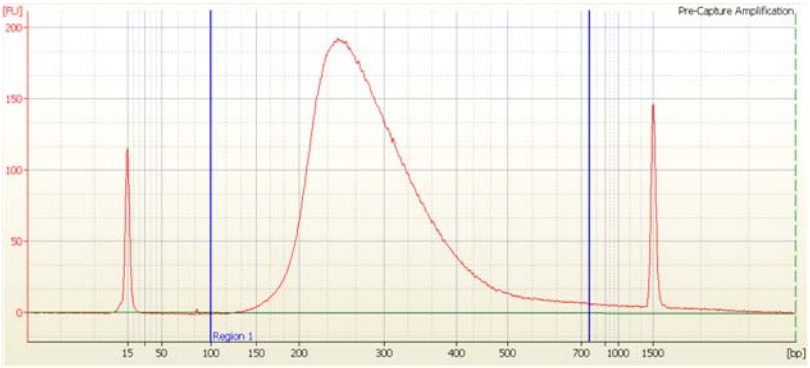


Figure 8 Analysis of amplified library DNA using a DNA 1000 assay.

Option 2: Analysis using the Agilent 2200 TapeStation and D1000 ScreenTape

Use a D1000 ScreenTape (p/n 5067-5582) and associated reagent kit (p/n 5067-5583) to analyze the amplified libraries. For more information to do this step, see the *Agilent 2200 TapeStation User Manual* at www.genomics.agilent.com.

1. Seal the DNA sample plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 1.0 sec.
2. Vortex the plate to mix samples in each well, then centrifuge the plate for 30 seconds to drive the well contents off the walls and plate seal.
3. Prepare the TapeStation samples as instructed in the *Agilent 2200 TapeStation User Manual*. Use 1 µL of each amplified library DNA sample diluted with 3 µL of D1000 sample buffer for the analysis.

CAUTION Make sure that you thoroughly mix the combined DNA and D1000 sample buffer on a vortex mixer for 5 seconds for accurate quantitation.

4. Load the sample plate or tube strips from step 3, the D1000 ScreenTape, and loading tips into the 2200 TapeStation as instructed in the *Agilent 2200 TapeStation User Manual*. Start the run.
5. Verify that the electropherogram shows the peak of DNA fragment size positioned between 225 to 275 bp. A sample electropherogram is shown in Figure 9.

Stopping Point If you do not continue to the next step, seal the library DNA sample plate and store at 4°C overnight or at –20°C for prolonged storage.

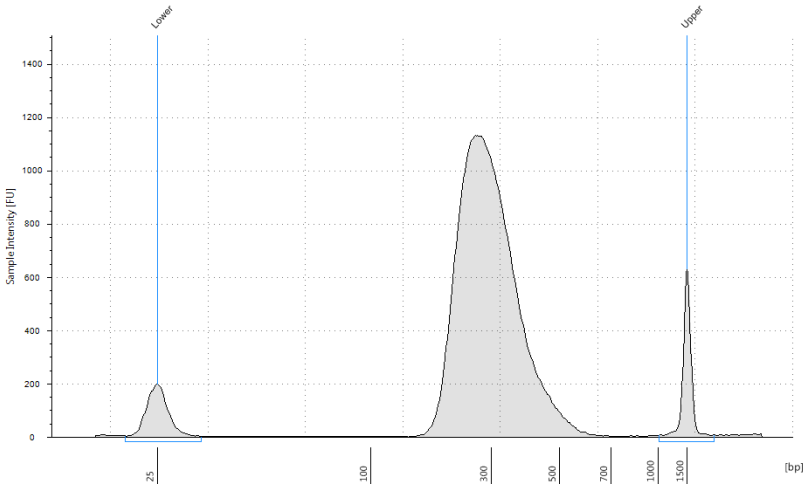


Figure 9 Analysis of amplified library DNA using the 2200 TapeStation.

4 Sample Preparation (200 ng DNA Samples)

This section contains instructions for the preparation of gDNA libraries from samples containing 200 ng of DNA. For higher input (3 μg) DNA samples, see the protocol on page 35.

This section contains instructions for gDNA library preparation specific to the Illumina paired-read sequencing platform and to automated processing using the Agilent NGS Workstation. For each sample to be sequenced, individual library preparations, hybridizations, and captures are performed in separate wells of a 96-well plate. The samples are then tagged by PCR with an index sequence. Depending on the target size of the SureSelect capture, multiple samples can be pooled and sequenced in a single lane using the Illumina-specified index tags that are provided with SureSelect^{XT} target enrichment kits.

Step 1. Shear DNA

For each DNA sample to be sequenced, prepare 1 library.

1 Use the Qubit dsDNA BR Assay to determine the concentration of your gDNA sample. Make sure the gDNA is of high quality (non-degraded, A_{260}/A_{280} is 1.8 to 2.0).

   Follow the instructions for the instrument.

2 Dilute 200 ng of high-quality gDNA with 1X Low TE Buffer in a 1.5-mL LoBind tube to a total volume of 50 µL.

3 Set up the Covaris E-Series or S-Series instrument.

   a Check that the water in the Covaris tank is filled with fresh deionized water to the appropriate fill line level according to the manufacturer's recommendations for the specific instrument model and sample tube or plate in use.

   b Check that the water covers the visible glass part of the tube.

   c On the instrument control panel, push the Degas button. Degas the instrument for at least 30 minutes, or according to the manufacturer's recommendations.

   d Set the chiller temperature to between 2°C to 5°C to ensure that the temperature reading in the water bath displays 5°C.

   e *Optional.* Supplement the circulated water chiller with ethylene glycol to 20% volume to prevent freezing.

   Refer to the Covaris instrument user guide for more details.

4 Put a Covaris microTube into the loading and unloading station.

   Keep the cap on the tube.

NOTE When using a Covaris E-series instrument to prepare multiple gDNA samples in the same experiment, you can also use the 96 microTube plate (see Table 4 on page 15) for the DNA shearing step.

5 Use a tapered pipette tip to slowly transfer the 50-µL DNA sample through the pre-split septa.

   Be careful not to introduce a bubble into the bottom of the tube.

6 Secure the microTube in the tube holder and shear the DNA with the settings in Table 31 or Table 32, depending on the Covaris instrument SonoLab software version used.

The target peak size is 150 to 200 bp.

Table 31 Shear settings for Covaris instruments using SonoLab software version 7 or newer

Setting	Value
Duty Factor	10%
Peak Incident Power (PIP)	175
Cycles per Burst	200
Treatment Time	360 seconds
Bath Temperature	4° to 8° C

Table 32 Shear settings for Covaris instruments using SonoLab software prior to version 7

Setting	Value
Duty Cycle	10%
Intensity	5
Cycles per Burst	200
Time	6 cycles of 60 seconds each
Set Mode	Frequency sweeping
Temperature	4° to 7° C

7 Put the Covaris microTube back into the loading and unloading station.

8 While keeping the snap-cap on, insert a pipette tip through the pre-split septa, then slowly remove the sheared DNA.

9 Transfer the sheared DNA into the wells of a 96-well Eppendorf plate, column-wise for processing on the Agilent NGS Workstation, in well order A1 to H1, then A2 to H2, ending with A12 to H12.

NOTE

SureSelect Automated Library Prep and Capture System runs may include 1, 2, 3, 4, 6, or 12 columns of the plate. See Using the Agilent NGS Workstation for SureSelect Target Enrichment for additional sample placement considerations.

10 Seal the plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 1.0 sec.

11 Centrifuge the plate for 30 seconds to drive the well contents off the walls and plate seal and to remove air bubbles.

Stopping Point If you do not continue to the next step, store the sample plate at 4°C overnight or at –20°C for prolonged storage.

CAUTION

The Sample Preparation protocol for 200 ng gDNA samples does not include the post-shear purification step that is included in the Sample Preparation protocol for 3 µg gDNA samples.

If you wish to analyze the sheared DNA fragment size prior to library preparation, use the optional protocol on page 75. Otherwise, proceed directly to "Step 3. Modify DNA ends for target enrichment" on page 78.

Step 2. Assess sample quality (optional)

Analysis of the sheared DNA samples prior to library preparation is optional. If you elect to include this step, follow the instructions below.

Option 1: Analysis using the 2100 Bioanalyzer and High Sensitivity DNA Assay

Use the Bioanalyzer High Sensitivity DNA Assay to analyze the 200-ng sheared DNA samples. See the *High Sensitivity DNA Kit Guide* at www.genomics.agilent.com for more information on doing this step.

1. Set up the 2100 Bioanalyzer as instructed in the reagent kit guide.
2. Vortex the plate to mix samples in each well, then centrifuge the plate for 30 seconds to drive the well contents off the walls and plate seal.
3. Prepare the chip, samples and ladder as instructed in the reagent kit guide, using 1 µL of each sample for the analysis.
4. Load the prepared chip into the 2100 Bioanalyzer and start the run within five minutes after preparation.
5. Verify that the electropherogram shows the peak of DNA fragment size positioned between 120 to 150 bp. A sample electropherogram is shown in Figure 10.

Stopping Point If you do not continue to the next step, seal the plate and store at 4°C overnight or at –20°C for prolonged storage.

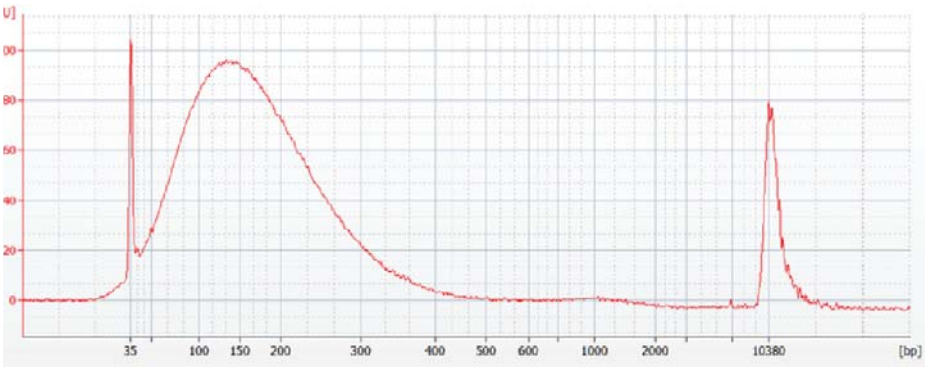


Figure 10 Analysis of sheared DNA using a High Sensitivity DNA Bioanalyzer assay.

Option 2: Analysis using the Agilent 2200 TapeStation and High Sensitivity D1000 ScreenTape

Use a High Sensitivity D1000 ScreenTape (p/n 5067-5584) and reagent kit (p/n 5067-5585) to analyze the 200-ng sheared DNA samples. For more information to do this step, see the *Agilent 2200 TapeStation User Manual* at www.genomics.agilent.com.

1 Seal the sheared DNA sample plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 1.0 sec.

2 Vortex the plate to mix samples in each well, then centrifuge the plate for 30 seconds to drive the well contents off the walls and plate seal.

3 Prepare the TapeStation samples as instructed in the *Agilent 2200 TapeStation User Manual*. Use 2 µL of each indexed DNA sample diluted with 2 µL of High Sensitivity D1000 sample buffer for the analysis.

CAUTION Make sure that you thoroughly mix the combined DNA and sample buffer on a vortex mixer for 5 seconds for accurate quantitation.

4 Load the sample plate or tube strips from step 3, the High Sensitivity D1000 ScreenTape, and loading tips into the 2200 TapeStation as instructed in the *Agilent 2200 TapeStation User Manual*. Start the run.

5 Verify that the electropherogram shows the peak of DNA fragment size positioned between 120 to 150 bp. A sample electropherogram is shown in Figure 11.

Stopping Point If you do not continue to the next step, seal the sheared DNA sample plate and store at 4°C overnight or at –20°C for prolonged storage.

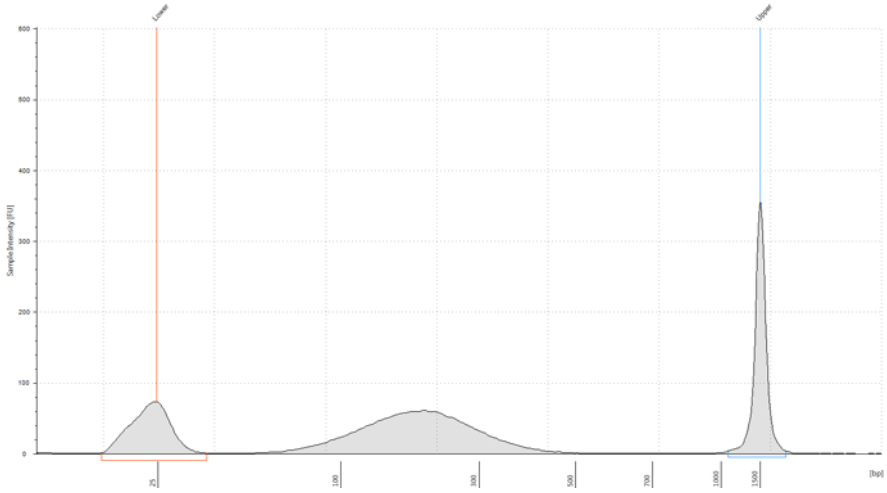


Figure 11 Analysis of sheared DNA using the 2200 TapeStation.

Step 3. Modify DNA ends for target enrichment

In this step, the Agilent NGS Workstation completes the DNA end modification steps required for SureSelect target enrichment, including GA end-repair, A-tailing, and adaptor ligation. After the appropriate modification steps, the Agilent NGS Workstation purifies the prepared DNA using AMPure XP beads.

Before starting the run, you need to prepare master mixes (with overage) for each step, without the DNA sample. Master mixes for runs that include 1, 2, 3, 4, 6, and 12 columns (including overage) are shown in each table.

Prepare each master mix on ice.

CAUTION

The Library Prep automation protocol for 200 ng gDNA samples differs from the 3 µg gDNA protocol in the amount of SureSelect Adaptor Oligo Mix used in the adaptor ligation master mix. Be sure to use the master mix preparation table provided on page 80 for 200 ng DNA samples.

Prepare the workstation

1. Clear the Labware MiniHub and BenchCel of all plates and tip boxes.
2. Pre-set the temperature of Bravo deck position 6 to 4°C using the Inheco Multi TEC control touchscreen, as described in Setting the Temperature of Bravo Deck Heat Blocks. Bravo deck position 6 corresponds to CPAC 2, position 2 on the Multi TEC control touchscreen.
3. Turn on the ThermoCube, set to 0°C, at position 9 of the Bravo deck. Be sure that the chiller reservoir contains at least 300 mL of 25% ethanol.

Prepare the SureSelect DNA end-repair master mix

4 Prepare the appropriate volume of end-repair master mix, according to Table 33. Mix well using a vortex mixer and keep on ice.

Table 33 Preparation of End-Repair Master Mix

SureSelect XT Reagent	Volume for 1 Library	Volume for 1 Column	Volume for 2 Columns	Volume for 3 Columns	Volume for 4 Columns	Volume for 6 Columns	Volume for 12 Columns
Nuclease-free water	35.2 µL	448.8 µL	748.0 µL	1047.2 µL	1346.4 µL	1944.8 µL	3889.6 µL
10X End-Repair Buffer	10.0 µL	127.5 µL	212.5 µL	297.5 µL	382.5 µL	552.5 µL	1105.0 µL
dNTP mix	1.6 µL	20.4 µL	34.0 µL	47.6 µL	61.2 µL	88.4 µL	176.8 µL
T4 DNA polymerase	1.0 µL	12.8 µL	21.3 µL	29.8 µL	38.3 µL	55.3 µL	110.5 µL
Klenow DNA polymerase	2.0 µL	25.5 µL	42.5 µL	59.5 µL	76.5 µL	110.5 µL	221.0 µL
T4 Polynucleotide Kinase	2.2 µL	28.1 µL	46.8 µL	65.5 µL	84.2 µL	121.6 µL	243.1 µL
Total Volume	**52 µL**	**663 µL**	**1105 µL**	**1547 µL**	**1989 µL**	**2873 µL**	**5746 µL**

Prepare the A-tailing master mix

5 Prepare the appropriate volume of A-tailing master mix, according to Table 34. Mix well using a vortex mixer and keep on ice.

Table 34 Preparation of A-Tailing Master Mix

SureSelect XT Reagent	Volume for 1 Library	Volume for 1 Column	Volume for 2 Columns	Volume for 3 Columns	Volume for 4 Columns	Volume for 6 Columns	Volume for 12 Columns
Nuclease-free water	11.0 µL	187.0 µL	280.5 µL	374.0 µL	467.5 µL	654.5 µL	1262.3 µL
10x Klenow Polymerase Buffer	5.0 µL	85.0 µL	127.5 µL	170.0 µL	212.5 µL	297.5 µL	573.8 µL
dATP	1.0 µL	17.0 µL	25.5 µL	34.0 µL	42.5 µL	59.5 µL	114.8 µL
Exo (–) Klenow	3.0 µL	51.0 µL	76.5 µL	102.0 µL	127.5 µL	178.5 µL	344.3 µL
Total Volume	**20 µL**	**340 µL**	**510 µL**	**680 µL**	**850 µL**	**1190 µL**	**2295 µL**

Prepare the adaptor ligation master mix

6 Prepare the appropriate volume of adaptor ligation master mix, according to Table 35. Mix well using a vortex mixer and keep on ice.

Table 35 Preparation of Adaptor Ligation Master Mix **(use only for the 200 ng DNA input workflow)**

SureSelect XT Reagent	Volume for 1 Library	Volume for 1 Column	Volume for 2 Columns	Volume for 3 Columns	Volume for 4 Columns	Volume for 6 Columns	Volume for 12 Columns
Nuclease-free water	24.5 µL	312.4 µL	520.6 µL	728.9 µL	937.1 µL	1353.6 µL	2707.3 µL
5X T4 DNA Ligase Buffer	10.0 µL	127.5 µL	212.5 µL	297.5 µL	382.5 µL	552.5 µL	1105.0 µL
SureSelect Adaptor Oligo Mix*	1.0 µL	12.8 µL	21.3 µL	29.8 µL	38.3 µL	55.3 µL	110.5 µL
T4 DNA Ligase	1.5 µL	19.1 µL	31.9 µL	44.6 µL	57.4 µL	82.9 µL	165.8 µL
Total Volume	**37.0 µL**	**471.8 µL**	**786.3 µL**	**1100.8 µL**	**1415.3 µL**	**2044.3 µL**	**4088.5 µL**

* Previously labeled as InPE Adaptor Oligo Mix.

Prepare the master mix source plate

7 In a Nunc DeepWell plate, prepare the master mix source plate containing the master mixes prepared in steps 3 to 5. Add the volumes indicated in Table 36 of each master mix to all wells of the indicated column of the Nunc DeepWell plate. Keep the master mixes on ice during the aliquoting steps. The final configuration of the master mix source plate is shown in Figure 12.

Table 36 Preparation of the Master Mix Source Plate for LibraryPrep_XT_ILM_v1.5.1.rst

Master Mix Solution	Position on Source Plate	Volume of Master Mix added per Well of Nunc Deep Well Source Plate					
		1-Column Runs	2-Column Runs	3-Column Runs	4-Column Runs	6-Column Runs	12-Column Runs
End Repair Master Mix	Column 1 (A1-H1)	76.4 µL	131.6 µL	186.9 µL	242.1 µL	352.6 µL	711.8 µL
A-Tailing Master Mix	Column 2 (A2-H2)	40.0 µL	61.3 µL	82.5 µL	103.8 µL	146.3µL	284.4 µL
Adaptor Ligation Master Mix	Column 3 (A3-H3)	54.3 µL	93.7 µL	133.0 µL	172.3 µL	250.9 µL	506.4 µL

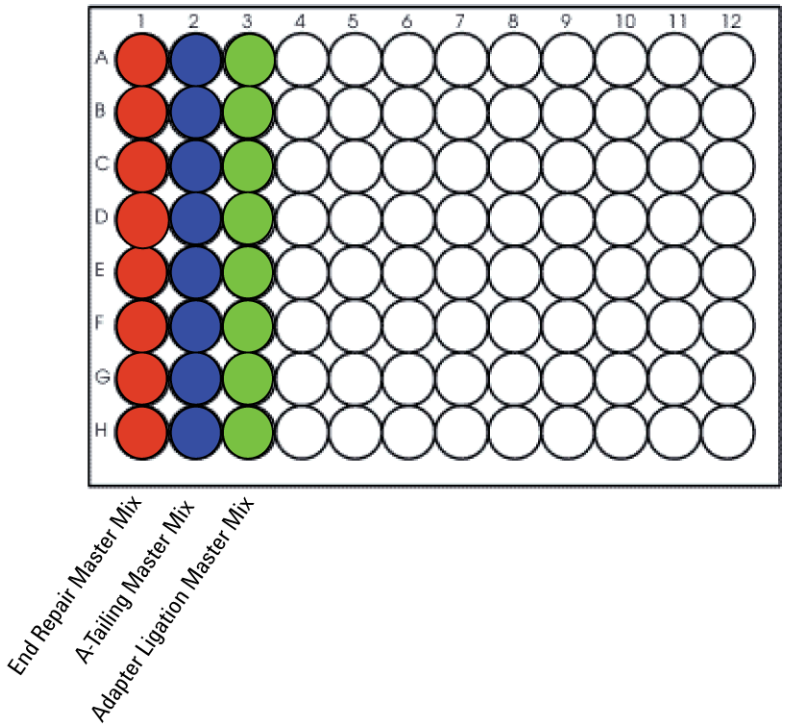


Figure 12 Configuration of the master mix source plate for LibraryPrep_XT_ILM_v1.5.1.rst

8 Seal the master mix source plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 1.0 sec.

9 Centrifuge the plate for 30 seconds to drive the well contents off the walls and plate seal and to eliminate any bubbles. Keep the master mix source plate on ice.

NOTE

The presence of bubbles in source plate solutions may cause inaccurate volume transfer by the Bravo liquid handling platform. Ensure that the source plate is sealed and centrifuged prior to use in a run.

Prepare the purification reagents

10 Verify that the AMPure XP bead suspension is at room temperature. *Do not freeze the beads at any time.*

11 Mix the bead suspension well so that the reagent appears homogeneous and consistent in color.

12 Prepare a separate Nunc DeepWell source plate for the beads by adding 370 µL of homogeneous AMPure XP beads per well, for each well to be processed.

13 Prepare a Thermo Scientific reservoir containing 20 mL of nuclease-free water.

14 Prepare a separate Thermo Scientific reservoir containing 150 mL of freshly-prepared 70% ethanol.

Load the Agilent NGS Workstation

15 Load the Labware MiniHub according to Table 37, using the plate orientations shown in Figure 13.

Table 37 Initial MiniHub configuration for LibraryPrep_XT_ILM_v1.5.1.rst

Vertical Shelf Position	Cassette 1	Cassette 2	Cassette 3	Cassette 4
Shelf 5 (Top)	Empty Nunc DeepWell plate	Empty Nunc DeepWell plate	Empty Nunc DeepWell plate	Empty
Shelf 4	Empty	Empty Eppendorf plate	Empty Eppendorf plate	Empty
Shelf 3	Empty	Empty	Empty	Empty Eppendorf plate
Shelf 2	Empty tip box	Nuclease-free water reservoir from step 13	AMPure XP beads in Nunc DeepWell plate from step 12	Empty
Shelf 1 (Bottom)	New tip box	70% ethanol reservoir from step 14	Empty	Empty tip box

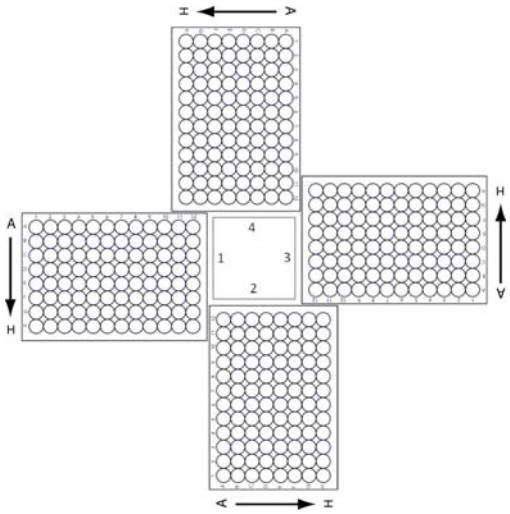


Figure 13 Agilent Labware MiniHub plate orientation. For Thermo Scientific reservoirs, place the notched corner facing the center of the hub.

16 Load the Bravo deck according to Table 38.

Table 38 Initial Bravo deck configuration for LibraryPrep_XT_ILM_v1.5.1.rst

Location	Content
1	Empty waste reservoir (Axygen 96 Deep Well Plate, square wells)
6	Empty Eppendorf plate
7	Eppendorf plate containing sheared gDNA samples (unsealed)
9	DNA End Modification Master Mix Source Plate (unsealed) seated on silver Nunc DeepWell insert

17 Load the BenchCel Microplate Handling Workstation according to Table 39.

Table 39 Initial BenchCel configuration for LibraryPrep_XT_ILM_v1.5.1.rst

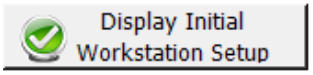
No. of Columns Processed	Rack 1	Rack 2	Rack 3	Rack 4
1	2 Tip boxes	Empty	Empty	Empty
2	4 Tip boxes	Empty	Empty	Empty
3	5 Tip boxes	Empty	Empty	Empty
4	7 Tip boxes	Empty	Empty	Empty
6	10 Tip boxes	Empty	Empty	Empty
12	11 Tip boxes	8 Tip boxes	Empty	Empty

Run VWorks runset LibraryPrep_XT_ILM_v1.5.1.rst

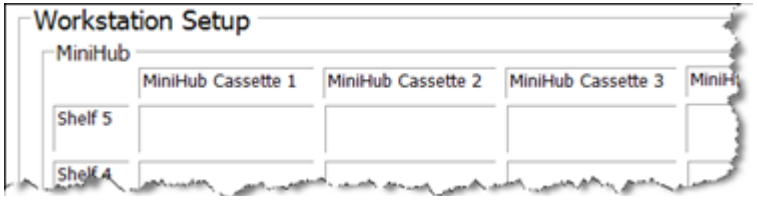
18 On the SureSelect setup form, under **Select Protocol to Run**, select **LibraryPrep_XT_ILM_v1.5.1.rst.**

19 Select the number of columns of samples to be processed. Runs must include 1, 2, 3, 4, 6, or 12 columns.

20 Click **Display Initial Workstation Setup**.

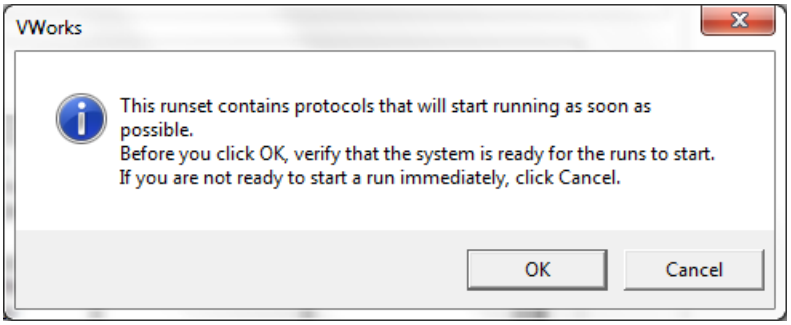


21 Verify that the NGS workstation has been set up as displayed in the Workstation Setup region of the form.



22 When verification is complete, click **Run Selected Protocol**.

23 When ready to begin the run, click **OK** in the following window.



Running the LibraryPrep_XT_ILM_v1.5.1.rst runset takes approximately 3.5 hours. Once complete, the purified, adaptor-ligated DNA samples are located in the Eppendorf plate at position 7 of the Bravo deck.

Stopping Point If you do not continue to the next step, seal the plate and store at 4°C overnight or at –20°C for prolonged storage.

Step 4. Amplify adaptor-ligated libraries

In this step, the Agilent NGS Workstation completes the liquid handling steps for amplification of the adaptor-ligated DNA samples. Afterward, you transfer the PCR plate to a thermal cycler for amplification.

CAUTION To avoid cross-contaminating libraries, set up PCR master mixes in a dedicated clean area or PCR hood with UV sterilization and positive air flow.

Prepare the workstation

1. Turn on the ThermoCube, set to 0°C, at position 9 of the Bravo deck. Be sure that the chiller reservoir contains at least 300 mL of 25% ethanol.
2. Leave tip boxes on shelves 1 and 2 in cassette 1 of the Labware MiniHub from the previous LibraryPrep_XT_ILM_v1.5.1.rst run. Otherwise, clear the remaining positions of the MiniHub and BenchCel of plates and tip boxes.
3. Pre-set the temperature of Bravo deck position 6 to 4°C using the Inheco Multi TEC control touchscreen, as described in Setting the Temperature of Bravo Deck Heat Blocks. Bravo deck position 6 corresponds to CPAC 2, position 2 on the Multi TEC control touchscreen.

Prepare the pre-capture PCR master mix and master mix source plate

4 Prepare the appropriate volume of pre-capture PCR Master Mix, according to Table 40. Mix well using a vortex mixer and keep on ice.

Table 40 Preparation of Pre-Capture PCR Master Mix **(use only for the 200 ng DNA input workflow)**

SureSelect^{XT} Reagent	Volume for 1 Library	Volume for 1 Column	Volume for 2 Columns	Volume for 3 Columns	Volume for 4 Columns	Volume for 6 Columns	Volume for 12 Columns
Nuclease-free water	6.0 µL	76.5 µL	127.5 µL	178.5 µL	229.5 µL	331.5 µL	663.0 µL
Herculase II 5X Reaction Buffer*	10.0 µL	127.5 µL	212.5 µL	297.5 µL	382.5 µL	552.5 µL	1105 µL
dNTP mix*	0.5 µL	6.4 µL	10.6 µL	14.9 µL	19.1 µL	27.6 µL	55.3 µL
SureSelect Primer† (Forward)	1.25 µL	15.9 µL	26.6 µL	37.2 µL	47.8 µL	69.1 µL	138.1 µL
SureSelect Indexing Pre-Capture PCR (Reverse) Primer‡	1.25 µL	15.9 µL	26.6 µL	37.2 µL	47.8 µL	69.1 µL	138.1 µL
Herculase II Polymerase	1.0 µL	12.8 µL	21.3 µL	29.8 µL	38.3 µL	55.3 µL	110.5 µL
Total Volume	**20 µL**	**255 µL**	**425 µL**	**595 µL**	**765 µL**	**1105 µL**	**2210 µL**

* Included with the Herculase II Fusion DNA Polymerase. *Do not use the buffer or dNTP mix from any other kit.*

† Included in SureSelect XT Library Prep Kit ILM.

‡ Included in SureSelect XT Automation ILM Module Box 2. Ensure that the correct primer is selected from Box 2 at this step (do not use the SureSelect Indexing Post-Capture PCR (Forward) Primer).

5 Using the same Nunc DeepWell master mix source plate that was used for the LibraryPrep_XT_ILM_v1.5.1.rst run, add the volume of PCR Master Mix indicated in Table 41 to all wells of column 4 of the master mix source plate. The final configuration of the master mix source plate is shown in Figure 14.

Table 41 Preparation of the Master Mix Source Plate for Pre-CapturePCR_XT_ILM_200ng_v1.5.1.pro

Master Mix Solution	Position on Source Plate	Volume of Master Mix added per Well of Nunc Deep Well Source Plate					
		1-Column Runs	2-Column Runs	3-Column Runs	4-Column Runs	6-Column Runs	12-Column Runs
Pre-Capture PCR Master Mix	Column 4 (A4-H4)	29.4 µL	50.6 µL	71.9 µL	93.1 µL	135.6 µL	273.8 µL

NOTE If you are using a new DeepWell plate for the pre-capture PCR source plate, leave columns 1 to 3 empty and add the PCR Master Mix to column 4 of the new plate.

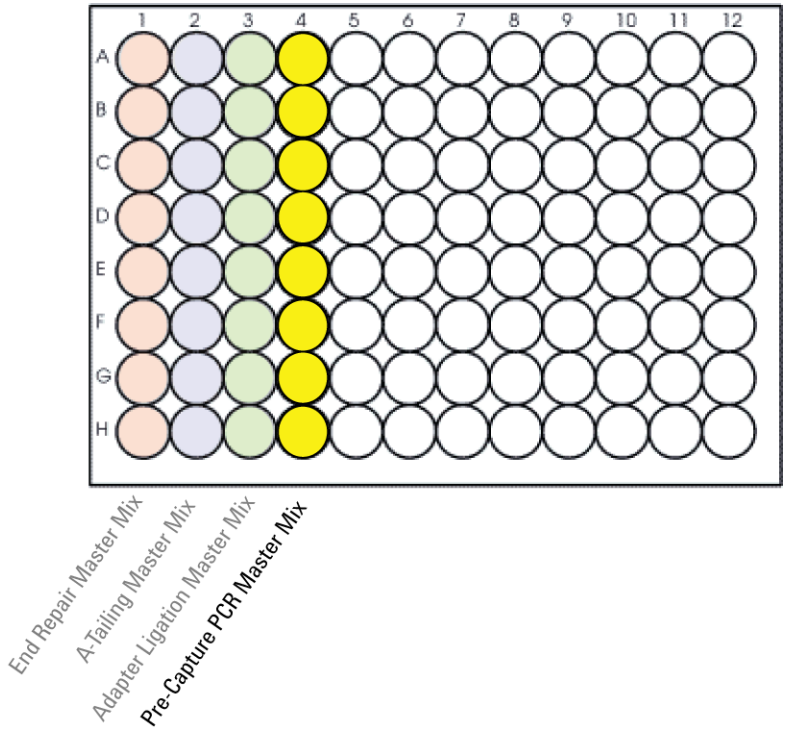


Figure 14 Configuration of the master mix source plate for Pre-CapturePCR_XT_ILM_200ng_v1.5.1.pro. Columns 1-3 were used to dispense master mixes during the previous protocol.

6 Seal the master mix source plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 1.0 sec.

7 Centrifuge the plate for 30 seconds to drive the well contents off the walls and plate seal and to eliminate any bubbles.

NOTE

The presence of bubbles in source plate solutions may cause inaccurate volume transfer by the Bravo liquid handling platform. Ensure that the source plate is sealed and centrifuged prior to use in a run.

Load the Agilent NGS Workstation

8 Load the Labware MiniHub according to Table 42, using the plate orientations shown in Figure 13.

Table 42 Initial MiniHub configuration for Pre-CapturePCR_XT_ILM_200ng_v1.5.1.pro

Vertical Shelf Position	Cassette 1	Cassette 2	Cassette 3	Cassette 4
Shelf 5 (Top)	Empty	Empty	Empty	Empty
Shelf 4	Empty	Empty	Empty	Empty
Shelf 3	Empty	Empty	Empty	Empty
Shelf 2	Waste tip box*	Empty	Empty	Empty
Shelf 1 (Bottom)	Clean tip box*	Empty	Empty	Empty tip box

* The waste tip box (Cassette 1, Shelf 2) and clean tip box (Cassette 1, Shelf 1) are retained from the LibraryPrep_XT_ILM_v1.5.1.rst run and reused here.

NOTE If you are using a new box of tips on shelf 1 of cassette 1, first remove the tips from columns 1 to 3 of the tip box. Any tips present in columns 1 to 3 of the tip box may be inappropriately loaded onto the Bravo platform pipette heads and may interfere with automated processing steps.

9 Load the Bravo deck according to Table 43.

Table 43 Initial Bravo deck configuration for Pre-CapturePCR_XT_ILM_200ng_v1.5.1.pro

Location	Content
6	Empty PCR plate seated on red insert (PCR plate type must be specified on setup form under step 2)
7	Adaptor-ligated DNA samples in Eppendorf plate
9	Master mix plate containing PCR Master Mix in Column 4 (unsealed)

10 Load the BenchCel Microplate Handling Workstation according to Table 44.

Table 44 Initial BenchCel configuration for Pre-CapturePCR_XT_ILM_200ng_v1.5.1.pro

No. of Columns Processed	Rack 1	Rack 2	Rack 3	Rack 4
1	1 Tip box	Empty	Empty	Empty
2	1 Tip box	Empty	Empty	Empty
3	1 Tip box	Empty	Empty	Empty
4	1 Tip box	Empty	Empty	Empty
6	1 Tip box	Empty	Empty	Empty
12	1 Tip box	Empty	Empty	Empty

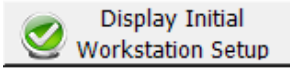
Run VWorks protocol Pre-CapturePCR_XT_ILM_200ng_v1.5.1.pro

11 On the SureSelect setup form, under **Select Protocol to Run**, select **Pre-CapturePCR_XT_ILM_200ng_v1.5.1.pro.**

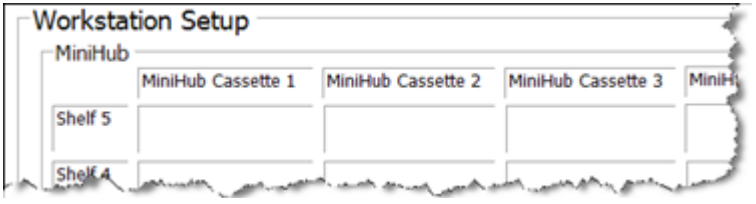
12 Under **Select PCR plate labware for Thermal Cycling**, select the specific type of PCR plate used at position 6 of the Bravo deck.

13 Select the number of columns of samples to be processed. Runs must include 1, 2, 3, 4, 6, or 12 columns.

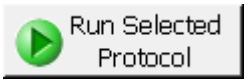
14 Click **Display Initial Workstation Setup**.



15 Verify that the NGS workstation has been set up as displayed in the Workstation Setup region of the form.

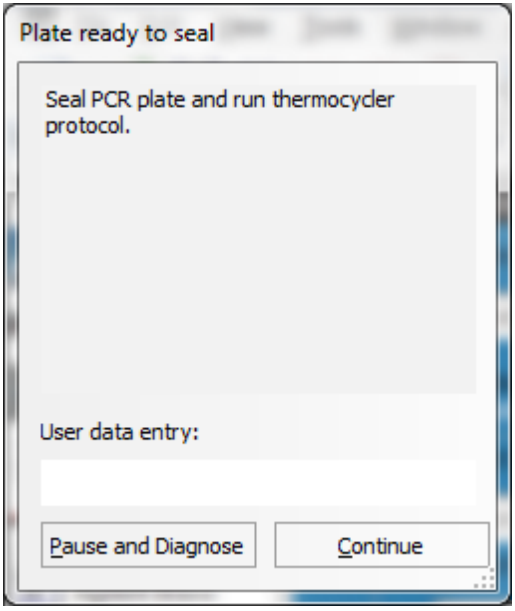


16 When verification is complete, click **Run Selected Protocol**.



Running the Pre-CapturePCR_XT_ILM_200ng_v1.5.1.pro protocol takes approximately 15 minutes. Once complete, the PCR-ready samples, containing prepped DNA and PCR master mix, are located in the PCR plate at position 6 of the Bravo deck.

17 When you see the following prompt, remove the PCR plate from position 6 of the Bravo deck and seal the plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 3.0 seconds.



18 Centrifuge the plate for 30 seconds to drive the well contents off the walls and plate seal and to eliminate air bubbles.

19 Transfer the PCR plate to a thermal cycler and run the PCR amplification program shown in Table 45.

Table 45 Pre-Capture PCR cycling program **(use only for the 200 ng DNA input workflow)**

Segment	Number of Cycles	Temperature	Time
1	1	98°C	2 minutes
2	10	98°C	30 seconds
		65°C	30 seconds
		72°C	1 minute
3	1	72°C	10 minutes
4	1	4°C	Hold

Step 5. Purify amplified DNA using AMPure XP beads

In this step, the Agilent NGS Workstation transfers AMPure XP beads and amplified adaptor-ligated DNA to a Nunc DeepWell plate and then collects and washes the bead-bound DNA.

Prepare the workstation and reagents

1 Clear the Labware MiniHub and BenchCel of all plates and tip boxes.

2 Verify that the AMPure XP bead suspension is at room temperature. (If necessary, allow the bead solution to come to room temperature for at least 30 minutes.) *Do not freeze the beads at any time*.

3 Mix the bead suspension well so that the reagent appears homogeneous and consistent in color.

4 Prepare a Nunc DeepWell source plate for the beads by adding 95 µL of homogeneous AMPure XP beads per well, for each well to be processed.

5 Prepare a Thermo Scientific reservoir containing 15 mL of nuclease-free water.

6 Prepare a separate Thermo Scientific reservoir containing 45 mL of freshly-prepared 70% ethanol.

7 Load the Labware MiniHub according to Table 46, using the plate orientations shown in Figure 13.

Table 46 Initial MiniHub configuration for AMPureXP_XT_ILM_v1.5.1.pro:Pre-Capture PCR

Vertical Shelf Position	Cassette 1	Cassette 2	Cassette 3	Cassette 4
Shelf 5 (Top)	Empty Nunc DeepWell plate	Empty	Empty	Empty
Shelf 4	Empty	Empty	Empty	Empty
Shelf 3	Empty	Empty Eppendorf Plate	Empty	Empty
Shelf 2	Empty	Nuclease-free water reservoir from step 5	AMPure XP beads in Nunc DeepWell plate from step 4	Empty
Shelf 1 (Bottom)	Empty	70% ethanol reservoir from step 6	Empty	Empty tip box

8 Load the Bravo deck according to Table 47.

Table 47 Initial Bravo deck configuration for AMPureXP_XT_ILM_v1.5.1.pro:Pre-Capture PCR

Location	Content
1	Empty waste reservoir (Axygen 96 Deep Well Plate, square wells)
9	Amplified DNA libraries in PCR plate seated in red insert (PCR plate type must be specified on setup form under step 2)

9 Load the BenchCel Microplate Handling Workstation according to Table 48.

Table 48 Initial BenchCel configuration for AMPureXP_XT_ILM_v1.5.1.pro:Pre-Capture PCR

No. of Columns Processed	Rack 1	Rack 2	Rack 3	Rack 4
1	1 Tip box	Empty	Empty	Empty
2	1 Tip box	Empty	Empty	Empty
3	2 Tip boxes	Empty	Empty	Empty
4	2 Tip boxes	Empty	Empty	Empty
6	3 Tip boxes	Empty	Empty	Empty
12	6 Tip boxes	Empty	Empty	Empty

Run VWorks protocol *AMPureXP_XT_ILM_v1.5.1.pro:Pre-Capture PCR*

10 On the SureSelect setup form, under **Select Protocol to Run**, select **AMPureXP_XT_ILM_v1.5.1.pro:Pre-Capture PCR**.

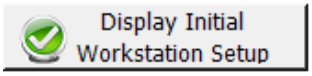
NOTE

AMPureXP purification protocols are used during multiple steps of the SureSelect automation workflow. Be sure to select the correct workflow step when initiating the automation protocol.

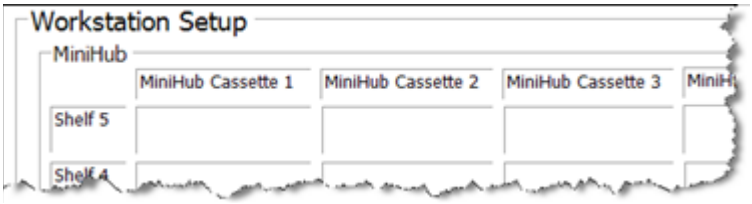
11 Under **Select PCR plate labware for Thermal Cycling**, select the specific type of PCR plate containing the amplified libraries at position 9.

12 Select the number of columns of samples to be processed. Runs must include 1, 2, 3, 4, 6, or 12 columns.

13 Click **Display Initial Workstation Setup**.



14 Verify that the NGS workstation has been set up as displayed in the Workstation Setup region of the form.



15 When verification is complete, click **Run Selected Protocol**.

The purification protocol takes approximately 45 minutes. When complete, the purified DNA samples are in the Eppendorf plate located on Bravo deck position 7.

Step 6. Assess Library DNA quantity and quality

The hybridization protocol in the following section requires 750 ng of each amplified DNA library. Measure the concentration of each library using one of the methods detailed below. Once DNA concentration for each sample is determined, calculate the volume of the library to be used for hybridization using the following formula:

Volume (µL) = 750 ng/concentration (ng/µL)

Option 1: Analysis using the Agilent 2100 Bioanalyzer and DNA 1000 Assay

Use a Bioanalyzer DNA 1000 chip and reagent kit. For more information to do this step, see the *Agilent DNA 1000 Kit Guide* at www.genomics.agilent.com.

1. Set up the 2100 Bioanalyzer as instructed in the reagent kit guide.
2. Seal the sample plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 1.0 sec.
3. Vortex the plate to mix samples in each well, then centrifuge the plate for 30 seconds to drive the well contents off the walls and plate seal.
4. Prepare the chip, samples and ladder as instructed in the reagent kit guide, using 1 µL of each sample for the analysis.
5. Load the prepared chip into the 2100 Bioanalyzer and start the run within five minutes after preparation.
6. Verify that the electropherogram shows the peak of DNA fragment size positioned between 225 to 275 bp. A sample electropherogram is shown in Figure 15.
7. Determine the concentration of the library (ng/µL) by integrating under the peak.

Stopping Point If you do not continue to the next step, seal the plate and store at 4°C overnight or at –20°C for prolonged storage.

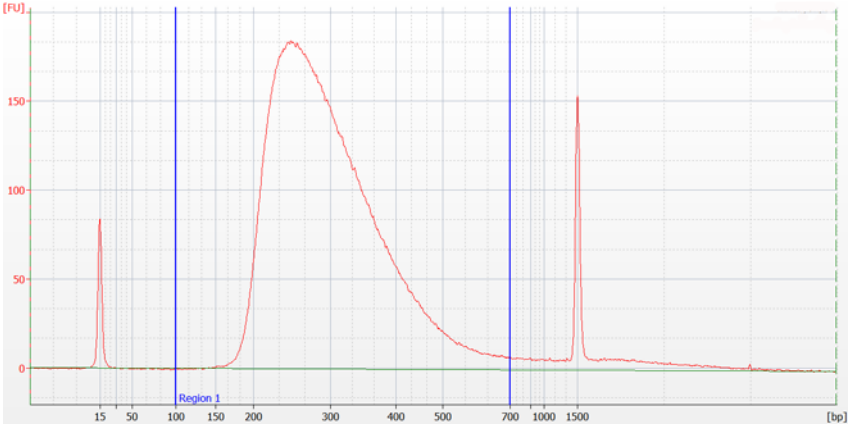


Figure 15 Analysis of amplified library DNA using a DNA 1000 assay.

Option 2: Analysis using the Agilent 2200 TapeStation and D1000 ScreenTape

Use a D1000 ScreenTape (p/n 5067-5582) and associated reagent kit (p/n 5067-5583) to analyze the amplified libraries. For more information to do this step, see the *Agilent 2200 TapeStation User Manual* at www.genomics.agilent.com.

1. Seal the DNA sample plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 1.0 sec.
2. Vortex the plate to mix samples in each well, then centrifuge the plate for 30 seconds to drive the well contents off the walls and plate seal.
3. Prepare the TapeStation samples as instructed in the *Agilent 2200 TapeStation User Manual*. Use 1 µL of each amplified library DNA sample diluted with 3 µL of D1000 sample buffer for the analysis.

CAUTION Make sure that you thoroughly mix the combined DNA and D1000 sample buffer on a vortex mixer for 5 seconds for accurate quantitation.

4. Load the sample plate or tube strips from step 3, the D1000 ScreenTape, and loading tips into the 2200 TapeStation as instructed in the *Agilent 2200 TapeStation User Manual*. Start the run.
5. Verify that the electropherogram shows the peak of DNA fragment size positioned between 225 to 275 bp. A sample electropherogram is shown in Figure 16.

Stopping Point If you do not continue to the next step, seal the library DNA sample plate and store at 4°C overnight or at –20°C for prolonged storage.

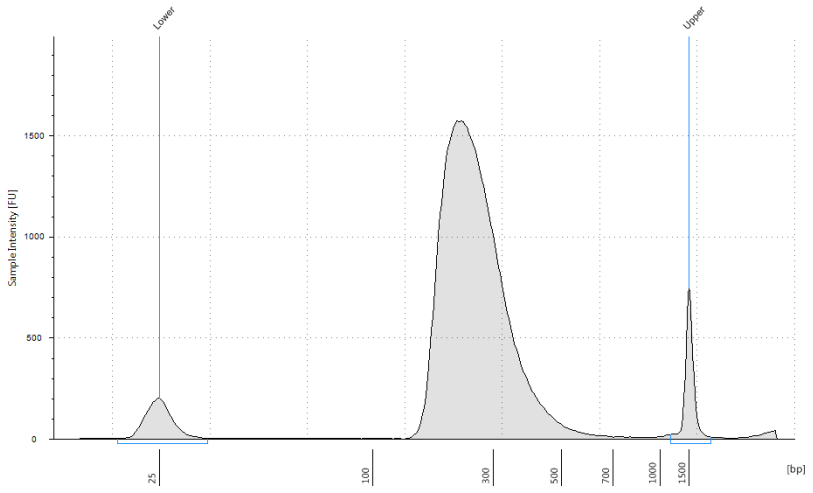


Figure 16 Analysis of amplified library DNA using the 2200 TapeStation.

5 Hybridization

This chapter describes the steps to combine the prepped library with the blocking agents and the SureSelect capture library. Each DNA library sample must be hybridized and captured individually prior to addition of the indexing tag by PCR.

CAUTION The ratio of SureSelect capture library to prepped library is critical for successful capture.

Step 1. Aliquot prepped DNA samples for hybridization

For each sample library prepared, do one hybridization and capture. Do not pool samples at this stage.

Each hybridization reaction will contain 750 ng of the prepped gDNA sample. Before starting the hybridization step, you must create a table containing instructions for the Agilent NGS Workstation indicating the volume of each sample required for a 750-ng aliquot.

1 Create a .csv (comma separated value) file with the headers shown in Figure 17. The header text must not contain spaces. The table may be created using a spreadsheet application, such as Microsoft Excel software, and then saved in .csv format. The file must include rows for all 96 wells of the plate.

2 Enter the information requested in the header for each DNA sample.

- In the SourceBC field, enter the sample plate description or barcode. The SourceBC field contents must be identical for all rows.
- In the SourceWell and DestinationWell fields, enter each well position for the plate. SourceWell and DestinationWell field contents must be identical for a given sample.
- In the Volume field, enter the volume (in µL) equivalent to 750 ng DNA for each sample. These values are determined from the concentration values obtained from Bioanalyzer or TapeStation traces in the previous section. For all empty wells on the plate, enter the value 0, as shown in Figure 17; **do not delete rows for empty wells**.

	A	B	C	D
1	**SourceBC**	**SourceWell**	**DestinationWell**	**Volume**
2	SamplePlateXYZ	A1	A1	5.35
3	SamplePlateXYZ	B1	B1	4.28
4	SamplePlateXYZ	C1	C1	4.76
5	SamplePlateXYZ	D1	D1	5.19
6	SamplePlateXYZ	E1	E1	5.49
7	SamplePlateXYZ	F1	F1	4.86
8	SamplePlateXYZ	G1	G1	5.05
9	SamplePlateXYZ	H1	H1	4.37
10	SamplePlateXYZ	A2	A2	0
11	SamplePlateXYZ	B2	B2	0
12	SamplePlateXYZ	C2	C2	0
13	SamplePlateXYZ	D2	D2	0

Figure 17 Sample spreadsheet for 750-ng sample aliquot for 1-column run.

NOTE

You can find a sample spreadsheet in the directory **C: > VWorks Workspace > NGS Option B > XT Illumina_1.5.1> Aliquot Library Input Files > 750ng_transfer_full_plate_template_xlsx.**

The 750ng_transfer_full_plate_template.xlsx file may be copied and used as a template for creating the .csv files for each Aliquot_Libraries_v1.5.1.pro run. If you are using the sample file as a template for runs with fewer than 12 columns, be sure to retain rows for all 96 wells, and populate the Volume column with 0 for unused wells.

3 Load the .csv file onto the PC containing the VWorks software into a suitable folder, such as **C: > VWorks Workspace > NGS Option B > XT Illumina_1.5.1 > Aliquot Library Input Files.**

4 Turn on the chiller, set to 0°C, at position 9 of the Bravo deck. Be sure that the chiller reservoir contains at least 300 mL of 25% ethanol.

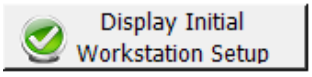
5 Load the Bravo deck according to Table 49.

Table 49 Initial Bravo deck configuration for Aliquot_Libraries_v1.5.1.pro

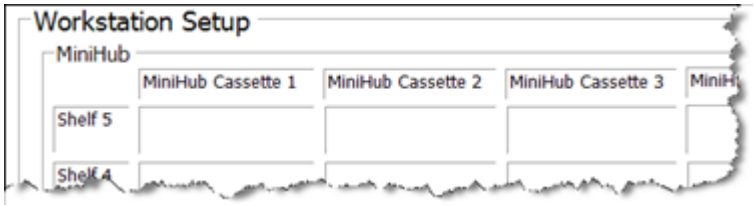
Location	Content
5	Empty Eppendorf plate
6	Empty tip box
8	New tip box
9	Prepped library DNA in Eppendorf plate

6 On the SureSelect setup form, under **Select Protocol to Run**, select **Aliquot_Libraries_v1.5.1.pro.**

7 Click **Display Initial Workstation Setup**.

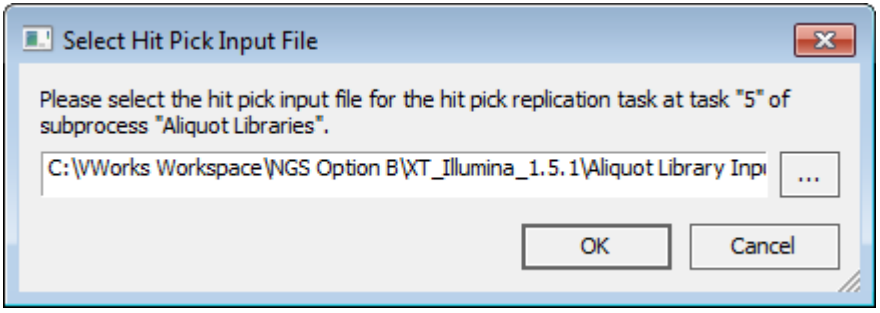


8 Verify that the NGS workstation has been set up as displayed in the Workstation Setup region of the form.



9 When verification is complete, click **Run Selected Protocol**.

10 When prompted by the dialog below, browse to the .csv file created for the source plate of the current run, and then click **OK** to start the run.



The library aliquoting protocol takes approximately 1 hour for 96 samples. When complete, the 750-ng samples are in the PCR plate located on Bravo deck position 5.

11 Remove the 750-ng sample plate from the Bravo deck and use a vacuum concentrator to dry the sample at ≤ 45°C.

12 Reconstitute each dried sample with 3.4 µL of nuclease-free water to bring the final concentration to 221 ng/µL. Pipette up and down along the sides of each well for optimal recovery.

13 Seal the plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 1.0 sec.

14 Vortex the plate for 30 seconds to ensure complete reconstitution, then centrifuge the plate for 1 minute to drive the well contents off the walls and plate seal.

Step 2. Hybridize DNA Samples to the Capture Library

In this step, the Agilent NGS Workstation completes the liquid handling steps to prepare for hybridization. Afterward, you transfer the sample plate to a thermal cycler, held at 65°C, to allow hybridization of the prepared DNA samples to one or more Capture Libraries.

Prepare the workstation

1. Clear the Labware MiniHub and BenchCel of all plates and tip boxes.
2. Gently wipe down the Labware MiniHub, Bravo decks, and BenchCel with a NucleoClean decontamination wipe.
3. Turn on the chiller, set to 0°C, at position 9 of the Bravo deck. Be sure that the chiller reservoir contains at least 300 mL of 25% ethanol.
4. Place the silver Nunc DeepWell plate insert on position 6 of the Bravo deck. This insert is required to facilitate heat transfer to DeepWell source plate wells during the Hybridization protocol.

Prepare the SureSelect Block master mix

5 Prepare the appropriate volume of SureSelect Block master mix, on ice, as indicated in Table 50.

Table 50 Preparation of SureSelect Block Master Mix

SureSelect^{XT} Reagent	Volume for 1 Library	Volume for 1 Column	Volume for 2 Columns	Volume for 3 Columns	Volume for 4 Columns	Volume for 6 Columns	Volume for 12 Columns
Nuclease-free water	6.0 µL	76.5 µL	127.5 µL	178.5 µL	229.5 µL	331.5 µL	663.0 µL
SureSelect Indexing Block 1 (green cap)	2.5 µL	31.9 µL	53.1 µL	74.4 µL	95.6 µL	138.1 µL	276.3 µL
SureSelect Block 2 (blue cap)	2.5 µL	31.9 µL	53.1 µL	74.4 µL	95.6 µL	138.1 µL	276.3 µL
SureSelect ILM Indexing Block 3 (brown cap)	0.6 µL	7.7 µL	12.8 µL	17.9 µL	23.0 µL	33.2 µL	66.3 µL
Total Volume	**11.6 µL**	**147.9 µL**	**246.5 µL**	**345.1 µL**	**443.7 µL**	**640.9 µL**	**1281.9 µL**

Prepare one or more Capture Library master mixes

6 Prepare the appropriate volume of SureSelect capture library master mix for each of the capture libraries that will be used for hybridization as indicated in Table 51 to Table 54. Mix the components by pipetting. Keep the master mixes on ice during preparation and aliquoting.

NOTE

Each row of the prepped gDNA sample plate may be hybridized to a different Capture Library. However, capture libraries of different sizes require different post-capture amplification cycles. Plan experiments such that similar-sized libraries are hybridized on the same plate.

For runs that use a single capture library for all rows of the plate, prepare the master mix as described in Step a (Table 51 or Table 52) below.

For runs that use different capture libraries for individual rows, prepare each master mix as described in Step b (Table 53 or Table 54) below.

a **For runs that use a single capture library for all rows**, prepare the Capture Library Master Mix as listed in Table 51 or Table 52, based on the Mb target size of your design.

Table 51 Preparation of Capture Library Master Mix for target sizes <3.0 Mb, 8 rows of wells

Target size <3.0 Mb							
SureSelect^{XT} Reagent	**Volume for 1 Library**	**Volume for 1 Column**	**Volume for 2 Columns**	**Volume for 3 Columns**	**Volume for 4 Columns**	**Volume for 6 Columns**	**Volume for 12 Columns**
Nuclease-free water	4.5 µL	76.5 µL	114.8 µL	153.0 µL	191.3 µL	306.0 µL	592.9 µL
RNase Block (purple cap)	0.5 µL	8.5 µL	12.8 µL	17.0 µL	21.3 µL	34.0 µL	65.9 µL
Capture Library	2.0 µL	34.0 µL	51.0 µL	68.0 µL	85.0 µL	136.0 µL	263.5 µL
Total Volume	**7.0 µL**	**119.0 µL**	**178.6 µL**	**238.0 µL**	**297.6 µL**	**476.0 µL**	**922.3 µL**

Table 52 Preparation of Capture Library Master Mix for target sizes >3.0 Mb, 8 rows of wells

Target size >3.0 Mb							
SureSelect^{XT} Reagent	**Volume for 1 Library**	**Volume for 1 Column**	**Volume for 2 Columns**	**Volume for 3 Columns**	**Volume for 4 Columns**	**Volume for 6 Columns**	**Volume for 12 Columns**
Nuclease-free water	1.5 µL	25.5 µL	38.3 µL	51.0 µL	63.8 µL	102.0 µL	197.6 µL
RNase Block (purple cap)	0.5 µL	8.5 µL	12.8 µL	17.0 µL	21.3 µL	34.0 µL	65.9 µL
Capture Library	5.0 µL	85.0 µL	127.5 µL	170.0 µL	212.5 µL	340.0 µL	658.8 µL
Total Volume	**7.0 µL**	**119.0 µL**	**178.6 µL**	**238.0 µL**	**297.6 µL**	**476.0 µL**	**922.3 µL**

b **For runs that use different capture libraries in individual rows**, prepare a Capture Library Master Mix for each capture library as listed in Table 53 or Table 54, based on the Mb target size of your design. The volumes listed in Table 53 and Table 54 are for a single row of sample wells. If a given capture library will be hybridized in multiple rows, multiply each of the values below by the number of rows assigned to that capture library.

Table 53 Preparation of Capture Library Master Mix for target sizes <3.0 Mb, single row of wells

Target size <3.0 Mb							
SureSelect^{XT} Reagent	Volume for 1 Library	Volume for 1 Column	Volume for 2 Columns	Volume for 3 Columns	Volume for 4 Columns	Volume for 6 Columns	Volume for 12 Columns
Nuclease-free water	4.5 µL	9.0 µL	13.8 µL	18.6 µL	23.3 µL	37.7 µL	73.5 µL
RNase Block (purple cap)	0.5 µL	1.0 µL	1.5 µL	2.1 µL	2.6 µL	4.2 µL	8.2 µL
Capture Library	2.0 µL	4.0 µL	6.1 µL	8.3 µL	10.4 µL	16.8 µL	32.7 µL
Total Volume	**7.0 µL**	**14.0 µL**	**21.4 µL**	**28.9 µL**	**36.3 µL**	**58.6 µL**	**114.4 µL**

Table 54 Preparation of Capture Library Master Mix for target sizes >3.0 Mb, single row of wells

Target size >3.0 Mb							
SureSelect^{XT} Reagent	Volume for 1 Library	Volume for 1 Column	Volume for 2 Columns	Volume for 3 Columns	Volume for 4 Columns	Volume for 6 Columns	Volume for 12 Columns
Nuclease-free water	1.5 µL	3.0 µL	4.6 µL	6.2 µL	7.8 µL	12.6 µL	24.5 µL
RNase Block (purple cap)	0.5 µL	1.0 µL	1.5 µL	2.1 µL	2.6 µL	4.2 µL	8.2 µL
Capture Library	5.0 µL	10.0 µL	15.3 µL	20.6 µL	25.9 µL	41.9 µL	81.7 µL
Total Volume	**7.0 µL**	**14.0 µL**	**21.4 µL**	**28.9 µL**	**36.3 µL**	**58.6 µL**	**114.4 µL**

Prepare the Hybridization Buffer master mix

7 Prepare the appropriate volume of Hybridization Buffer Master Mix, at room temperature, as indicated in Table 55.

Table 55 Preparation of Hybridization Buffer Master Mix

SureSelect^{XT} Reagent	Volume for 1 Column	Volume for 2 Columns	Volume for 3 Columns	Volume for 4 Columns	Volume for 6 Columns	Volume for 12 Columns
SureSelect Hyb 1 (orange cap or bottle)	140.9 µL	197.3 µL	250.0 µL	310.1 µL	422.8 µL	789.3 µL
SureSelect Hyb 2 (red cap)	5.6 µL	7.9 µL	10.0 µL	12.4 µL	16.9 µL	31.6 µL
SureSelect Hyb 3 (yellow cap or bottle)	56.4 µL	78.9 µL	100.0 µL	124.0 µL	169.1 µL	315.7 µL
SureSelect Hyb 4 (black cap or bottle)	73.3 µL	102.6 µL	130.0 µL	161.2 µL	219.9 µL	410.4 µL
Total Volume	**276.2 µL**	**386.7 µL**	**490.0 µL**	**607.7 µL**	**828.7 µL**	**1547 µL**

8 If precipitate forms, warm the hybridization buffer at 65°C for 5 minutes.

Prepare the master mix source plate

9 In a Nunc DeepWell plate, prepare the master mix source plate containing the master mixes prepared in step 5 to step 7 at room temperature. Add the volumes indicated in Table 56 of each master mix to each well of the indicated column of the Nunc DeepWell plate. When using multiple capture libraries in a run, add each Capture Library Master Mix to the appropriate row(s) of the Nunc DeepWell plate. The final configuration of the master mix source plate is shown in Figure 18.

Table 56 Preparation of the Master Mix Source Plate for Hybridization_v1.5.1.pro

Master Mix Solution	Position on Source Plate	Volume of Master Mix added per Well of Nunc Deep Well Source Plate					
		1-Column Runs	2-Column Runs	3-Column Runs	4-Column Runs	6-Column Runs	12-Column Runs
Block Master Mix	Column 1 (A1-H1)	17.0 µL	29.4 µL	41.7 µL	54.0 µL	78.7 µL	158.8 µL
Capture Library Master Mix	Column 2 (A2-H2)	14.0 µL	21.4 µL	28.9 µL	36.3 µL	58.6 µL	114.4 µL
Hybridization Buffer Master Mix	Column 3 (A3-H3)	30.5 µL	44.3 µL	57.2 µL	71.9 µL	99.5 µL	189.3 µL

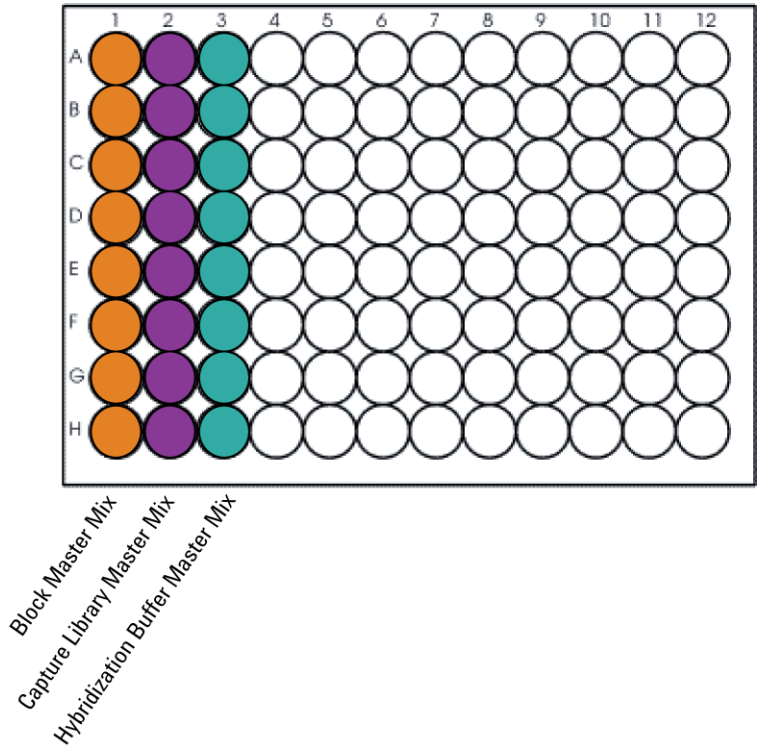


Figure 18 Configuration of the master mix source plate for Hybridization_v1.5.1.pro.

10 Seal the master mix source plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 1.0 sec.

11 Centrifuge the plate for 30 seconds to drive the well contents off the walls and plate seal and to eliminate any bubbles. Keep the master mix plate at room temperature.

Load the Agilent NGS Workstation

12 Load the Labware MiniHub according to Table 57, using the plate orientations shown in Figure 3.

Table 57 Initial MiniHub configuration for Hybridization_v1.5.1.pro

Vertical Shelf Position	Cassette 1	Cassette 2	Cassette 3	Cassette 4
Shelf 5 (Top)	Empty	Empty	Empty	Empty
Shelf 4	Empty	Empty	Empty	Empty
Shelf 3	Empty	Empty	Empty	Empty
Shelf 2	Empty	Empty	Empty	Empty tip box
Shelf 1 (Bottom)	Empty	Empty	Empty	Empty

13 Load the Bravo deck according to Table 58.

Table 58 Initial Bravo deck configuration for Hybridization_v1.5.1.pro

Location	Content
4	Empty PCR plate seated in red insert (PCR plate type must be specified on setup form under step 2)
5	Empty Eppendorf plate
6	Hybridization Master Mix source plate (unsealed) seated on silver Nunc DeepWell insert
8	Empty tip box
9	750-ng aliquots of prepped gDNA (reconstituted at 221 ng/µL), in Eppendorf plate (unsealed)

14 Load the BenchCel Microplate Handling Workstation according to Table 59.

Table 59 Initial BenchCel configuration for Hybridization_v1.5.1.pro

No. of Columns Processed	Rack 1	Rack 2	Rack 3	Rack 4
1	2 Tip boxes	Empty	Empty	Empty
2	2 Tip boxes	Empty	Empty	Empty
3	2 Tip boxes	Empty	Empty	Empty
4	2 Tip boxes	Empty	Empty	Empty
6	3 Tip boxes	Empty	Empty	Empty
12	4 Tip boxes	Empty	Empty	Empty

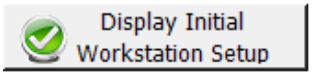
Run VWorks protocol Hybridization_v1.5.1.pro

15 On the SureSelect setup form, under **Select Protocol to Run**, select **Hybridization_v1.5.1.pro.**

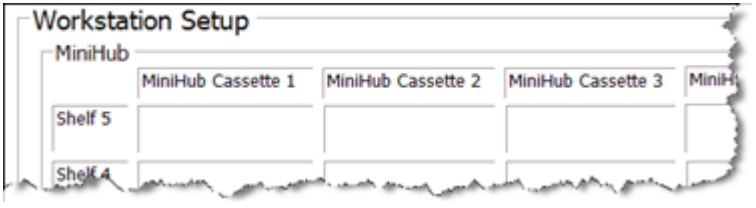
16 Under **Select PCR plate labware for Thermal Cycling**, select the specific type of PCR plate used at position 4 of the Bravo deck.

17 Select the number of columns of samples to be processed. Runs must include 1, 2, 3, 4, 6, or 12 columns.

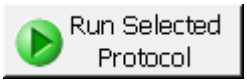
18 Click **Display Initial Workstation Setup**.



19 Verify that the NGS workstation has been set up as displayed in the Workstation Setup region of the form.

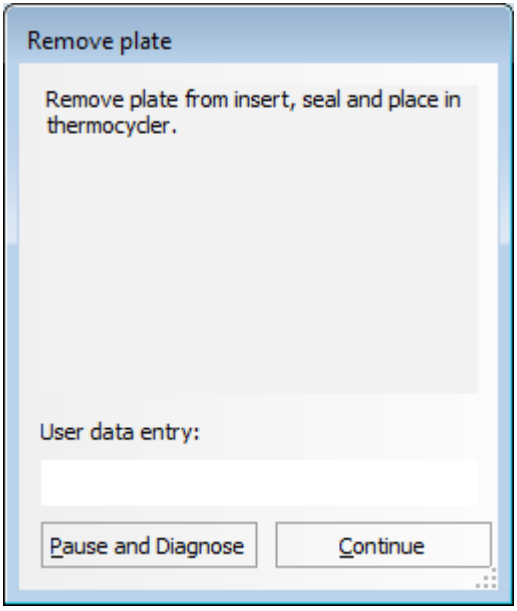


20 When verification is complete, click **Run Selected Protocol**.



The Agilent NGS Workstation transfers SureSelect Block Master Mix to the prepped gDNA-containing wells of the sample plate. When this process is complete, you will be prompted to transfer the plate to the thermal cycler for sample denaturation prior to hybridization.

21 When prompted by VWorks as shown below, remove the PCR plate from position 4 of the Bravo deck, leaving the red insert in place.



22 Seal the sample plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 3.0 sec.

23 Transfer the sealed plate to a thermal cycler and run the following program shown in Table 60. After transferring the plate, click **Continue** on the VWorks screen.

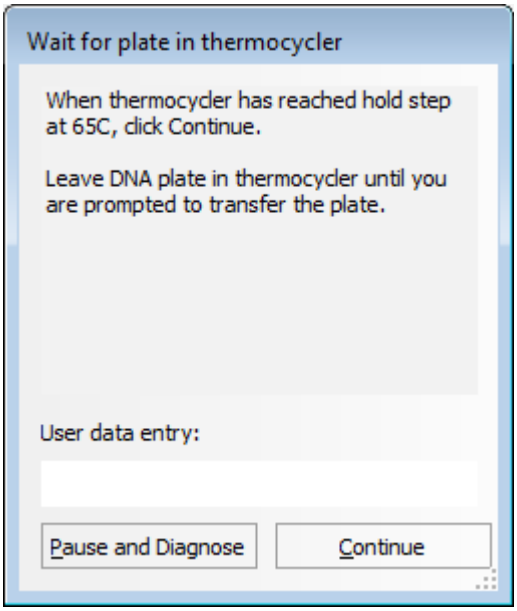
Table 60 Thermal cycler program used for sample denaturation prior to hybridization

Step	Temperature	Time
Step 1	95°C	5 minutes
Step 2	65°C	Hold

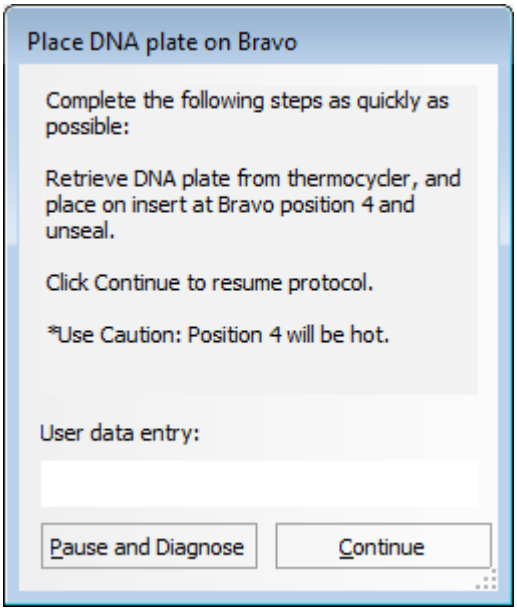
While the sample plate incubates on the thermal cycler, the Agilent NGS Workstation combines aliquots of the Capture Library Master Mix and Hybridization Buffer Master Mix.

CAUTION You must complete step 24 to step 28 quickly, and immediately after being prompted by the VWorks software. It is important that sample temperature remains approximately 65°C during transfers between the Agilent NGS Workstation and thermal cycler.

24 When the workstation has finished aliquoting the Capture Library and Hybridization Buffer master mixes, you will be prompted by VWorks as shown below. When the thermal cycler reaches the 65°C hold step, click **Continue**. Leave the sample plate in the thermal cycler until you are notified to move it.



25 When prompted by VWorks as shown below, quickly remove the sample plate from the thermal cycler, unseal the plate carefully to avoid splashing, and transfer the plate to position 4 of the Bravo deck, seated in the red insert. Click **Continue**.

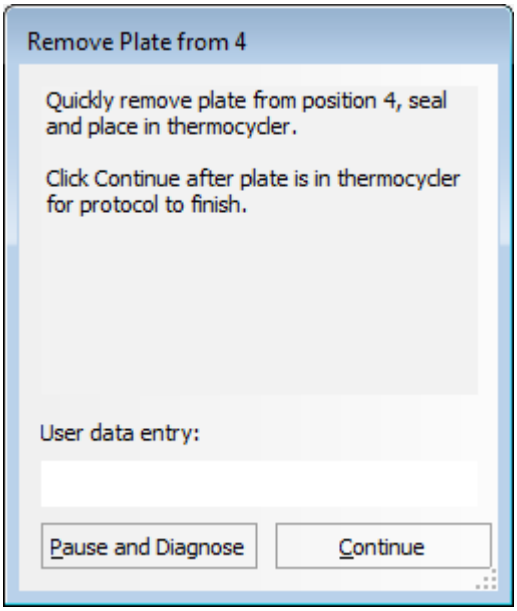


WARNING **Bravo deck position 4 will be hot.**

Use caution when handling components that contact heated deck positions.

The Agilent NGS Workstation transfers the capture library-hybridization buffer mixture to the wells of the PCR plate, containing the mixture of prepped gDNA samples and blocking agents.

26 When prompted by VWorks as shown below, quickly remove the PCR sample plate from Bravo deck position 4, leaving the red insert in place.



27 Seal the sample plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 3.0 sec.

28 Quickly transfer the plate back to the thermal cycler, held at 65°C. After transferring the plate, click **Continue** on the VWorks screen.

29 To finish the VWorks protocol, click **Continue** in the **Unused Tips** and **Empty Tip box** dialogs, and click **Yes** in the **Protocol Complete** dialog.

CAUTION The temperature of the plate in the thermal cycler should be held at 65°C using a heated lid at 105°C. The lid of the thermal cycler is hot and can cause burns. Use caution when working near the lid.

30 Incubate the hybridization mixture in the thermal cycler for 16 or 24 hours at 65°C with a heated lid at 105°C.

NOTE If you are using the SureCycler 8800 thermal cycler for this step, be sure to set up the incubation using a compression mat (see Table 4 on page 15 for ordering information).

Step 3. Capture the hybridized DNA

In this step, the gDNA-capture library hybrids are captured using streptavidin-coated magnetic beads. This step is run immediately after the 16 or 24-hour hybridization period.

This step is automated by the NGS workstation using the SureSelectCapture&Wash_v1.5.1.rst runset, with a total duration of approximately 3 hours. A workstation operator must be present to complete two actions during the runset, at the time points in the table below. The times provided are approximate; each action is completed in response to a VWorks prompt at the appropriate time in the runset.

Table 61

Operator action	Approximate time after run start
Transfer hybridization reactions from thermal cycler to NGS workstation	<5 minutes
Remove PCR plate from red aluminum insert	5-10 minutes

Prepare the workstation

1 Clear the Labware MiniHub and BenchCel of all plates and tip boxes.

2 Gently wipe down the Labware MiniHub, Bravo decks, and BenchCel with a NucleoClean decontamination wipe.

3 Pre-set the temperature of Bravo deck position 4 to 66°C using the Inheco Multi TEC control touchscreen, as described in Setting the Temperature of Bravo Deck Heat Blocks. Bravo deck position 4 corresponds to CPAC 2, position 1 on the Multi TEC control touchscreen.

Prepare the Dynabeads streptavidin beads

4 Vigorously resuspend the Dynabeads MyOne Streptavidin T1 magnetic beads on a vortex mixer. The beads settle during storage.

5 Wash the magnetic beads.

a In a conical vial, combine the components listed in Table 62. The volumes below include the required overage.

Table 62 Components required for magnetic bead washing procedure

Reagent	Volume for 1 Library	Volume for 1 Column	Volume for 2 Columns	Volume for 3 Columns	Volume for 4 Columns	Volume for 6 Columns	Volume for 12 Columns
Dynabeads MyOne Streptavidin T1 bead suspension	50 µL	425 µL	825 µL	1225 µL	1.65 mL	2.5 mL	5.0 mL
SureSelect Binding Buffer	0.2 mL	1.7 mL	3.3 mL	4.9 mL	6.6 mL	10 mL	20 mL
Total Volume	**0.25 mL**	**2.125 mL**	**4.125 mL**	**6.125 mL**	**8.25 mL**	**12.5 mL**	**25 mL**

b Mix the beads on a vortex mixer for 5 seconds.

c Put the vial into a magnetic device, such as the Dynal magnetic separator.

d Remove and discard the supernatant.

e Repeat step a through step d for a total of 3 washes. (Retain the beads after each wash and combine with a fresh aliquot of the indicated volume of SureSelect Binding Buffer.)

6 Resuspend the beads in SureSelect Binding buffer, according to Table 63 below.

Table 63 Preparation of magnetic beads for SureSelect Capture&Wash_v1.5.1.rst

Reagent	Volume for 1 Library	Volume for 1 Column	Volume for 2 Columns	Volume for 3 Columns	Volume for 4 Columns	Volume for 6 Columns	Volume for 12 Columns
SureSelect Binding Buffer	0.2 mL	1.7 mL	3.3 mL	4.9 mL	6.6 mL	10 mL	20 mL

7 Prepare a Nunc DeepWell source plate for the washed streptavidin bead suspension. For each well to be processed, add 200 µL of the homogeneous bead suspension to the Nunc DeepWell plate.

8 Place the streptavidin bead source plate at position 5 of the Bravo deck.

Prepare capture and wash solution source plates

9 Prepare a Thermo Scientific reservoir containing 15 mL of nuclease-free water.

10 Prepare an Eppendorf source plate labeled *Wash #1*. For each well to be processed, add 160 µL of SureSelect Wash Buffer 1.

11 Prepare a Nunc DeepWell source plate labeled *Wash #2*. For each well to be processed, add 1150 µL of SureSelect Wash Buffer 2.

12 Place the silver Nunc DeepWell plate insert on position 6 of the Bravo deck. This insert is required to facilitate heat transfer to DeepWell source plate wells during the Capture&Wash runset.

13 Place the *Wash #2* source plate on the insert at position 6 of the Bravo deck. Make sure the plate is seated properly on the silver DeepWell insert.

Load the Agilent NGS Workstation

14 Load the Labware MiniHub according to Table 64, using the plate orientations shown in Figure 3.

Table 64 Initial MiniHub configuration for SureSelect Capture&Wash_v1.5.1.rst

Vertical Shelf Position	Cassette 1	Cassette 2	Cassette 3	Cassette 4
Shelf 5 (Top)	Empty	Empty	Empty	Empty
Shelf 4	Empty	Empty	Empty	Empty
Shelf 3	Empty Eppendorf plate	Empty	*Wash #1* Eppendorf source plate	Empty
Shelf 2	Empty	Nuclease-free water reservoir	Empty	Empty
Shelf 1 (Bottom)	Empty	Empty	Empty	Empty tip box

15 Load the Bravo deck according to Table 65 (positions 5 and 6 should already be loaded).

Table 65 Initial Bravo deck configuration for SureSelectCapture&Wash_v1.5.1.rst

Location	Content
1	Empty waste reservoir (Axygen 96 Deep Well Plate, square wells)
4	Empty red insert
5	Dynabeads streptavidin bead DeepWell source plate
6	*Wash #2* DeepWell source plate seated on silver Nunc DeepWell insert

16 Load the BenchCel Microplate Handling Workstation according to Table 66.

Table 66 Initial BenchCel configuration for SureSelectCapture&Wash_v1.5.1.rst

No. of Columns Processed	Rack 1	Rack 2	Rack 3	Rack 4
1	2 Tip boxes	Empty	Empty	Empty
2	3 Tip boxes	Empty	Empty	Empty
3	4 Tip boxes	Empty	Empty	Empty
4	5 Tip boxes	Empty	Empty	Empty
6	7 Tip boxes	Empty	Empty	Empty
12	10 Tip boxes	3 Tip boxes	Empty	Empty

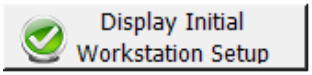
Run VWorks runset SureSelectCapture&Wash_v1.5.1.rst

17 On the SureSelect setup form, under **Select Protocol to Run**, select **SureSelectCapture&Wash_v1.5.1.rst.**

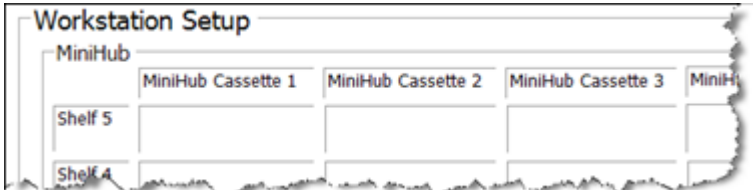
18 Under **Select PCR plate labware for Thermal Cycling**, select the specific type of PCR plate used for hybridization. This plate will be transferred from the thermal cycler to Bravo deck position 4 when prompted by VWorks.

19 Select the number of columns of samples to be processed. Runs must include 1, 2, 3, 4, 6, or 12 columns.

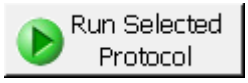
20 Click **Display Initial Workstation Setup**.



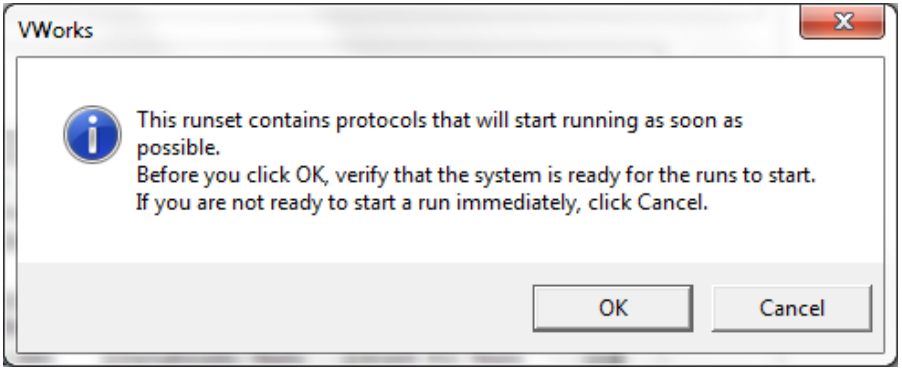
21 Verify that the NGS workstation has been set up as displayed in the Workstation Setup region of the form.



22 When verification is complete, click **Run Selected Protocol**.

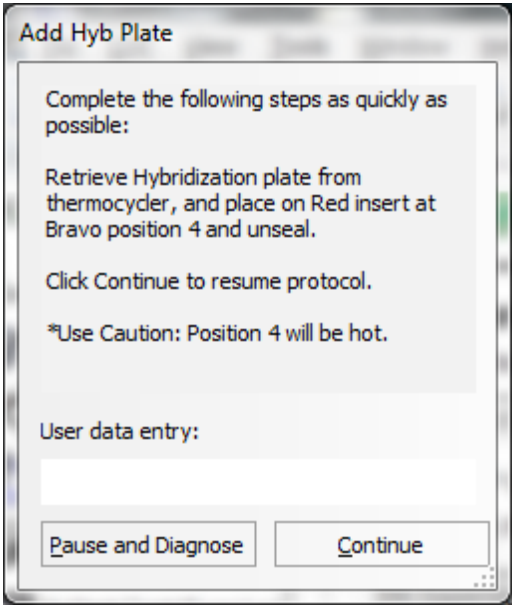


23 When ready to begin the run, click **OK** in the following window. If the temperature of Bravo deck position 4 was not pre-set to 66°C, the runset will pause while position 4 reaches temperature.



CAUTION It is important to complete step 24 quickly and carefully. Transfer the sample plate to the Bravo platform quickly to retain the 65°C sample temperature. Unseal the plate without tilting or jerking the plate to avoid sample splashing. Make sure that the Agilent NGS Workstation is completely prepared, with deck platforms at temperature and all components in place, before you transfer the sample plate to the Bravo deck.

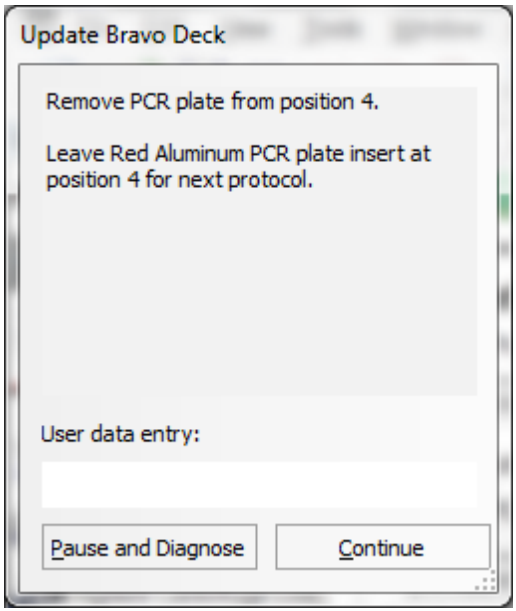
24 When prompted by VWorks as shown below, quickly remove the PCR plate, containing the hybridization reactions held at 65°C, from the thermal cycler. Unseal the plate carefully to avoid splashing, and quickly transfer the plate to position 4 of the Bravo deck, seated in the red insert. Click **Continue** to resume the runset.



WARNING **Bravo deck position 4 will be hot.**

Use caution when handling components that contact heated deck positions.

25 When prompted by VWorks as shown below, remove the PCR plate from position 4 of the Bravo deck, leaving the red aluminum insert in place. When finished, click **Continue** to resume the runset.



The remainder of the SureSelectCapture&Wash_v1.5.1.rst runset takes approximately 2 hours. Once the runset is complete, the captured, bead-bound DNA samples are located in the Eppendorf plate at position 9 of the Bravo deck

When the runset is complete, seal the plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 1.0 sec and store the plate on ice while setting up the next automation protocol.

NOTE Captured DNA is retained on the streptavidin beads during the post-capture amplification step.

6 Indexing

This chapter describes the steps to add index tags by amplification, purify, assess quality and quantity of the libraries, and pool indexed samples for multiplexed sequencing.

Step 1. Amplify the captured libraries to add index tags

In this step, the Agilent NGS Workstation completes the liquid handling steps for PCR-based addition of indexing tags to the SureSelect-enriched DNA samples. After the PCR plate is prepared by the Agilent NGS Workstation, you transfer the plate to a thermal cycler for amplification.

The size of your Capture Library determines the amplification cycle number used for indexing. Plan your experiments for amplification of samples prepared using Capture Libraries of similar sizes on the same plate. See Table 75 on page 141 for cycle number recommendations.

CAUTION

This chapter includes instructions for kits containing two different sets of indexing primers. **Verify that you are referencing the information appropriate for your kit version before you proceed.**

Kits with revised index configuration (typically received December, 2014 or later) include 96 different primers with 8-bp indexes A01 through H12 supplied in Library Prep Kit p/n 5500-0133 in a blue plate format. Refer to Table 86 on page 158 for the nucleotide sequences of the 8-bp indexes.

Kits with original index configuration (typically received before December, 2014) include 16 different primers with 6-bp indexes 1–16 supplied in Library Prep Kit p/n 5500-0075 in format of clear-capped tubes. Refer to Table 91 on page 162 for the nucleotide sequences of the 6-bp indexes.

The 8-bp index primers are provided at a lower concentration than the 6-bp index primers. Make sure you are adding the amount appropriate for your primer type when completing step 4 on page 134.

Assign indexes to DNA samples

Select the appropriate indexing primer for each sample.

Use a different index primer for each sample to be sequenced in the same lane. The number of samples that may be combined per lane depends on the sequencing platform performance and the Capture Library size.

As a guideline, Agilent recommends analyzing 100X amount of sequencing data compared to the Capture Library size for each sample. Specific examples of sequence data requirement recommendations are provided in Table 67. Calculate the number of indexes that can be combined per lane based on these guidelines.

Table 67 Sequencing data requirement guidelines

Capture Library Size	Recommended Amount of Sequencing Data per Sample*
1 kb up to 0.5 Mb	0.1 to 50 Mb
0.5 Mb up to 2.9 Mb	50 to 290 Mb
3 Mb up to 5.9 Mb	300 to 590 Mb
6 Mb up to 11.9 Mb	600 to 1190 Mb
12 Mb up to 24 Mb	1.2 to 2.4 Gb
Human All Exon v5	4 Gb
Human All Exon v5 + UTRs	6 Gb
Human All Exon 50 Mb	5 Gb
Human DNA Kinome	320 Mb
Mouse All Exon	5 Gb

* Agilent recommends analyzing 100X amount of sequencing data compared to the Capture Library size for each sample. Pool samples according to your expected sequencing output.

Prepare the workstation

1 Turn on the ThermoCube, set to 0°C, at position 9 of the Bravo deck. Be sure that the chiller reservoir contains at least 300 mL of 25% ethanol.

2 Clear the Labware MiniHub and BenchCel of plates and tip boxes.

3 Pre-set the temperature of Bravo deck positions 4 and 6 to 4°C using the Inheco Multi TEC control touchscreen, as described in Setting the Temperature of Bravo Deck Heat Blocks. Bravo deck position 4 corresponds to CPAC 2, position 1 and Bravo deck position 6 corresponds to CPAC 2, position 2 on the Multi TEC control touchscreen.

Prepare indexing primers and PCR master mix

CAUTION Do not use amplification enzymes other than Herculase II Fusion DNA Polymerase. Other enzymes have not been validated.

CAUTION To avoid cross-contaminating libraries, set up PCR master mixes in a dedicated clean area or PCR hood with UV sterilization and positive air flow.

4 Prepare the indexing primers in the amplification protocol PCR plate. In each well of the PCR plate, combine the specific indexing primer assigned to the sample well with water, using the amounts of each reagent shown in Table 68. Keep the plate on ice.

Table 68 Preparation of PCR plate with indexing primers

Reagent	8-bp Indexes A01–H12 (obtained from blue plate)	6-bp Indexes 1–16 (obtained from clear-capped tubes)
Nuclease-free water	4.0 µL	8.0 µL
Indexing PCR primer (reverse)	5.0 µL	1.0 µL
Total Volume	**9.0 µL**	**9.0 µL**

5 Prepare the appropriate volume of PCR master mix, according to Table 69. Mix well using a vortex mixer and keep on ice.

Table 69 Preparation of PCR Master Mix for Post-CaptureIndexing_XT_ILM_v1.5.1.pro

SureSelect^{XT} Reagent	Volume for 1 Library	Volume for 1 Column	Volume for 2 Columns	Volume for 3 Columns	Volume for 4 Columns	Volume for 6 Columns	Volume for 12 Columns
Nuclease-free water	14.5 µL	184.9 µL	308.1 µL	431.4 µL	554.6 µL	801.1 µL	1602.3 µL
Herculase II 5X Reaction Buffer*	10.0 µL	127.5 µL	212.5 µL	297.5 µL	382.5 µL	552.5 µL	1105.0 µL
SureSelect Indexing Post-Capture PCR (Forward) Primer†	1.0 µL	12.8 µL	21.3 µL	29.8 µL	38.3 µL	55.3 µL	110.5 µL
dNTP mix*	0.5 µL	6.4 µL	10.6 µL	14.9 µL	19.1 µL	27.6 µL	55.3 µL
Herculase II polymerase	1.0 µL	12.8 µL	21.3 µL	29.8 µL	38.3 µL	55.3 µL	110.5 µL
Total Volume	**27.0 µL**	**344.3 µL**	**573.8 µL**	**803.3 µL**	**1032.8 µL**	**1491.8 µL**	**2983.5 µL**

* Included with the Herculase II Fusion DNA Polymerase. *Do not use the buffer or dNTP mix from any other kit.*

† Included in SureSelect XT Automation ILM Module Box 2.

6 Using the same Nunc DeepWell master mix source plate that was used for the Hybridization_v1.5.1.pro protocol, add the volume of PCR master mix indicated in Table 70 to all wells of column 4 of the master mix source plate. The final configuration of the master mix source plate is shown in Figure 19.

Table 70 Preparation of the Master Mix Source Plate for Post-CaptureIndexing_XT_ILM_v1.5.1.pro

Master Mix Solution	Position on Source Plate	Volume of Master Mix added per Well of Nunc Deep Well Source Plate					
		1-Column Runs	2-Column Runs	3-Column Runs	4-Column Runs	6-Column Runs	12-Column Runs
PCR Master Mix	Column 4 (A4-H4)	39.7 µL	68.3 µL	97.0 µL	125.7 µL	183.1 µL	369.6 µL

NOTE If you are using a new DeepWell plate for the post-capture PCR source plate (for example, when amplifying the second half of the captured DNA sample), leave columns 1 to 3 empty and add the PCR Master Mix to column 4 of the new plate.

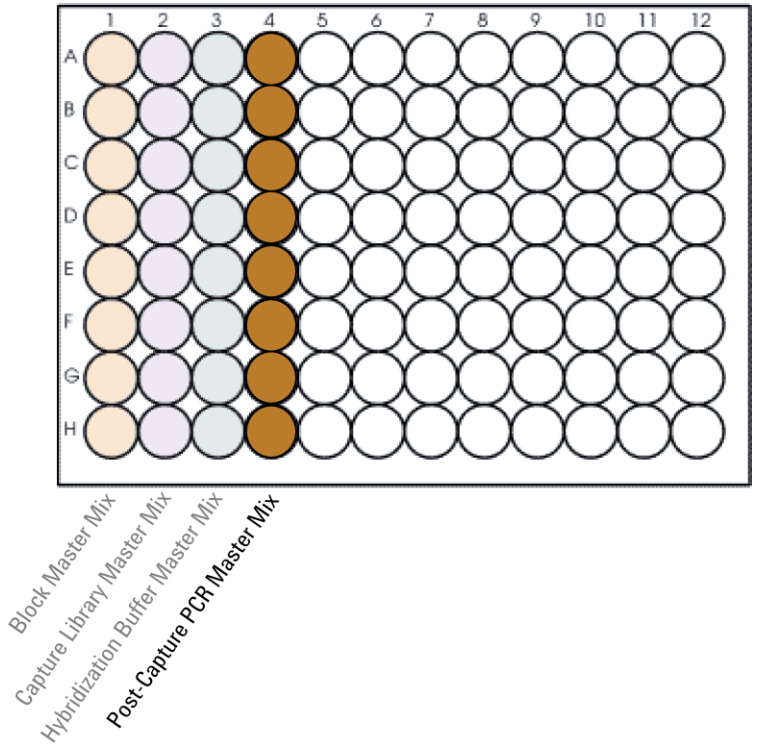


Figure 19 Configuration of the master mix source plate for Post-CaptureIndexing_XT_ILM_v1.5.1.pro. Columns 1–3 were used to dispense master mixes for the Hybridization_v1.5.1.pro protocol.

7 Seal the master mix source plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 1.0 sec.

8 Centrifuge the plate for 30 seconds to drive the well contents off the walls and plate seal and to eliminate any bubbles.

Load the Agilent NGS Workstation

9 Load the Labware MiniHub according to Table 71, using the plate orientations shown in Figure 3.

Table 71 Initial MiniHub configuration for Post-CaptureIndexing_XT_ILM_v1.5.1.pro

Vertical Shelf Position	Cassette 1	Cassette 2	Cassette 3	Cassette 4
Shelf 5 (Top)	Empty	Empty	Empty	Empty
Shelf 4	Empty	Empty	Empty	Empty
Shelf 3	Empty	Empty	Empty	Empty
Shelf 2	Empty tip box	Empty	Empty	Empty
Shelf 1 (Bottom)	New tip box	Empty	Empty	Empty tip box

10 Load the Bravo deck according to Table 72.

Table 72 Initial Bravo deck configuration for Post-CaptureIndexing_XT_ILM_v1.5.1.pro

Location	Content
4	Captured DNA bead suspensions in Eppendorf twin.tec plate (unsealed)
6	Diluted indexing primers in PCR plate seated in red insert (PCR plate type must be specified on setup form under step 2)
9	Master mix plate containing PCR Master Mix in Column 4 (unsealed) seated on silver Nunc DeepWell insert

11 Load the BenchCel Microplate Handling Workstation according to Table 73.

Table 73 Initial BenchCel configuration for Post-CaptureIndexing_XT_ILM_v1.5.1.pro

No. of Columns Processed	Rack 1	Rack 2	Rack 3	Rack 4
1	1 Tip box	Empty	Empty	Empty
2	1 Tip box	Empty	Empty	Empty
3	1 Tip box	Empty	Empty	Empty
4	1 Tip box	Empty	Empty	Empty
6	1 Tip box	Empty	Empty	Empty
12	1 Tip box	Empty	Empty	Empty

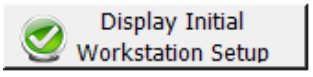
Run VWorks protocol Post-CaptureIndexing_XT_ILM_v1.5.1.pro

12 On the SureSelect setup form, under **Select Protocol to Run**, select **Post-CaptureIndexing_XT_ILM_v1.5.1.pro.**

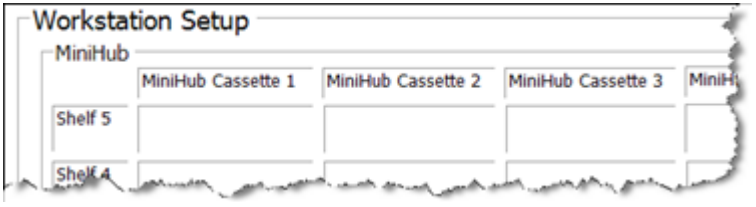
13 Under **Select PCR plate labware for Thermal Cycling**, select the specific type of PCR plate used at position 6 of the Bravo deck.

14 Select the number of columns of samples to be processed. Runs must include 1, 2, 3, 4, 6, or 12 columns.

15 Click **Display Initial Workstation Setup**.



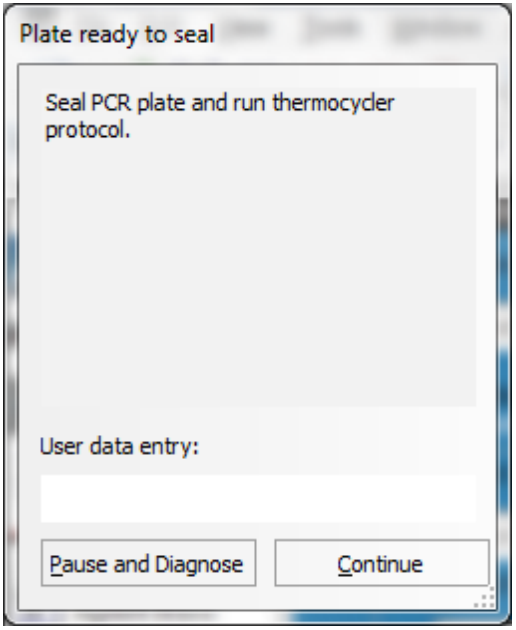
16 Verify that the NGS workstation has been set up as displayed in the Workstation Setup region of the form.



17 When verification is complete, click **Run Selected Protocol**.

Running the Post-CaptureIndexing_XT_ILM_v1.5.1.pro protocol takes approximately 15 minutes. Once complete, the PCR-ready samples, containing captured DNA and PCR master mix, are located in the PCR plate at position 6 of the Bravo deck. The Eppendorf plate containing the remaining bead-bound captured DNA samples, which may be stored for future use at 4°C overnight, or at –20°C for longer-term storage, is located at position 4 of the Bravo deck.

18 When you see the following prompt, remove the PCR plate from position 6 of the Bravo deck and seal the plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 3.0 seconds.



19 Centrifuge the plate for 30 seconds to drive the well contents off the walls and plate seal and to eliminate air bubbles.

20 Transfer the PCR plate to a thermal cycler and run the PCR amplification program shown in Table 74 using the cycle number specified in Table 75.

Table 74 Post-Capture PCR cycling program

Segment	Number of Cycles	Temperature	Time
1	1	98°C	2 minutes
2	10 to 16 Cycles see Table 75 for recommendations based on Capture Library size	98°C	30 seconds
		57°C	30 seconds
		72°C	1 minute
3	1	72°C	10 minutes
4	1	4°C	Hold

Table 75 Recommended cycle number based on Capture Library size

Size of Capture Library	Cycles
<0.5 Mb	16 cycles
0.5 to 1.49 Mb	14 cycles
> 1.5 Mb	12 cycles
All Exon and Exome libraries	10 to 12 cycles
OneSeq libraries (all designs)	10 cycles

NOTE

Amplify the captured DNA using a minimal number of PCR cycles. If yield is too low or non-specific high molecular weight products are observed, adjust the number of cycles accordingly with the remaining captured DNA template.

Step 2. Purify the amplified indexed libraries using Agencourt AMPure XP beads

In this step, the Agilent NGS Workstation transfers AMPure XP beads to the indexed DNA sample plate and then collects and washes the bead-bound DNA.

Prepare the workstation and reagents

1. Clear the Labware MiniHub and BenchCel of all plates and tip boxes.
2. Gently wipe down the Labware MiniHub, Bravo decks, and BenchCel with a Nucleoclean decontamination wipe.
3. Turn on the ThermoCube, set to 0°C, at position 9 of the Bravo deck. Be sure that the chiller reservoir contains at least 300 mL of 25% ethanol.
4. Let the AMPure XP beads come to room temperature for at least 30 minutes. *Do not freeze the beads at any time.*
5. Mix the bead suspension well so that the reagent appears homogeneous and consistent in color.
6. Prepare a Nunc DeepWell source plate containing AMPure XP beads. For each well to be processed, add 95 µL of homogeneous AMPure XP beads per well to the Nunc DeepWell plate.
7. Prepare a Thermo Scientific reservoir containing 15 mL of nuclease-free water.
8. Prepare a separate Thermo Scientific reservoir containing 45 mL of freshly-prepared 70% ethanol.

9 Load the Labware MiniHub according to Table 76, using the plate orientations shown in Figure 3.

Table 76 Initial MiniHub configuration for AMPureXP_XT_ILM_v1.5.1.pro:Post-Capture PCR

Vertical Shelf Position	Cassette 1	Cassette 2	Cassette 3	Cassette 4
Shelf 5 (Top)	Empty Nunc DeepWell plate	Empty	Empty	Empty
Shelf 4	Empty	Empty	Empty	Empty
Shelf 3	Empty	Empty Eppendorf Plate	Empty	Empty
Shelf 2	Empty	Nuclease-free water reservoir from step 7	AMPure XP beads in Nunc DeepWell plate from step 6	Empty
Shelf 1 (Bottom)	Empty	70% ethanol reservoir from step 8	Empty	Empty tip box

10 Load the Bravo deck according to Table 77.

Table 77 Initial Bravo deck configuration for AMPureXP_XT_ILM_v1.5.1.pro:Post-Capture PCR

Location	Content
1	Empty waste reservoir (Axygen 96 Deep Well Plate, square wells)
9	Indexed library samples in PCR plate seated in red insert (PCR plate type must be specified on setup form under step 2)

11 Load the BenchCel Microplate Handling Workstation according to Table 78.

Table 78 Initial BenchCel configuration for AMPureXP_XT_ILM_v1.5.1.pro:Post-Capture PCR

No. of Columns Processed	Rack 1	Rack 2	Rack 3	Rack 4
1	1 Tip box	Empty	Empty	Empty
2	1 Tip box	Empty	Empty	Empty
3	2 Tip boxes	Empty	Empty	Empty
4	2 Tip boxes	Empty	Empty	Empty
6	3 Tip boxes	Empty	Empty	Empty
12	6 Tip boxes	Empty	Empty	Empty

Run VWorks protocol AMPureXP_XT_ILM_v1.5.1.pro:Post-Capture PCR

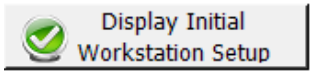
12 On the SureSelect setup form, under **Select Protocol to Run**, select **AMPureXP_XT_ILM_v1.5.1.pro:Post-Capture PCR**.

NOTE AMPureXP purification protocols are used during multiple steps of the SureSelect automation workflow. Be sure to select the correct workflow step when initiating the automation protocol.

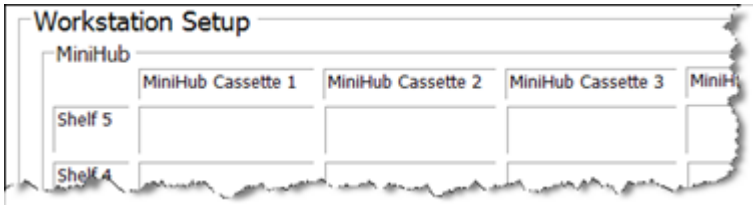
13 Under **Select PCR plate labware for Thermal Cycling**, select the specific type of PCR plate containing the indexed libraries at position 9.

14 Select the number of columns of samples to be processed. Runs must include 1, 2, 3, 4, 6, or 12 columns.

15 Click **Display Initial Workstation Setup**.



16 Verify that the NGS workstation has been set up as displayed in the Workstation Setup region of the form.



17 When verification is complete, click **Run Selected Protocol**.

The purification protocol takes approximately 45 minutes. When complete, the amplified DNA samples are in the Eppendorf plate located on Bravo deck position 7.

Step 3. Assess indexed DNA quality

Option 1: Analysis using the 2100 Bioanalyzer and High Sensitivity DNA Assay

1 Set up the 2100 Bioanalyzer as instructed in the *High Sensitivity DNA Kit Guide* at www.genomics.agilent.com.

NOTE Version B.02.07 or higher of the Agilent 2100 Expert Software is required for High Sensitivity DNA Assay Kit runs.

2 Seal the sample plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 1.0 sec.

3 Vortex the plate to mix samples in each well, then centrifuge the plate for 30 seconds to drive the well contents off the walls and plate seal.

4 Prepare the chip, samples and ladder as instructed in the reagent kit guide, using 1 µL of each sample for the analysis.

NOTE For some samples, Bioanalyzer results are improved by diluting 1 µL of the sample in 9 µL of 10 mM Tris, 1 mM EDTA prior to analysis. Be sure to mix well by vortexing at 2000 rpm on the IKA vortex supplied with the Bioanalyzer before analyzing the diluted samples.

5 Load the prepared chip into the 2100 Bioanalyzer and start the run within five minutes after preparation.

6 Verify that the electropherogram shows the peak of DNA fragment size positioned between 250 to 350 bp. A sample electropherogram is shown in Figure 20.

Stopping Point If you do not continue to the next step, seal the plate and store at 4°C overnight or at –20°C for prolonged storage.

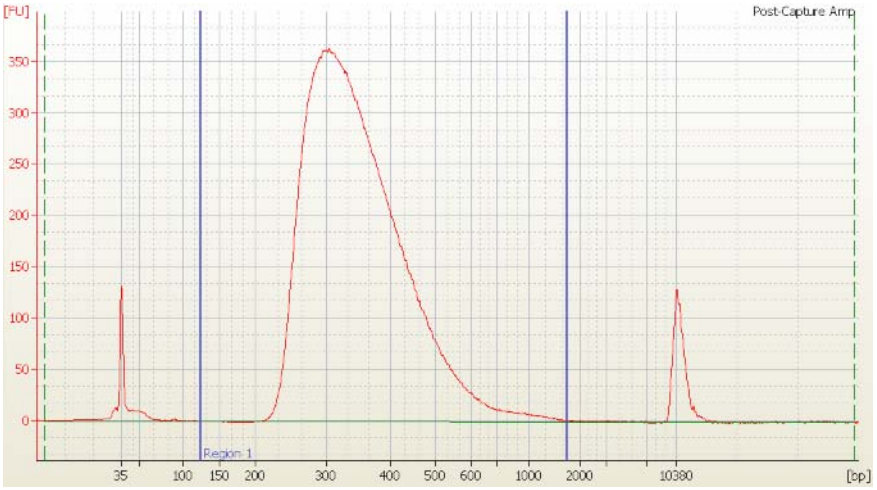


Figure 20 Analysis of indexed DNA using the High Sensitivity DNA Assay.

Option 2: Analysis using the Agilent 2200 TapeStation and High Sensitivity D1000 ScreenTape

Use a High Sensitivity D1000 ScreenTape (p/n 5067-5584) and reagent kit (p/n 5067-5585) to analyze the indexed DNA. For more information to do this step, see the *Agilent 2200 TapeStation User Manual* at www.genomics.agilent.com.

1 Seal the DNA sample plate using the PlateLoc Thermal Microplate Sealer, with sealing settings of 165°C and 1.0 sec.

2 Vortex the plate to mix samples in each well, then centrifuge the plate for 30 seconds to drive the well contents off the walls and plate seal.

3 Prepare the TapeStation samples as instructed in the *Agilent 2200 TapeStation User Manual*. Use 2 µL of each indexed DNA sample diluted with 2 µL of High Sensitivity D1000 sample buffer for the analysis.

CAUTION Make sure that you thoroughly mix the combined DNA and High Sensitivity D1000 sample buffer on a vortex mixer for 5 seconds for accurate quantitation.

4 Load the sample plate or tube strips from step 3, the High Sensitivity D1000 ScreenTape, and loading tips into the 2200 TapeStation as instructed in the *Agilent 2200 TapeStation User Manual*. Start the run.

5 Verify that the electropherogram shows the peak of DNA fragment size positioned between 250 to 350 bp. A sample electropherogram is shown in Figure 21.

Stopping Point If you do not continue to the next step, seal the indexed DNA sample plate and store at 4°C overnight or at –20°C for prolonged storage.

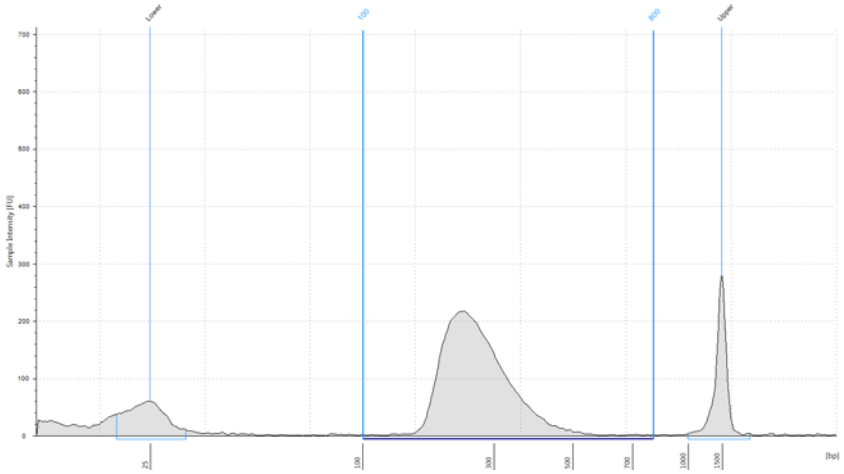


Figure 21 Analysis of indexed DNA using the 2200 TapeStation.

Step 4. Quantify each index-tagged library by QPCR

Refer to the protocol that is included with the Agilent QPCR NGS Library Quantification Kit (p/n G4880A) for more details to do this step.

1. Use the Agilent QPCR NGS Library Quantification Kit (for Illumina) to determine the concentration of each index-tagged captured library.
2. Prepare a standard curve using the quantification standard included in the kit, according to the instructions provided in the user guide.
3. Dilute each index-tagged captured library such that it falls within the range of the standard curve.

   Typically this corresponds to approximately a 1:1000 to 1:10,000 dilution of the captured DNA.
4. Prepare the QPCR master mix with Illumina adaptor-specific PCR primers according to instructions provided in the kit.
5. Add an aliquot of the master mix to PCR tubes and add template.
6. On a QPCR system, such as the Mx3005p, run the thermal profile outlined in the QPCR NGS Library Quantification kit user guide. Use the SYBR Green instrument setting.
7. Use the standard curve to determine the concentration of each unknown index-tagged library, in nM.

   The concentration will be used to accurately pool samples for multiplexed sequencing.

NOTE In most cases, the cycle numbers in Table 75 will produce an adequate yield for sequencing without introducing bias or non-specific products. If yield is too low or non-specific products are observed, adjust the number of cycles accordingly with the remaining captured DNA template.

Step 5. Pool samples for Multiplexed Sequencing

1 Combine the libraries such that each index-tagged sample is present in equimolar amounts in the pool. For each library, use the formula below to determine the amount of indexed sample to use.

$$\text{Volume of Index} = \frac{V(f) \times C(f)}{\# \times C(i)}$$

where *V(f)* is the final desired volume of the pool,

C(f) is the desired final concentration of all the DNA in the pool

is the number of indexes, and

C(i) is the initial concentration of each indexed sample.

Table 79 shows an example of the amount of 4 index-tagged samples (of different concentrations) and Low TE needed for a final volume of 20 µL at 10 nM.

Table 79 Example of index volume calculation for a total volume of 20 µL

Component	V(f)	C(i)	C(f)	#	Volume to use (µL)
Sample 1	20 µL	20 nM	10 nM	4	2.5
Sample 2	20 µL	10 nM	10 nM	4	5
Sample 3	20 µL	17 nM	10 nM	4	2.9
Sample 4	20 µL	25 nM	10 nM	4	2
Low TE					7.6

2 Adjust the final volume of the pooled library to the desired final concentration.

- If the final volume of the combined index-tagged samples is less than the desired final volume, V(f), add Low TE to bring the volume to the desired level.
- If the final volume of the combined index-tagged samples is greater than the final desired volume, V(f), lyophilize and reconstitute to the desired volume.

3 If you store the library before sequencing, add Tween 20 to 0.1% v/v and store at -20°C short term.

Guidelines for sequencing sample preparation and run setup

Use the appropriate Illumina Paired-End Cluster Generation Kit to do cluster amplification.

Refer to the instructions that are included with the Illumina Paired-End Cluster Generation Kit. The optimal seeding concentration for SureSelect^{XT} libraries is 6 to 8 pM, depending on the desired output and data quality.

Sequencing run setup guidelines for 8-bp indexes

For libraries prepared using kits with 8-bp indexes, sequencing runs must be set up to perform an 8-bp index read. For the HiSeq platform, use the *Cycles* settings shown in Table 80. Cycle number settings can be specified on the *Run Configuration* screen of the instrument control software interface after choosing *Custom* from the index type selection buttons.

For complete 8-bp index sequence information, see Table 86 on page 158.

Table 80 HiSeq platform Run Configuration screen Cycle Number settings*

Run Segment	Cycle Number
Read 1	100
Index 1 (i7)	9
Index 2 (i5)	0
Read 2	100

* Settings apply to v3.0 SBS chemistry.

7 Reference

This chapter contains reference information, including component kit contents and index sequences.

CAUTION This chapter contains two sets of index sequence and kit content information. The first section covers kits with 8-bp indexes supplied in Library Prep Kit p/n 5500-0133 (typically received December, 2014 or later). The second section covers kits with 6-bp indexes supplied in Library Prep Kit 5500-0075 (typically received before December, 2014). **Verify that you are referencing the information appropriate for your kit version before you proceed.**

Reference Information for Kits with Revised Index Configuration (8-bp indexes with indexing primers in blue plate)

Use the reference information in this section if your kit includes Library Prep Kit p/n 5500-0133. If your kit does not include this component kit, see page 159 for kit content and indexing primer information.

Kit Contents

Each SureSelect^{XT} Automation Reagent Kit contains the following component kits:

Table 81 SureSelect^{XT} Automation Reagent Kit Contents-Revised Index Configuration

Product	Storage Condition	96 Reactions	480 Reactions
SureSelect XT Library Prep Kit ILM	–20°C	5500-0133	5 x 5500-0133
SureSelect Target Enrichment Box 1	Room Temperature	5190-8646	5 x 5190-8646
SureSelect XT Automation ILM Module Box 2	–20°C	5190-3730	5190-3732

NOTE SureSelect capture libraries and reagents must be used within one year of receipt.

The contents of each of the component kits listed in Table 81 are described in the tables below.

Table 82 SureSelect XT Library Prep Kit ILM Content-Revised Index Configuration

Kit Component	Format
10X End Repair Buffer	tube with clear cap
10X Klenow Polymerase Buffer	tube with blue cap
5X T4 DNA Ligase Buffer	tube with green cap
T4 DNA Ligase	tube with red cap
Exo(–) Klenow	tube with red cap
T4 DNA Polymerase	tube with purple cap
Klenow DNA Polymerase	tube with yellow cap
T4 Polynucleotide Kinase	tube with orange cap
dATP	tube with green cap
dNTP Mix	tube with green cap
SureSelect Adaptor Oligo Mix	tube with brown cap
SureSelect Primer (forward primer)	tube with brown cap
SureSelect^{XT} Indexes, 8 bp reverse primers*	SureSelect 8bp Indexes A01 through H12, provided in blue 96-well plate†

* See Table 86 on page 158 for index sequences.

† See Table 85 on page 157 for a plate map.

Table 83 SureSelect Target Enrichment-Box 1 Content

Kit Component	Format
SureSelect Hyb 1	tube with orange cap
SureSelect Hyb 2	tube with red cap
SureSelect Hyb 4	tube with black cap
SureSelect Binding Buffer	bottle
SureSelect Wash Buffer 1	bottle
SureSelect Wash Buffer 2	bottle

Table 84 SureSelect XT Automation ILM Module Box 2 Content

Kit Component	96 Reactions	480 Reactions
SureSelect Hyb 3	tube with yellow cap	bottle
SureSelect Indexing Block 1	tube with green cap	tube with green cap
SureSelect Block 2	tube with blue cap	tube with blue cap
SureSelect ILM Indexing Block 3	tube with brown cap	tube with brown cap
SureSelect RNase Block	tube with purple cap	tube with purple cap
SureSelect Indexing Pre-Capture PCR (Reverse) Primer	tube with clear cap	tube with clear cap
SureSelect Indexing Post-Capture PCR (Forward) Primer	tube with orange cap	tube with orange cap

Table 85 Plate map for SureSelect 8bp Indexes A01 through H12, provided in blue plate in Library Prep kit p/n 5500-0133

	1	2	3	4	5	6	7	8	9	10	11	12
A	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	A12
B	B01	B02	B03	B04	B05	B06	B07	B08	B09	B10	B11	B12
C	C01	C02	C03	C04	C05	C06	C07	C08	C09	C10	C11	C12
D	D01	D02	D03	D04	D05	D06	D07	D08	D09	D10	D11	D12
E	E01	E02	E03	E04	E05	E06	E07	E08	E09	E10	E11	E12
F	F01	F02	F03	F04	F05	F06	F07	F08	F09	F10	F11	F12
G	G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12
H	H01	H02	H03	H04	H05	H06	H07	H08	H09	H10	H11	H12

Nucleotide Sequences of SureSelect^{XT} 8-bp Indexes

Each index is 8 nt in length. See page 152 for sequencing run setup requirements for sequencing libraries using 8-bp indexes.

Table 86 SureSelect^{XT} Indexes, for indexing primers provided in blue 96-well plate

Index	Sequence	Index	Sequence	Index	Sequence	Index	Sequence
A01	ATGCCTAA	A04	AACTCACC	A07	ACGTATCA	A10	AATGTTGC
B01	GAATCTGA	B04	GCTAACGA	B07	GTCTGTCA	B10	TGAAGAGA
C01	AACGTGAT	C04	CAGATCTG	C07	CTAAGGTC	C10	AGATCGCA
D01	CACTTCGA	D04	ATCCTGTA	D07	CGACACAC	D10	AAGAGATC
E01	GCCAAGAC	E04	CTGTAGCC	E07	CCGTGAGA	E10	CAACCACA
F01	GACTAGTA	F04	GCTCGGTA	F07	GTGTTCTA	F10	TGGAACAA
G01	ATTGGCTC	G04	ACACGACC	G07	CAATGGAA	G10	CCTCTATC
H01	GATGAATC	H04	AGTCACTA	H07	AGCACCTC	H10	ACAGATTC
A02	AGCAGGAA	A05	AACGCTTA	A08	CAGCGTTA	A11	CCAGTTCA
B02	GAGCTGAA	B05	GGAGAACA	B08	TAGGATGA	B11	TGGCTTCA
C02	AAACATCG	C05	CATCAAGT	C08	AGTGGTCA	C11	CGACTGGA
D02	GAGTTAGC	D05	AAGGTACA	D08	ACAGCAGA	D11	CAAGACTA
E02	CGAACTTA	E05	CGCTGATC	E08	CATACCAA	E11	CCTCCTGA
F02	GATAGACA	F05	GGTGCGAA	F08	TATCAGCA	F11	TGGTGGTA
G02	AAGGACAC	G05	CCTAATCC	G08	ATAGCGAC	G11	AACAACCA
H02	GACAGTGC	H05	CTGAGCCA	H08	ACGCTCGA	H11	AATCCGTC
A03	ATCATTCC	A06	AGCCATGC	A09	CTCAATGA	A12	CAAGGAGC
B03	GCCACATA	B06	GTACGCAA	B09	TCCGTCTA	B12	TTCACGCA
C03	ACCACTGT	C06	AGTACAAG	C09	AGGCTAAC	C12	CACCTTAC
D03	CTGGCATA	D06	ACATTGGC	D09	CCATCCTC	D12	AAGACGGA
E03	ACCTCCAA	E06	ATTGAGGA	E09	AGATGTAC	E12	ACACAGAA
F03	GCGAGTAA	F06	GTCGTAGA	F09	TCTTCACA	F12	GAACAGGC
G03	ACTATGCA	G06	AGAGTCAA	G09	CCGAAGTA	G12	AACCGAGA
H03	CGGATTGC	H06	CCGACAAC	H09	CGCATACA	H12	ACAAGCTA

Reference Information for Kits with Original Index Configuration (6-bp indexes with indexing primers in 16 tubes)

Use the reference information in this section if your kit includes Library Prep Kit p/n 5500-0075. If your kit does not include this component kit, see page 154 for kit content and indexing primer information.

Kit Contents

Each SureSelect^{XT} Automation Reagent Kit contains the following component kits:

Table 87 SureSelect XT Automation Reagent Kit Contents-Original Index Configuration

Product	Storage Condition	96 Reactions	480 Reactions
SureSelect XT Library Prep Kit ILM	–20°C	5500-0075	5 x 5500-0075
SureSelect Target Enrichment Box 1	Room Temperature	5190-4394	5190-4395
SureSelect XT Automation ILM Module Box 2	–20°C	5190-3730	5190-3732

NOTE

SureSelect capture libraries and reagents must be used within one year of receipt.

The contents of each of the component kits listed in Table 87 are described in the tables below.

Table 88 SureSelect XT Library Prep Kit ILM Content-Original Index Configuration

Kit Component	Format
10X End Repair Buffer	tube with clear cap
10X Klenow Polymerase Buffer	tube with blue cap
5X T4 DNA Ligase Buffer	tube with green cap
T4 DNA Ligase	tube with red cap
Exo(–) Klenow	tube with red cap
T4 DNA Polymerase	tube with purple cap
Klenow DNA Polymerase	tube with yellow cap
T4 Polynucleotide Kinase	tube with orange cap
dATP	tube with green cap
dNTP Mix	tube with green cap
SureSelect Adaptor Oligo Mix	tube with brown cap
SureSelect Primer (forward primer)	tube with brown cap
PCR Primer Index 1 through Index 16 (reverse primers containing 6-bp index sequences)*	16 tubes with clear caps

* See Table 91 on page 162 for index sequences.

Table 89 SureSelect Target Enrichment Box 1 Content

Kit Component	96 Reactions	480 Reactions
SureSelect Hyb 1	tube with orange cap	bottle
SureSelect Hyb 2	tube with red cap	tube with red cap
SureSelect Hyb 4	tube with black cap	bottle
SureSelect Binding Buffer	bottle	bottle
SureSelect Wash Buffer 1	bottle	bottle
SureSelect Wash Buffer 2	bottle	bottle
SureSelect Elution Buffer*	bottle	bottle
SureSelect Neutralization Buffer*	bottle	bottle

* The provided SureSelect Elution Buffer and Neutralization Buffer are not used in the workflow described in this manual.

Table 90 SureSelect XT Automation ILM Module Box 2 Content

Kit Component	96 Reactions	480 Reactions
SureSelect Hyb 3	tube with yellow cap	bottle
SureSelect Indexing Block 1	tube with green cap	tube with green cap
SureSelect Block 2	tube with blue cap	tube with blue cap
SureSelect ILM Indexing Block 3	tube with brown cap	tube with brown cap
SureSelect RNase Block	tube with purple cap	tube with purple cap
SureSelect Indexing Pre-Capture PCR (Reverse) Primer	tube with clear cap	tube with clear cap
SureSelect Indexing Post-Capture PCR (Forward) Primer	tube with orange cap	tube with orange cap

Nucleotide Sequences of SureSelect^{XT} 6-bp Indexes

Refer to the sequence information in Table 91 only if your kit includes Library Prep kit p/n 5500-0075, with indexing primers provided in 16 clear-capped tubes (original kit configuration).

Table 91 SureSelect^{XT} Indexes 1-16

Index Number	Sequence
1	ATCACG
2	CGATGT
3	TTAGGC
4	TGACCA
5	ACAGTG
6	GCCAAT
7	CAGATC
8	ACTTGA
9	GATCAG
10	TAGCTT
11	GGCTAC
12	CTTGTA
13	AAACAT
14	CAAAAG
15	GAAACC
16	AAAGCA

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In This Book

This guide contains information to run the SureSelect^{XT} Automated Target Enrichment for Illumina Paired-End Multiplexed Sequencing protocol using a SureSelect^{XT} Automated Reagent Kit (HSQ or MSQ) and automation protocols provided with the Agilent NGS Workstation Option B.

Version G.2, April 2015

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Agilent Technologies

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High-Throughput Sequencing Facility: Next-Generation Sequencing Production companion to the Illumina HiSeq 2500 Rapid Run System

Step I. Denature and Dilute Library Pools for On-Instrument Clustering

For successful sequencing, each pool of indexed exome libraries should be denatured and diluted to proper loading concentration.

Prior to denaturation, pools are verified for appropriate size and concentration using the Qubit dsDNA High Sensitivity Assay and the BioRad Experion Automated Electrophoresis System. The Qubit uses fluorometry to verify the quality and concentration of the pooled libraries. The Experion uses electrophoresis to verify the quality and size of the pooled libraries.

Qubit Fluorometer Protocol

Reagent/Sample Prep:

1) Remove Qubit standard reagents (0 ng/µL and 10 ng/µL) from the 4° fridge. Pull Qubit reagent kit containing buffer + dye from the drawer but keep in a dark place until ready for use (dye is light sensitive). Briefly vortex and spin down reagents and pools.

2) Prepare pools and reagents as follows (for X number of pools):
   a) For X number of pools, add 4. This accounts for each standard, control, and overage (i.e. 8 pools + 4 standards = 12 total pools).
   b) To make the Qubit working stock mix, perform protocol as follows:
      i) X total pools * 199 = _____ buffer.
      ii) X total pools * 1 = _____ dye.
   c) Calculate amount of working stock mix and sample to equal 200 µL for X number of pools and 2 standards.
      i) 190 µL mix + 10 µL standard = 200 µL.
      ii) 198 µL mix + 2 µL sample = 200 µL.

Qubit Results:

3) Vortex mixture of sample and working stock for 2-3 seconds, then incubate at room temperature for 2 minutes.
4) Take samples and standards to Qubit machine and select 'DNA Concentration – High Sensitivity.'
5) Select "New Calibration."
6) Run 0 ng/µL standard to calibrate machine, then run 10 ng/µL to finish calibration.
7) Run control to verify calibration.
8) Run each sample in order, recording each reading.
9) Run 10 ng/µL standard.
10) Run 0 ng/mL followed by 100 ng/mL and record results.
11) Turn off Qubit machine.

Experion Electrophoresis Protocol

Reagent Prep:

1) Remove Experion DNA 1K Analysis Kit reagents from the 4° fridge. Allow them to equilibrate to room temperature for 15 minutes, and then briefly vortex and spin down reagents and pools.

2) Prepare gel-stain solution by adding 12.5 µl DNA stain (blue cap) to a tube of 250 µl DNA 1K gel (green cap). Vortex the GS for 10 sec, and then spin it down briefly in a microcentrifuge.
3) Transfer the gel-stain solution to a spin filter, and label and date the tube.
4) Centrifuge the spin filter for 15 min at 2,400 × g. Inspect the tubes to ensure all of the gel has passed through the filters, and then discard the filters. Blue staining of the filter membrane is normal.
5) Wrap the tube of GS in aluminum foil to protect the stain from light.
6) A gel-stain solution preparation is sufficient for use with at least four DNA chips. Use it within 1 month. Keep prepared gel-stain solution at room temperature and covered with foil until ready for use. If the GS was already prepared, equilibrate it as detailed above.

Sample and Ladder Prep (for X number of pools):

7) Pipet 5 µl DNA loading buffer (yellow cap) into X number of tubes for X number of pools and one ladder.
8) Pipet 1 µl DNA ladder into the tube labeled L with loading buffer.
9) Pipet 1 µl pools into tubes with loading buffer and spin them down briefly.

Prime the Chip:

10) Pipet 9 µl gel-stain solution into the highlighted well labeled GS on the chip. Insert the pipet tip vertically and to the bottom of the well when dispensing. Dispense slowly to the first stop on the pipet, and do not expel air at the end of the pipetting step.
11) On the priming station, set the pressure setting to C and the time setting to 3.
12) Open the Experion priming station and place the chip on the chip platform.
13) Close the priming station by pressing down on the lid. The lid should snap closed.
14) Press Start. Priming requires 60 sec. An audible signal and “Ready” message indicate that priming is complete.
15) Open the priming station and remove the chip.

Load the Chip:

16) Pipet 9 µl gel-stain solution into the 3 other wells labeled GS on the chip.
17) Pipet 6 µl DNA loading buffer and pool mix into each sample well.
18) Pipet 6 µl DNA loading buffer and ladder into the well labeled L.
19) Pipet 6 µl water for blanks into sample wells.
20) Inspect all wells for bubbles by holding the chip above a light-colored background and looking through the wells. Dislodge any bubbles at the bottom of a well with a clean pipet tip or by removing and reloading the solution.
21) Place the loaded chip into the Experion electrophoresis station and start the run within 5 min from when first primed.

Experion Results:

22) In the Experion software toolbar, click New Run. In the New Run screen, from the Assay pull-down list, select DNA 1K.
23) Enter a name for the run in the Run Prefix field. Pool names can be entered at this point or after the run. Then click Start Run.
24) After the run (~30 minutes for a full chip), evaluate the run and record the sizes of each pool.

Molarity of the pools are then calculated, and once the pools are verified to be good quality (peak size ~250 - 450bp; molarity >2nM), the pools proceed to denaturation.

Denaturation of Library Pools

1) Adjust the concentration of prepared pools to 2nM using Tris-Cl 10 mM, pH 8.5 with 0.1% Tween 20, and then briefly vortex and spin down.
2) Prepare a fresh dilution of 1 mL of 0.1N NaOH by combining the following volumes in a microcentrifuge tube, and then briefly vortex and spin down:
   a) Laboratory-grade water (900µL)
   b) Stock 1N NaOH (100µL)
3) Combine the following volumes in a microcentrifuge tube, and then briefly vortex and spin down:
   a) 2nM pool (10µL)
   b) 0.1N NaOH (10 µL)
4) Incubate for 5 minutes at room temperature to denature the pool into single strands.
5) Add 980 µL pre-chilled HT1 (Hybridization Buffer) to 20 µL denatured pool to result in a 20pM pool.
6) Place the denatured pool on ice until ready to proceed to final dilution.

Dilution of Denatured Pools

7) Determine the final loading concentration (usually 6pM, but differs based on estimated cluster density success).
8) Combine the following volumes in a microcentrifuge tube (for 6pM loading concentration), and then briefly vortex and spin down:
   a) 20pM denatured pool (126µL)
   b) pre-chilled HT1 (294µL)
9) Place the diluted denatured pool on ice until ready to add PhiX control spike-in.

Denaturation, Dilution, and Addition of a PhiX Control Spike-In

Illumina recommends a low-concentration PhiX control spike-in at 1% to allow direct assessment of error rates for each lane. A PhiX spike-in is important for unbalanced or low-diversity libraries.

10) Combine the following volumes to dilute the PhiX library to 2nM, and then briefly vortex and spin down:
   a) 10 nM PhiX library (2µL)
   b) 10 mM Tris-Cl, pH 8.5 with 0.1% Tween 20 (8µL)
11) Combine the following volumes of 2nM PhiX library and 0.1N NaOH in a microcentrifuge tube to result in a 1nM PhiX library, and then briefly vortex and spin down:
   a) 2nM PhiX library (10µL)
   b) 0.1N NaOH (10µL)
12) Incubate for 5 minutes at room temperature to denature the PhiX library into single strands.
13) Add 980µL prechilled HT1 to 20µL denatured PhiX library to result in a 20pM PhiX library. The denatured 20pM PhiX library can be stored up to 3 weeks at -25°C to -15°C. After 3 weeks, cluster numbers tend to decrease.
14) Dilute Denatured PhiX Library by combining the following volumes to dilute to 6pM:
   a) 20 pM denatured PhiX library (300µL)
   b) Prechilled HT1 (700µL)

15) Combine the following volumes of diluted denatured PhiX control and diluted denatured pool in a 1.5 ml or 1.7 ml Eppendorf tube, and then briefly vortex and spin down:
    a) Prepared library (416µL)
    b) PhiX control (4µL)
16) Set the combined spiked-diluted-denatured pool and PhiX solutions aside on ice until ready to load onto the HiSeq2500.

Step 2: Prepare Reagents

1) Remove Illumina's TruSeq Rapid SBS Kit (200 cycles) and TruSeq Rapid PE Cluster Kit from -20°C storage.
2) Thaw reagents in a room temperature deionized water bath for about 90 minutes. Alternatively, thaw reagents at 4°C for up to 16 hours.
3) The Cleavage Reagent Mastermix should be thawed in a separate room temperature deionized water bath for 90 minutes, or 4°C for up to 16 hours. Always replace your gloves after handling the Cleavage Reagent Mastermix to avoid contamination to other reagents.
4) Invert each bottle several times to mix.
5) Inspect the reagents for ice crystals. Make sure that the reagents have thawed completely.
6) Load the reagent bottles with color-coded labels that match their positions in the reagent racks.
7) Load 25 ml of laboratory-grade water in positions 2, 4, 8 and 19 in the reagent racks.
8) Set reagents aside on ice or 4°C until ready to load them onto the instrument. Set the Cleavage Reagent Mastermix aside on ice separately to prevent cross-contamination.

Step 3: Perform a Rapid Run

To ensure the fluidics of the intended HiSeq2500 instrument are clean and running properly, a pre-run volume check is performed. Once the instrument passes, it is ready for loading all the reagents and the spiked-diluted-denatured pools.

Pre-Run Volume Check

1) From the Welcome screen, select Rapid Run, then Sequence | New Run.
2) The Volume Check screen opens. When prompted by the software to perform a volume check, select Yes.
3) Place waste tubes 1, 2, 3, 6, 7, and 8 for the current flow cell in a 1 liter bottle filled with deionized water. Placing the tubes in deionized water prevents damage to the reagent pumps.
4) Place waste tubes 4 and 5 into separate empty 15 ml conical tubes.
5) Load laboratory-grade water into empty sterile tubes in all positions on an empty reagent racks, and the library/pool position for the current (previously used) flow cell. Make sure that a used rapid flow cell is loaded on the instrument.
6) Close the loading station.

7) Select the Water loaded and template loading station closed checkbox and select Next.
8) Enter the ID of the used flow cell and select Next.
9) Select Pump to confirm flow.
10) Select Next. The volume check begins.
11) When the volume check is complete, the expected volume is 9.5 ml ±10% for each tube.
12) Return all waste tubes to the waste bottle and select Next.

Run Configuration

13) Proceed without connecting to BaseSpace, by selecting None and then selecting Next.
14) Select the Save to an output folder checkbox, select Browse to navigate to a preferred network location, and select Next.
15) Scan the flow cell ID (barcode) of the flow cell to be sequenced.
16) Enter an experiment name and select Next. The experiment name appears on each screen to help identify the run in progress.
17) [Optional] Select the Confirm First Base checkbox and select Next. A first base report is generated automatically for each run. Selecting this option opens the first base report before proceeding with the run.
18) Select Flow Cell Recipe Format for 100 x 7 x 100 run
    a) Single Index—Performs a single-read or paired-end run with 1 indexing read.
    b) Paired End
    c) Enter the number of cycles for Read 1 and Read 2 (100 cycles each)
19) Select On-Board Cluster Generation to perform clustering on-instrument and select Next.
20) For paired-end runs, scan or enter the reagent kit ID for the cluster kit.
21) Scan or enter the SBS reagent kit ID and select the SBS reagent kit for the run: 200 cycles.
22) Review the run parameters on the Review screen and select Next to proceed or select Back to change parameters.

Pre-Run Setup

23) Load the reagents and spiked-diluted-denatured pools into the HiSeq2500 instrument and close loading station.
24) Select the Reagents and Template loaded, and reagents and template loading station closed checkboxes. Select Next.
25) To load a flow cell:
    a) Open the flow cell compartment door.
    b) Slowly move the flow cell lever to position 1 to disengage the manifolds.
    c) Slowly move the flow cell lever to position 0 to disengage the vacuum seal and release the flow cell.
    d) Lift the used flow cell from the flow cell holder and discard it.
    e) Remove the new flow cell from the flow cell container using a pair of plastistats.
    f) Rinse the flow cell with laboratory-grade water and dry it with a lens cleaning tissue.
    g) Rinse the flow cell with ethanol and dry it with a lens cleaning tissue.

h) Hold the edges of the clustered flow cell with 2 fingers. Make sure that the inlet and outlet ports are facing up.
i) Wipe each side of the flow cell with a single sweeping motion. Repeat, refolding the lens cleaning tissue with each pass, until the flow cell is clean and dry.
j) Visually inspect the flow cell holder to make sure that it is free of lint and the vacuum holes are free of obstructions.
k) Place the flow cell on the flow cell holder with the inlet and outlet ports facing down and the barcode on the right. Make sure that the arrow on the left edge of the flow cell, which indicates flow direction, points towards the instrument.
l) Gently slide the flow cell towards the top and right guide pins until it stops.
m) Remove your hand from the flow cell before engaging the vacuum switch to prevent possible alignment drift over time.
n) Slowly move the flow cell lever to position 1 to engage the vacuum and secure the flow cell into position. When the flow cell lever is green, the vacuum is engaged.
o) Wait for about 5 seconds, and then slowly move the flow cell lever to position 2. When the flow cell lever is solid green, the manifolds are in position and the flow cell is ready for use.
p) Make sure that the Vacuum Engaged checkbox is selected on the load sequencing flow cell screen.

26) To confirm proper flow, select solution 5 (USB) from the drop-down list.
27) Make sure that the following default values are entered:
    a) Volume: 250
    b) Aspirate Rate: 1500
    c) Dispense Rate: 2000
28) Make sure that waste outlet tubes 1, 2, 3, 6, 7, and 8 are in a bottle of clean water and that tubes 4 and 5 are in the waste container.
29) Select Pump.
30) Visually inspect the flow cell for bubbles passing through the lanes and leaks near the manifolds.
31) If excessive bubbles are present, check the manifold gaskets for obstructions, and repeat the process.
32) Select solution 6 (USB) to avoid depleting USB from position 5.
33) Reduce the aspirate rate to 1000, and pump another 250 µl of USB to the flow cell.
34) After you have confirmed proper flow, select Next to proceed.
35) Make sure that the flow cell lever is green, and then close the flow cell compartment door.
36) Confirm that the Vacuum Engaged and Door Closed checkboxes are selected, and then select Next.

Initiate Run

37) Select Start to start the sequencing run.
38) If the Confirm First Base option was selected during run setup, the first base confirmation dialog box opens automatically after imaging of the first cycle is complete. The run pauses at this step.
39) Review the First Base Report from the confirmation dialog box.
40) If the results are satisfactory, select Continue.

41) When the run is complete, perform a Post-Run Wash.

Sources

HiSeq 2500 System User Guide. Part # 15035786 Rev. D, November 2014, Illumina.

Experion DNA 1K and DNA 12K Analysis Kits Instruction Manual. Bulletin 10006453 Rev B, Bio-Rad Laboratories, Inc.

Qubit dsDNA HS Assay Kits. MAN0002326, MP32851, Revision: B.0, 5 March 2015, Thermo Fisher Scientific Inc.

UNC HEALTH CARE	Policy Name/Procedure	**Custom DNA Sequencing**
	Author/ Revision Date	**Ferrin Wheeler, Revision date 08/12/15**
	Date this Version Effective	**August 2015**

This is a general procedure for sequence-based testing of a known/familial mutation.

Assay Design

Identify the exon and flanking intronic sequence that harbors the known mutation using Ensembl, UCSC Genome Browser or NCBI. Typically, amplification of the entire exon will be the best assay design, with primers placed in the flanking introns. Choose primers by hand or use a primer design website such as Primer3 (http://bioinfo.ut.ee/primer3/). Ideal amplicon size is between 200 and 400 bp. In most cases, PCR primers will also be used as sequencing primers. Ideal primer Tm values are 55-60 ºC, with GC content of 50%.

PCR Amplification

For previously unanalyzed specimens: In addition to the patient sample, include a positive control (family member with mutation, when available), normal, and no template control in every run.

For specimens run to confirm the presence of a previously identified mutation (such as information from a research assay): In addition to the patient sample, include a no template control in every run.

PCR Master Mix	1 Reaction (in µl)
Platinum Blue PCR Master mix	22
Forward Primer (10 µM)	1
Reverse Primer (10 µM)	1
DNA or Water	1

94ºC for 2 min.

94ºC for 30 sec.
55ºC for 30 sec.
72ºC for 1 min.
} X 35

4ºC hold

Check Gel

Run 5 µl of PCR product on an e-gel and proceed if DNA samples amplified and the water control is blank.

Purification of PCR Product

Add 2 µl l of ExoSAP-IT to 5 µl of PCR product.

Incubate in PE 9700 or MJ thermocycler using **EXOSAP-IT** program:
37 °C for 30 minutes, then 80 °C for 15 minutes. Product can be stored at –20°C if necessary.

Cycle Sequencing Reaction

1. The sequencing reaction is set up on ice as follows:

Sequencing Reaction	12.5 ul reaction*
ExoSAP-IT treated PCR product	1.125
Primer (10 µM)	0.75
Autoclaved ddH_20	5.625
BigDye Terminator	5

* All volumes are in microliters.

2. Amplify in 9700 thermocycler using the following program:
9700 user: cx; program: cx26-seq

96°C for 5 sec.

96°C for 10 sec.
50°C for 5 sec.
60°C for 4 min.
} X 25

60°C for 10 min.

4°C hold

Samples can be stored at –20°C if not used immediately

Purification of Cycle Sequencing Product

1. Separate the required number of 8-well strips from the package, cut the bottom plugs and remove the foil covers.

2. Place the strips in the wash trays, making sure to balance the trays, using old strips if

necessary. Centrifuge at 750 g for 2 minutes.

3. Place the strips into the ABI 3130 Genetic Analyzer 96-well plate, in the wells to be used for the run. If the run is larger than 16 samples, use two plates for the run so the spins are balanced. If not, use old columns for the balance plate in the centrifuge.

4. Using a P20 pipette set at 15 µl, add the sequencing reaction to the center of the column.
   Be careful to keep track, taking care to add one sample to each tube, in the correct order. Keep the empty reaction tubes in the order used to set up the sample sheet.

5. Secure samples with parafilm and centrifuge at 750gfor 2 minutes.

6. Runs are done in groups of 16 (two rows of 8 from the 96-well plate). If fewer than 16 samples are to be run, the remaining wells are filled with 20 µl of water. Add the water to the side of the well, to avoid having air trapped below.

7. Cover the plate(s) with the 96-well septa and place the sample tray(s) in a 9700 thermocycler at 95 °C for 3 minutes to denature the samples.

ABI 3130 Operation

1. Turn on the computer before turning on the instrument.

2. Open **3130 Data Collection** from the desktop, click the **Plate View** tab and click **New** or **Edit**. This process is analogous to creating a sample sheet and will identify which sample is in each well. Name the plate with the date followed, if necessary, by a letter suffix (a, b, c, etc), and select **Sequencing**.

3. Use the **Plate Editor** as a template for the plate and enter sample information for the wells containing samples. If a full plate is not needed, mark on the plate which wells have been used so the remaining wells can be used for subsequent runs. Enter the following information using the pull-down window when present:

   Sample Name: Sample and primer (i.e., MO10-exonF). Spaces are not allowed in this window.
   Comment: Gene name and exon number
   Results Group: Whatever folder you want the results to be saved to
   Instrument Protocol: SeqE
   Analysis Protocol: SeqE

   When the plate record is complete, click **OK**. The plate record will now appear in the **Plate View** tab as a Pending Plate Record.

4. Place the 96-well plate, with the 96-well septa in place, into the plate base and fit the plate retainer over the top. Make sure the plate retainer holes are aligned with the holes in the septa. Place the entire plate assembly onto the autosampler.

5. When the plate is in place, the plate position indicator in the **Plate** View will change from gray to yellow, indicating it is ready to be linked to a pending plate record.

6. Click on the appropriate plate record in **Pending Plate Records** and then click the plate position indicator that contains the plate to be linked.

7. Once the plate has been linked to a plate record, the green **Run Instrument** button on the toolbar is enabled. Click the **Run** button to start run. The run will be separated into groups of 16.

8. During a run **Status View** can be used to monitor the run. **Array View** and **Capillary View** can be viewed but these windows should not be left open as they can cause unrecoverable screen update problems.

Run Analysis

1. Open ABI Sequencing Analysis Software from the **Start** menu:
   Start
   Applied Biosystems
   Sequence Analysis 5.2
   Sequencing analysis

2. In the **Sample Manager** window, click **Add Files**. Under Desktop, select **Shortcut to Data Extractor**. Select the appropriate run and select **Add All**.

3. Individual files can be viewed and printed.

Gene	Gene MIM	Phenotype	Phenotype MIM	Outcome Considered	Severity	Likelihood	Intervention Considered	Efficacy	Acceptability	Knowledge	Total
ACADM	607008	Acyl-CoA Dehydrogenase, Medium Chain, Deficiency of	201450	Death from Hypoglycemic Crises	3	3	Avoid Fasting, frequent feeding, emergency letter	3	3	3	**15**
ACADVL	609575	VLCAD deficiency	201475	Hypoglycemic Crises	3	3	Prevention of fasting, dietary restriction of long chain fatty acids; carnitine supplement	3	3	3	**15**
HADHA	600890	LCHAD deficiency	609016	Hypoglycemic Crises	3	3	Prevention of fasting, dietary restriction of long chain fatty acids;	3	3	3	**15**
IL2RG	308380	Severe Combined Immunodeficiency, X-linked	300400	Immunodeficiency	3	3	Hematopoietic Stem Cell Transplantation (HSCT)	3	3	3	**15**
JAK3	600173	SCID, AR, T-negative/B-positive type	600802	Immunodeficiency	3	3	Transplantation of Hematopoietic Stem Cells	3	3	3	**15**
ALDOB	612724	Fructose intolerance	229600	All Outcomes	2	3	Strict Dietary Restriction	3	3	3	**14**
CTNS	606272	Cystinosis	219800	kidney failure (renal Fanconi Syndrome)	2	3	cysteamine, monitoring to determine if/when renal transplant indicated	3	3	3	**14**
CYP21A2	613815	Adrenal hyperplasia, congenital, due to 21-hydroxylase deficiency	201910	Salt-wasting crises	2	3	Glucocorticoid/mineralocorticoid administration	3	3	3	**14**
ELN	130160	Supravalvar aortic stenosis	185500	SVAS induced heart failure	2	3	Echocardiogram	3	3	3	**14**
GAA	606800	Glycogen storage disease II (GSD2)	232300	HCM, respiratory distress, hypotonia	3	3	ERT, indivualized care for cardiomyopathy	3	2	3	**14**
HFE2	608374	Hemochromatosis, type 2A	602390	Multiple system iron overload (heart, liver)	2	3	Yearly ferritin -> phlebotomy	3	3	3	**14**
HSD3B2	613890	3-beta-hydroxysteroid dehydrogenase, type II, deficiency	201810	Salt Wasting Crises	3	2	Endocrine eval, IV saline, glucocorticoid/mineralocorticoid if indicated	3	3	3	**14**
INS	176730	Diabetes mellitus, permanent neonatal	606176	Hyperglycemia, ketoacidosis	3	3	Insulin	3	3	2	**14**
KCNE1	176261	Jervell and Lange-Nielsen syndrome 2 (recessive)	612347	Sudden death due to arrhythmia	3	3	ICD	3	2	3	**14**
MEFV (heterozygous)	608107	Familial Mediterranean fever, AD, Classic mutations associated with renal failure	134610	Renal Failure	2	3	Colchisine	3	3	3	**14**
MEFV (homozygous)	608107	Familial Mediterranean fever, AR, Classic mutations associated with renal failure	249100	Renal Failure	2	3	Colchisine	3	3	3	**14**
NAGS	608300	N-acetylglutamate synthase deficiency	237310	Hyperammonemic crisis	3	3	All Interventions	3	2	3	**14**
OTC	300461	Ornithine transcarbamylase deficiency (Males)	311250	Hyperammonemic crisis	3	3	Diet, sodium phenylacetate and sodium benzoate, illness management	3	2	3	**14**
PTPN11	176876	Noonan syndrome 1	163950	Congenital Heart Defects	3	3	ECG / no sports / ICD (HCM), pulmonary balloon valvuloplasty or	3	2	3	**14**
RB1	614041	Retinoblastoma	180200	Retinoblastoma	2	3	Funduscopic Exam	3	3	3	**14**
STAR	600617	Lipoid adrenal hyperplasia	201710	salt-wasting, failure to thrive	3	3	Hormone Replacement	3	3	2	**14**
TG	188450	Thyroid dyshormonogenesis 3	274700	Brain, neuron damage, MR, FTT, jaundice, cretinism	2	3	Thyroid hormone replacement (L-thyroxine)	3	3	3	**14**
TPO	606765	Thyroid dyshormonogenesis 2A	274500	Brain, neuron damage, MR, FTT, jaundice, cretinism	2	3	Thyroid hormone replacement (L-thyroxine)	3	3	3	**14**
ZAP70	176947	Selective T-cell defect	269840	recurrent bacterial, viral, and opportunistic infections	3	3	HSCT	3	3	2	**14**
ACAT1	607809	Alpha-Methylacetoacetic Aciduria	203750	Severe Metabolic Acidosis	2	3	Dietary: Avoidance of Fasting, Low Protein	3	3	2	**13**
ALDH7A1	107323	Pyridoxine-dependent epilepsy	266100	Epileptic encephalopathy	1	3	B6 supplementation	3	3	3	**13**
APC	611731	Familial Adenomatous Polyposis	175100	Colorectal Cancer	2	3	Colonoscopy	3	2	3	**13**
ASS1	603570	Citrullinemia	215700	Hyperammonemic Crisis	2	3	Diet, sodium phenylacetate and sodium benzoate	3	2	3	**13**
BTD	609019	Biotinidase Deficiency	253260	Developmental Delay	1	3	Biotin	3	3	3	**13**
CACNA1C	114205	Timothy syndrome	601005	Sudden death due to arrhythmia	3	3	EKG screening / avoidance of triggers / ICD	2	3	2	**13**
CASQ2	114251	Ventricular tachycardia, catecholaminergic polymorphic, 2 (recessive)	611938	Sudden death due to arrhythmia	3	3	Stress testing/avoidance of triggers/beta-blockers/ICD	2	3	2	**13**

CBS	613381	Homocystinuria, B6-responsive and nonresponsive types	236200	Risk for Thrombosis	2	3	Diet +/- pyridoxine, cystadane	3	2	3	**13**
CDH23	605516	Usher Syndrome, type 1D	601067	Usher Phenotype (Communication Deficits + RP)	2	3	Audiology and Vision Services	2	3	3	**13**
CDKN2A	600160	Pancreatic cancer/melanoma syndrome	606719	Melanoma	2	2	Skin Exam	3	3	3	**13**
CIB2	605564	Deafness, autosomal recessive 48	609439	Communication Deficits	1	3	All Interventions	3	3	3	**13**
CPS1	608307	Carbamoylphosphate synthetase I deficiency	237300	Hyperammonemic Crisis	2	3	Diet, sodium phenylacetate and sodium benzoate	3	2	3	**13**
DCLRE1C	605988	Severe Combined Immunodeficiency, Athabascan Type	602450	Death Secondary to Immune Deficiency	3	3	HSCT (Transplant)	3	2	2	**13**
DUOX2	606759	Thyroid dyshormonogenesis 6	607200	Brain, neuron damage, MR, FTT, jaundice, cretinism	2	3	Thyroid hormone replacement (L-thyroxine)	3	3	2	**13**
DUOXA2	612772	Thyroid dyshormonogenesis 5	274900	Brain, neuron damage, MR, FTT, jaundice, cretinism	2	3	Thyroid hormone replacement (L-thyroxine)	3	3	2	**13**
EDN3	131242	Waardenburg syndrome, type 4B	613265	Communication Deficits or Hirschsprung Disease	1	3	Audiology --> Hearing Aids --> Cochlear Implant or Screening -->	3	3	3	**13**
F10	613872	Factor X deficiency	227600	Bleeding	1	3	Fresh-frozen plasma or plasma-derived Prothrombin Complex concentrates (PCCs) with procedures	3	3	3	**13**
F8	300841	Hemophilia A	306700	Bleeding --> possible exsanguination	2	3	Factor replacement	3	2	3	**13**
F9	300746	Hemophilia B	306900	Bleeding --> possible exsanguination	2	3	Factor replacement	3	2	3	**13**
FAH	613871	Tyrosinemia, type I	276700	Liver failure, hepatocellular carcinoma	2	3	NTBC, dietary intervention	3	2	3	**13**
FBN1	134797	Marfan Syndrome	154700	Aortic Dissection	3	2	Annual Echocardiogram	2	3	3	**13**
G6PC	613742	Glycogen Storage Disease 1a	232200	Severe Hypoglycemia	2	3	Dietary (Low sugar), avoid fasting, uncooked cornstarch	3	2	3	**13**
GALT	606999	Galactosemia	230400	Death from liver failure or E.coli sepsis	2	3	Dietary Restriction	3	2	3	**13**
GBA	606463	Gaucher Disease, Type I	230800	All Outcomes	1	3	Enzyme Replacement Therapy	3	3	3	**13**
GCDH	608801	Glutaricaciduria, type I	231670	Metabolic crisis	2	3	Diet, carnitine, Anticipatory emergent management	2	3	3	**13**
GCH1	600225	Dystonia, DOPA-responsive, with or without hyperphenylalaninemia	128230	Dystonia	1	3	Oral dopa/carbidopa	3	3	3	**13**
GIF	609342	Intrinsic factor deficiency	261000	Pernicious Anemia	2	3	B12 Injections	3	3	2	**13**
GJB2	121011	Deafness, autosomal recessive 1A (DFNB1A)	220290	Communication Deficits	1	3	Audiology --> Cochlear Implants	3	3	3	**13**
HADH	601609	3-Hydroxyacyl-CoA Dehydrogenase Deficiency	231530	Profound Hypoglycemia in Infancy	2	3	Matabolic eval; Adequate Carbohydrate Source; Diazoxide	3	3	2	**13**
HADHB	143450	Trifunctional protein deficiency	609015	Hypotonia, Respiratory Failure, Cardiomyopathy, SIDS-like	3	3	Prevention of fasting, dietary restriction of long chain fatty acids;	2	3	2	**13**
HAMP	606464	Hemochromatosis, type 2B	613313	Severe iron overload	2	3	Phlebotomy	3	3	2	**13**
HBB	141900	Thalassemias, beta- (Major, AR)	613985	life threatening anemia	2	3	transfusions, iron chelation (desferoxamine)	3	2	3	**13**
HFE2	608374	Hemochromatosis, type 2A	602390	Severe iron overload	2	3	Yearly ferritin -> phlebotomy	3	3	2	**13**
HLCS	609018	Holocarboxylase synthetase deficiency	253270	Seizures	2	3	Biotin	3	3	2	**13**
IL7R	146661	Severe Combined Immunodeficiency, T-cell Negative, B-cell / Natural Killer Cell-Positive Type	608971	Death	3	3	BMT	3	2	2	**13**
IVD	607036	Isovaleric acidemia	243500	Encephalopathy with metabolic decompensation	3	2	Diet, supplements	3	2	3	**13**
JUP	173325	Naxos disease (recessive)	601214	Sudden death due to arrhythmia	3	3	Echo/MRI screening/no sports/ICD	2	3	2	**13**
KCNH2	152427	Romano-Ward Long QT syndrome 2	613688	Arrhythmia	3	2	EKG screening / avoidance of triggers / ICD	2	3	3	**13**
KCNQ1	607542	Romano-Ward Long QT syndrome 1	192500	Arrhythmia	3	2	EKG screening / avoidance of triggers / ICD	2	3	3	**13**
LDLR	606945	Familial hypercholesterolemia	143890	Hypercholesterolemia / Early MI	2	3	Cholesterol screening / Statins	2	3	3	**13**
LHX3	600577	Pituitary hormone deficiency, combined, 3	221750	Combined pituitary hormone deficiency (CPHD)	2	3	Hormone Replacement Therapy	3	3	2	**13**
MEN1	613733	Multiple endocrine neoplasia 1	131100	Multiple Endocrine Tumors	1	3	Biochemical Screening / Imaging --> Surgery	3	3	3	**13**

MITF	156845	Waardenburg Syndrome, type 2A	193510	Communication Deficits	1	3	Audiology --> Hearing Aid --> Cochlear Implant	3	3	3	**13**
MLH1	120436	Lynch syndrome	609310	Colorectal Cancer	2	3	Colonoscopy	3	2	3	**13**
MMAA	607481	Methylmalonic aciduria, vitamin B12-responsive	251100	Metabolic decompensation	2	2	Diet, B-12, illness management	3	3	3	**13**
MMAB	607568	Methylmalonic aciduria, vitamin B12-responsive, due to defect insynthesis of adenosylcobalamin. cblB	251110	Metabolic decompensation	2	3	Diet, B-12, illness management	2	3	3	**13**
MMACHC	609831	Methylmalonic aciduria and homocystinuria, cblC type	277400	infantile presentation (failure to thrive, poor feeding, and hypotonia with an	2	3	Diet, B-12, illness management	2	3	3	**13**
MPI	154550	CDG1b	602579	All Outcomes	1	3	All Interventions	3	3	3	**13**
MSH2	609309	Lynch syndrome	120435	Colorectal Cancer	2	3	Colonoscopy	3	2	3	**13**
MTHFR	607093	Homocystinuria due to MTHFR deficiency	236250	Risk for thrombosis	2	3	Folate supplementation, B6, B12, Betaine	3	3	2	**13**
MUT	609058	Methylmalonic aciduria, mut(0) type	251000	Metabolic decompensation	3	3	Dietary restriction and emergency letter	2	2	3	**13**
MUTYH	604933	Attenuated FAP / MUTYH-associated polyposis	608456	Colorectal Cancer	2	3	Colonoscopy	3	2	3	**13**
MYO7A	276903	Deafness, autosomal recessive, 2	600060	Communication Deficits	1	3	Audiology --> Cochlear Implants	3	3	3	**13**
MYO7A	276903	Usher Syndrome, type 1B	276900	Usher Phenotype (Communication Deficits + RP)	2	3	Audiology and Vision Services	2	3	3	**13**
OTC	300461	Ornithine transcarbamylase deficiency (Females)	311250	Hyperammonemic crisis	2	2	Diet, sodium phenylacetate and sodium benzoate. illness	3	3	3	**13**
OTOF	603681	Deafness, autosomal recessive 9	601071	Communication Deficits and/or Auditory Neuropathy	1	3	All Interventions	3	3	3	**13**
PAH	612349	Phenylketonuria	261600	Severe intellectual disability	2	3	Dietary restriction	3	2	3	**13**
PAX3	606597	Waardenburg Syndrome, type 1	193500	Communication Deficits	1	3	Audiology --> Hearing Aid --> Cochlear Implant	3	3	3	**13**
PCCA	232000	Propionicacidemia	606054	Encephalopathy, coma, seizures, developmental regression and	3	3	Avoidance of catabolic stressors and immediate treatment of metabolic	1	3	3	**13**
PCCB	232050	Propionicacidemia	606054	Encephalopathy, coma, seizures, developmental regression and	3	3	Avoidance of catabolic stressors and immediate treatment of metabolic	1	3	3	**13**
PCDH15	605514	Usher Syndrome, type 1F	602083	Usher Phenotype (Communication Deficits + RP)	2	3	Audiology and Vision Services	2	3	3	**13**
PDX1	600733	Pancreatic agenesis 1	260370	Neonatal diabetes	2	3	Insulin	3	3	2	**13**
PROC	612283	Thrombophilia due to protein C deficiency, autosomal recessive	612304	Thrombosis, PE	2	3	Hematological evaluation --> protein C or plasma if biochemical evidence of protein C deficiency	3	3	2	**13**
PROP1	601538	Pituitary hormone deficiency, combined, 2	262600	Growth failure/failure to thrive	1	3	screening--> hormone replacement	3	3	3	**13**
PTPN11	176876	Metachondromatosis	156250	Exostoses and enchondromatosis	1	3	Bi-annual clinical review, imaging	3	3	3	**13**
RAG1	179615	Severe combined immunodeficiency, B cell-negative	601457	Immunodeficiency, overwhelming infections	3	3	Bone marrow transplant	2	2	3	**13**
RAG2	179616	Severe combined immunodeficiency, B cell-negative	601457	Immunodeficiency, overwhelming infections	3	3	Bone marrow transplant	2	2	3	**13**
RET	164761	Multiple endocrine neoplasia IIA	171400	Medullary thyroid cancer	2	3	Thyroidectomy	3	2	3	**13**
RET	164761	Multiple endocrine neoplasia IIB	162300	Medullary thyroid cancer	2	3	Thyroidectomy	3	2	3	**13**
RET	164761	Familial Medullary Thyroid Cancer (FMTC)	155240	Medullary thyroid cancer	2	3	Thyroidectomy	3	2	3	**13**
SCN5A	600163	Romano-Ward Long QT syndrome 3	603830	Arrhythmia	3	2	EKG screening / avoidance of triggers / ICD	2	3	3	**13**
SLC19A3	606152	Thiamine metabolism dysfunction syndrome 2 (biotin- or thiamine-responsive encephalopathy type 2)	607483	Recurrent subacute encephalopathy	2	3	Oral biotin and thiamine	3	3	2	**13**
SLC25A20	212138	Carnitine-Acylcarnitine Translocase Deficiency	212138	Hypoglycemia --> Neurological Disorder	2	3	Low fat diet, avoidance of fasting	3	3	2	**13**
SLC37A4	602671	Glycogen Storage Disease Ib/Ic		All Outcomes	2	3	All Interventions	3	2	3	**13**
SOX10	602229	Waardenburg syndrome, type 4C	613266	Communication Deficits	1	3	Audiology --> Hearing Aids --> Cochlear Implant	3	3	3	**13**
SRY	480000	46XY sex reversal 1	400044	gonadoblastoma	2	2	surgical removal of gonads	3	3	3	**13**
TECTA	602574	Deafness, autosomal dominant 8/12 (DFNA12)	601543	Communication Deficits	1	3	Audiology --> Cochlear Implants	3	3	3	**13**
TFR2	604720	Hemochromatosis, type 3	604250	Intermediate iron overload	2	3	Phlebotomy	3	3	2	**13**

TSHB	188540	Hypothyroidism, congenital, nongoitrous 4	275100	ID and growth retardation	2	3	T4 treatment	2	3	3	**13**
UNC13D	608897	Hemophagocytic lymphohistiocytosis, familial, 3	608898	Severe Inflammation / Immune Dysfunction	2	3	Chemotherapy and Immunotherapy --> HSCT	3	2	3	**13**
USH1C	605242	Usher Syndrome, type 1C	276904	Usher Phenotype (Communication Deficits + RP)	2	3	Audiology and Vision Services	2	3	3	**13**
VHL	608537	von Hippel-Lindau syndrome	193300	Renal cancer / CNS hemangioblastomas / Pheochromocytoma	2	3	Annual renal imaging / biochemical screening	2	3	3	**13**
VWF	613160	von Willibrand disease, type 3	277480	Severe Mucocutaneous and Musculoskeletal Bleeding	2	3	Prophylactic Infusions of VWF/FVIII Concentrates	3	3	2	**13**
ACTG1	102560	Deafness, autosomal dominant 20/26	604717	Communication Deficits	1	3	All Interventions	3	3	2	**12**
ACVRL1	601284	Telangiectasia, hereditary hemorrhagic, type 2	600376	GI bleeding, CVA from cerebral AVMs, infectious complications	2	2	annual CBC, O2 sats, contrast echo, one-time head MRI. Don't	2	3	3	**12**
ADGRV1	602851	Usher Syndrome, type 2C	605472	Usher Phenotype (Communication Deficits + RP)	1	3	Audiology and Vision Services	2	3	3	**12**
AGL	610860	Glycogen Storage Disease III	232400	All Outcomes	1	3	All Interventions	2	3	3	**12**
AMS1	606844	Alstrom Syndrome	203800	Alstrom Syndrome (Communication deficits. visual impairments.	2	3	Referral to audiology and vision services. surveillance and monitoring	1	3	3	**12**
APOB	107730	Familial hypercholesterolemia due to ligand-defective APOB	144010	Hypercholesterolemia / Early MI	2	3	Cholesterol screening / statins	2	3	2	**12**
ASL	608310	Argininosuccinic aciduria	207900	Hyperammonemic crisis, chronic liver disease	2	3	Diet (Normal diet with arginine supplement or a diet in which protein	2	2	3	**12**
ATP6V1B1	192132	Renal tubular acidosis with deafness	267300	Renal Tubular Acidosis	1	3	Urine and Blood Tests, Alkaline Treatment	3	3	2	**12**
ATP7A	300011	Menkes Disease	309400	Low serum copper (seizures, neurological deficits. failure to thrive)	3	3	Copper histidine or Copper chloride injections	1	3	2	**12**
ATP7B	606882	Wilson Disease	277900	Liver Cirrhosis	2	2	Monitoring, low copper diet, chelation (if Cu levels elevated)	2	3	3	**12**
BCHE	177400	Increased sensitivity to choline ester anesthesia		Avoiding prolonged apnea after use of choline ester anesthesia	2	1	Avoidance of suxamethonium (succinylcholine)	3	3	3	**12**
BCKDHA	608348	Maple syrup urine disease, type Ia, Ib, and type II	248600	MSUD	2	3	Diet	2	2	3	**12**
BCKDHB	248611	Maple syrup urine disease, type Ib	248600	MSUD	2	3	Diet	2	2	3	**12**
BMPR1A	601299	Polyposis, juvenile intestinal	174900	GI cancer	2	2	CBC, annual colonoscopy (scored on this). In severe cases. colectomy	3	2	3	**12**
BRCA1	113705	Hereditary Breast and Ovarian Cancer	604370	Breast Cancer / Ovarian Cancer	2	3	Prophylactic Mastectomy / BSO	3	1	3	**12**
BRCA2	600185	Hereditary Breast and Ovarian Cancer	612555	Breast Cancer / Ovarian Cancer	2	3	Prophylactic Mastectomy / BSO	3	1	3	**12**
CARD11	607210	Immunodeficiency 11	615206	All Outcomes or SCIDs or Profound Combined Immunodeficiency	3	3	BMT / HSCT	3	2	1	**12**
CD3D	186790	Immunodeficiency 19	615617	SCIDs	3	3	Bone Marrow Transplant	3	2	1	**12**
CDH23	605516	Deafness, autosomal recessive 12 (DFNB12)	601386	Communication Deficits	1	3	All Interventions	3	3	2	**12**
CFTR	602421	Cystic Fibrosis	219700	Pulmonary Disease	2	3	Antibiotics, bronchodilaters, chest PT	2	2	3	**12**
CIB2	605564	Usher Syndrome, type IJ	614869	Usher Phenotype (Communication Deficits + RP)	2	3	Audiology and Vision Services	2	3	2	**12**
CISD2	611507	Wolfram syndrome 2	604928	Diabetes Mellitus / Insipidus	2	3	Surveillance --> Treatment of Manifestations	3	2	2	**12**
CLDN14	605608	Deafness, autosomal recessive 29	614035	Communication Deficits	1	3	All Interventions	3	3	2	**12**
COCH	603196	Deafness, autosomal dominant 9 (Non-syndromic deafness. dominant)	601369	All Outcomes	1	3	All Interventions	3	3	2	**12**
COL11A2	120290	Otospondylomegaepiphyseal Dysplasia	215150	Communication Deficits	1	3	Audiology --> Hearing Aids --> Cochlear Implant	3	3	2	**12**
COL11A2	120290	Stickler Syndrome, Type III	184840	Communication Deficits	1	3	Audiology --> Hearing Aids --> Cochlear Implant	3	3	2	**12**
COL1A2	120160	Osteogenesis imperfecta 3	259420	Progressive fractures, intracranial bleed	2	3	Bisphosphates	1	3	3	**12**
CORO1A	605000	Immunodeficiency 8	615401	Recurrent Infections	2	3	Bone Marrow Transplant	3	2	2	**12**
CTP1A	600528	CPT deficiency, hepatic, type IA	255120	Hypoketotic hypoglycemia, liver failure	3	2	Diet, avoidance of fasting	2	3	2	**12**
CYP27B1	609506	Vitamin D-dependent rickets, type I	264700	Bone disease	1	2	Vitamin D	3	3	3	**12**
DBT	248610	Maple syrup urine disease, type II	248600	MSUD	2	3	Diet	2	2	3	**12**
DNMT3B	602900	Immunodeficiency-centromeric instability-facial anomalies syndrome 1	242860	Immunodeficiency	2	3	IVIG; allogeneic stem cell transplant	3	2	2	**12**
DSP	125647	Dilated cardiomyopathy with woolly hair and keratoderma	605676	Heart failure	2	3	Echocardiogram	2	3	2	**12**
ENG	131195	Telangiectasia, hereditary hemorrhagic, type 1	187300	GI bleeding, CVA from cerebral AVMs, infectious complications	2	2	Annual CBC, O2 sats, contrast echo, one-time head MRI. Don't	2	3	3	**12**

ETFA	608053	Glutaric acidemia IIA	231680	Metabolic crisis	2	2	Riboflavin, carnitine, Anticipatory emergent management	2	3	3	**12**
ETFB	130410	Glutaric acidemia IIB	231680	Metabolic crisis	2	2	Riboflavin, carnitine, Anticipatory emergent management	2	3	3	**12**
ETFDH	231675	Glutaric acidemia IIC	231680	Metabolic crisis	2	2	Riboflavin, carnitine, Anticipatory emergent management	2	3	3	**12**
F13A1	134570	Factor XIIIA deficiency	613225	Intracranial Hemorrhage	2	2	Hematology Consult --> FFP	3	3	2	**12**
F13B	134580	Factor XIIIB deficiency	613235	Intracranial Hemorrhage	2	2	Hematology consult, assess factor level; rfXIII if low or for acute	3	3	2	**12**
F2	176930	Prothrombin Deficiency, congenital (Dysprothrombinemia, Hypoprothrombinemia)	613679	Intracranial Hemorrhage	2	2	Hematology consult, Fresh Frozen Plasma with Procedures / Trauma.	3	3	2	**12**
F5	612309	Factor V deficiency	227400	Bleeding	1	3	Hematology Consult --> FFP	3	3	2	**12**
F7	613878	Factor VII deficiency	227500	Bleeding --> hemorrhagic stroke	2	2	Factor replacement	3	2	3	**12**
FBP1	611570	Fructose bisphosphatase deficiency	229700	Hypoglycemia and metabolic acidosis	2	2	avoidance of fasting, high carbohydrate diet and avoid fructose/	3	3	2	**12**
FGFR3	134934	Muenke syndrome	602849	Craniosynostosis --> increased intracranial pressure	1	3	Physical exam --> imaging to screen --> surgery if necessary	3	2	3	**12**
G6PD	305900	hemolytic anemia due to G6PD deficiency	300908	neurological damage	1	2	photo therapy or transfusion , avoidance of oxidative stress	3	3	3	**12**
GATA2	137295	Immunodeficiency 21	137295	susceptibility to infection and myeloid malignancies	2	3	HSCT	3	2	2	**12**
GGCX	137167	Vitamin K-dependent coagulation defect	277450	Bleeding (Severe)	2	3	Oral Vitamin K	3	3	1	**12**
GIPC3	608792	Deafness, Autosomal Recessive 15	601869	Communication Deficits	1	3	All Interventions	3	3	2	**12**
GJB2	121011	Deafness, autosomal dominant 3A (DFNA3A)	601544	Communication Deficits	1	3	Audiology --> Hearing Aids	3	3	2	**12**
GJB6	604418	Non-Syndromic Deafness, Recessive 1B	612645	Communication Deficits	1	3	All Interventions	3	3	2	**12**
GPSM2	609245	Chudley-McCullough Syndrome	604213	Communication Deficits	1	3	Audiology --> Hearing Aids --> Cochlear Implants	3	3	2	**12**
GRXCR1	613283	Deafness, autosomal recessive 25	613285	Communication Deficits, with or without vestibular dysfunction	1	3	Audiology --> Hearing Aids	3	3	2	**12**
HBB	141900	Sickle cell anemia	603903	All Outcomes	2	2	All Interventions	2	3	3	**12**
HFE	613609	Hereditary hemochromatosis	235200	Multiple system iron overload (heart, liver)	2	1	Yearly ferritin -> phlebotomy	3	3	3	**12**
HFE - C282Y homozygous	613609	Hereditary hemochromatosis	235200	Multiple system iron overload (heart, liver)	2	1	Yearly ferritin -> phlebotomy	3	3	3	**12**
ILDR1	609739	Deafness, autosomal recessive 42	609646	Communication Deficits	1	3	All Interventions	3	3	2	**12**
IYD	612025	Thyroid dyshormonogenesis 4	274800	Brain, neuron damage, MR, FTT, jaundice, cretinism	1	3	Thyroid hormone replacement (L-thyroxine)	2	3	3	**12**
KCNJ2	600681	Andersen-Tawil syndrome; LQT 7	170390	Sudden death due to arrhythmia	3	3	EKG screening / avoidance of triggers / ICD	2	2	2	**12**
KCNQ4	603537	Deafness, autosomal dominant 2A	600101	Communication Deficits	1	3	All Interventions	3	3	2	**12**
KRIT1	604214	Cerebral Cavernous Malformations - 1	116860	Symptomatic CCM	2	3	Monitoring --> Surgery as indicated to remove the cerebral cavernous	2	3	2	**12**
LAMP2	309060	Glycogen Storage Disease IIb/Danon disease	300257	Cardiovascular irregularties (i.e. hypertrophic cardiomyopathy, dilated	2	3	Cardiac screening --> management	2	3	2	**12**
LDLRAP1	605747	Hypercholesterolemia, familial, autosomal recessive	603813	Hypercholesterolemia, early MI	2	3	Lipid monitoring, statins	2	3	2	**12**
LMNA	150330	Dilated cardiomyopathy 1A	115200	Arrhythmia or heart failure	3	2	Echo Screening / ICD	2	3	2	**12**
LRTOMT	612414	Deafness, autosomal recessive 63	611451	Communication Deficits	1	3	All Interventions	3	3	2	**12**
MARVELD2	610572	Deafness, autosomal recessive 49	610153	Communication Deficits	1	3	Audiology --> Cochlear Implants	3	3	2	**12**
MSH6	600678	Lynch syndrome	120435	Colorectal Cancer	2	2	Colonoscopy	3	2	3	**12**
MYH7	160760	Hypertrophic cardiomyopathy 1	192600	Arrhythmia	3	1	Echo Screening / no sports / ICD	3	3	2	**12**
MYO15A	602666	Deafness, autosomal recessive 3	600316	Communication Deficits	1	3		3	3	2	**12**
MYO6	600970	Non-Syndromic Deafness, Dominant 22	606346	Communication Deficits	1	3	All Interventions	3	3	2	**12**
NKX2-1	600635	Choreoathetosis, hypothyroidism, and neonatal respiratory distress	610978	Hypothyroidism	1	3	Thyroid Hormone Replacement	3	3	2	**12**
NPC1	607623	Niemann-Pick Disease, types C1 and D	257220	Progressive neurological involvement / deterioration	2	3	Miglustat	1	3	3	**12**
PALB2	610355	Fanconi anemia, complementation group N	610832	Bone marrow failure	2	3	Blood counts, annual bone marrow aspirate, gCSF	2	2	3	**12**
PAX8	167415	Hypothyroidism, congenital, due to thyroid dysgenesis or hypoplasia	218700	Low T4, high TSH, MR if untreated	2	2	L-thyroxine replacement	3	3	2	**12**

PCDH15	605514	Deafness, autosomal recessive 23	609533	Communication Deficits	1	3	All Interventions	3	3	2	**12**
PHKA2	300798	Glycogen Storage Disease, type IXa1/IXa2	306000	Severe Hypoglycemic Episode	2	2	Dietary Management	3	3	2	**12**
PIK3CD	602839	Immunodeficiency 14	615513	primary immunodeficiency	2	3	Immune evaluation -> antibiotics/immunoglobulin	2	3	2	**12**
PMS2	600259	Lynch syndrome	120435	Colorectal Cancer	2	2	Colonoscopy	3	2	3	**12**
POU1F1	173110	Pituitary hormone deficiency, combined, 1	613038	FTT, short stature, cretinism, MR	2	3	Hormone replacement: Levothyroxine. rGH subQ to 17y	2	3	2	**12**
POU3F4	300039	Deafness, X-linked 2	304400	Communication Deficits	1	3	All Interventions	3	3	2	**12**
POU4F3	602460	Deafness, autosomal dominant 15	602459	Communication Deficits	1	3	All Interventions	3	3	2	**12**
PROS1	176880	Thrombophilia due to protein S deficiency (AR)	614514	Thrombosis, multiple sites	3	3	FFP replacement	2	2	2	**12**
PROS1	176880	Thrombophilia due to protein S deficiency (AD)	612336	Thrombosis, PE	2	3	Prophyaxis and avoidance of immobility	2	2	3	**12**
PTEN	601728	PTEN hamartoma tumor syndrome	158350	Breast / Uterine / Thyroid Cancer	2	3	Mammography / Thyroid Ultrasound	2	3	2	**12**
PTPN11	176876	LEOPARD syndrome 1	151100	Heart Defects	2	3	echo screening / no sports / ICD	1	3	3	**12**
PTPN11	176876	Leukemia, juvenile myelomonocytic	607785	Myelomonocytic leukemia	3	3	Monitoring for Noonan syndrome --> HSCT	1	2	3	**12**
PTPRC	151460	Severe Combined Immunodeficiency, T cell-negative, B-cell / Natural Killer-Cell Positive	608971	Chronic Generalized Infection leading to Death	3	3	HSCT / BMT	3	2	1	**12**
PTPRQ	603317	Deafness, autosomal recessive 84A	613391	Communication Deficits	1	3	Audiology --> Hearing Aids	3	3	2	**12**
PYGM	608455	McArdle Disease	232600	All Outcomes	1	3	Controlled physical training and programed glucose intake	2	3	3	**12**
RAF1	164760	LEOPARD syndrome 2	611554	Hypertrophic cardiomyopathy	2	3	Echo, EKG	2	3	2	**12**
RAF1	164760	Noonan syndrome 5	611553	Hypertrophic cardiomyopathy	2	3	Echo, EKG	2	3	2	**12**
RYR1	180901	Malignant hyperthermia susceptibility	145600	Anesthesia-induced malignant hyperthermia	2	1	Avoidance of certain anesthetics, extreme heat	3	3	3	**12**
RYR2	180902	Catecholaminergic polymorphic ventricular tachycardia	604772	Sudden death due to arrhythmia	3	3	Beta blockers, ICD	2	2	2	**12**
SCN5A	600163	Brugada Syndrome	601144	Sudden Death due to Arrhythmia	3	2	EKG screening / avoidance of triggers / ICD	2	3	2	**12**
SERPINA1	107400	Emphysema-cirrhosis, due to AAT deficiency	613490	COPD / Emphysema, liver cirrhosis	2	3	Smoking avoidance, periodic monitoring	1	3	3	**12**
SLC19A2	603941	Thiamine-responsive megaloblastic anemia syndrome	249270	Megablastic anemia, hearing loss, dm	2	3	Thiamine at pharmacologic doses	2	3	2	**12**
SLC40A1	604653	Hemochromatosis, type 4	606069	Multiple system iron overload (heart, liver)	2	2	Phlebotomy	3	3	2	**12**
SLC46A1	611672	Folate malabsorption, hereditary	229050	Anemia, hypogammaglobulinemia (like SCID)	2	3	Reduced folate supplementation (injection more common)	3	2	2	**12**
SLC5A5	601843	Thyroid dyshormonogenesis 1	274400	Brain, neuron damage, MR, FTT, jaundice. cretinism	1	3	Thyroid hormone replacement (L-thyroxine)	2	3	3	**12**
SLC7A7	603593	Lysinuric protein intolerance	222700	GI symptoms, FTT, vomiting, diarrhea	1	3	Protein restriction, citrulline, nitrogen-scavenging drugs, lysine, carnitine	2	3	3	**12**
SPR	182125	Dystonia, dopa-responsive, due to sepiapterin reductase deficiency	612716	Dystonia	1	3	Oral l-dopa and 5-hydroxytryptophan	3	3	2	**12**
STRC	606440	Deafness, autosomal recessive 16	603720	Communication Deficits	1	3	All Interventions	3	3	2	**12**
TAZ	300394	Barth Syndrome	302060	Cardiac disease (Dilated cardiomyopathy and sudden ventricular	2	3	Cardiac evaluation with consideration of medical therapy, heart transplant	2	3	2	**12**
TECTA	602574	Deafness, autosomal recessive 21 (DFNB21)	603629	Communication Deficits	1	3	All Interventions	3	3	2	**12**
TGFB2	190220	Loeys-Dietz Syndrome 4	614816	Aortic Dissection	3	3	Annual Echocardiogram	1	3	2	**12**
TGFBR1	190181	Loeys-Dietz Syndrome 1	609192	Aortic Dissection	3	3	Annual Echocardiogram	1	3	2	**12**
TGFBR2	190182	Loeys-Dietz Syndrome 2	610168	Aortic Dissection	3	3	Annual Echocardiogram	1	3	2	**12**
TMC1	606706	Deafness, Autosomal Recessive 7	606706	Communication Deficits	1	3	All Interventions	3	3	2	**12**
TMIE	607237	Deafness, autosomal recessive 6	600971	Communication Deficits	1	3	Audiology --> Cochlear Implants	3	3	2	**12**
TMPRSS3	605511	Deafness, autosomal recessive 8/10	601072	Communication Deficits	1	3	All Interventions	3	3	2	**12**
TPRN	613354	Deafness, autosomal recessive 79	613307	Communication Deficits	1	3	All Interventions	3	3	2	**12**
TRIOBP	609761	Deafness, autosomal recessive 28	609823	Communication Deficits	1	3	Audiology --> Hearing Aids	3	3	2	**12**
TSHR	603372	Hypothyroidism, congenital, nongoitrous, 1	275200	ID and growth retardation	2	2	T4 treatment	2	3	3	**12**

TTPA	600415	Ataxia with isolated vitamin E deficiency	277460	Ataxia, progressive	1	3	Vitamin E	3	3	2	**12**
USH1G	607696	Usher Syndrome, type 1G	606943	Usher Phenotype (Communication Deficits + RP)	2	3	Audiology and Vision Services	2	3	2	**12**
USH2A	608400	Usher Syndrome, type 2A	276901	Usher Phenotype (Communication Deficits + RP)	1	3	Audiology and Vision Services	2	3	3	**12**
VWF	613160	von Willebrand disease, types 2A, 2B, 2M, and 2N	613554	Mild to Moderate Mucocutaneous Bleeding	1	2	Hematology --> Desmopressin	3	3	3	**12**
ABCA3	601615	Surfactant metabolism dysfunction, pulmonary, 3	610921	Repiratory Distress	3	3	Surfactant Replacement	1	1	3	**11**
ABCD1	300371	Adrenoleukodystrophy	300100	Adult myeloneuropathy with childhood onset adrenal insufficiency	2	3	biochemical screening and corticosteroid replacement	2	3	1	**11**
ABCG5	605459	Sitosterolemia	210250	All Outcomes	2	3	All Interventions	2	2	2	**11**
ACTA2	102620	Familial Thoracic Aortic Aneurysms	611788	Aortic Dissection	3	2	Annual Echocardiogram	2	3	1	**11**
AK2	103020	Reticular Dysgenesis	267500	SCID	3	3	HSCT	1	2	2	**11**
ARSB	611542	Mucopolysaccharidosis type VI (Maroteaux-Lamy)	253200		2	3	HSCT or ERT	2	2	2	**11**
BLM	604610	Bloom syndrome	210900	Colorectal cancer	2	2	Colonoscopy	3	2	2	**11**
CACNA1D	114206	Sinoatrial Node Dysfunction and Deafness	614896	Communication Deficits	1	3	Audiology --> Hearing Aids --> Cochlear Implant	3	3	1	**11**
CACNA1S	114208	Malignant hyperthermia susceptibility 5	601887	Anesthesia-induced malignant hyperthermia	2	1	Avoidance of Certain Anesthetics	3	3	2	**11**
CCDC50	611051	Deafness, autosomal dominant 44	607453	Communication Deficits	1	3	All Interventions	3	3	1	**11**
CD40LG	300386	Immunodeficiency, X-linked, with hyper-IgM	308230	Primary Immunodeficiency	2	3	BMT	2	2	2	**11**
CDC73	607393	Hyperparathyroidism, familial primary	145000	Parathyroid carcinoma	2	2	Biochemical screening	3	3	1	**11**
CDH23 / PCDH15	605514	Usher Syndrome, type ID / F Digenic	601067	Usher Phenotype (Communication Deficits + RP)	2	3	Audiology and Vision Services	2	3	1	**11**
CEACAM16	614591	?Deafness, autosomal dominant 4B	614614	Communication Deficits	1	3	Audiology --> Hearing Aids --> Cochlear Implant	3	3	1	**11**
CLRN1	606397	Usher Syndrome, type 3A	276902	Usher Phenotype (Communication Deficits + RP)	1	3	Audiology and Vision Services	2	3	2	**11**
COL11A2	120290	Deafness, autosomal recessive 53	609706	Communication Deficits	1	3	Audiology --> Hearing Aids	3	3	1	**11**
COL1A1	120150	Caffey Disease	114000	Hyperostosis and pain	1	3	Monitor for pain, early pain management with symptoms	2	3	2	**11**
COL3A1	120180	Ehlers-Danlos Syndrome - Vascular Type	130050	Arterial Dissection / Organ Rupture	3	3	Echocardiogram / MRA	1	3	1	**11**
CYP11B1	610613	Adrenal hyperplasia, congenital, due to 11-beta-hydroxylase deficiency	202010	Androgen excess, virilization, and hypertension	2	2	Glucocorticoid Administration	2	3	2	**11**
DFNA5	608798	Deafness, autosomal dominant 5	600994	Communication Deficits	0	3	All Interventions	3	3	2	**11**
DFNB59	610219	Non-Syndromic Deafness, Recessive	610220	Communication Deficits, with or without auditory neuropathy spectrum	1	3	All Interventions	3	3	1	**11**
DHCR7	602858	Smith-Lemli-Opitz syndrome	270400	Microcephaly, developmental delay, behavioral issues, congenital anomalies	2	3	Cholesterol supplementation	1	3	2	**11**
DIAPH1	602121	Deafness, autosomal dominant 1	124900	Communication Deficits	1	3	All Interventions	3	3	1	**11**
DOCK8	611432	Hyper-IgE recurrent infection syndrome, autosomal recessive	243700	Combined Immunodeficiency	2	2	HSCT	3	2	2	**11**
DSG2	125671	Arrhythmogenic right ventricular cardiomyopathy 10	610193	Sudden death due to arrhythmia	3	2	Echo screening/no sports/ICD	2	2	2	**11**
DSP	125647	Arrhythmogenic right ventricular cardiomyopathy 8	607450	Sudden death due to arrhythmia	3	2	Echo screening/no sports/ICD	2	2	2	**11**
EDNRB	131244	Waardenburg syndrome, type 4A	277580	Communication Deficits or Hirschsprung Disease	1	2	Audiology --> Hearing Aids --> Cochlear Implant or Screening -->	3	3	2	**11**
ESPN	606351	Deafness, autosomal recessive 36	609006	Communication Deficits with or without Vestibular Involvement	1	3	Audiology --> Hearing Aids	3	3	1	**11**
ESRRB	602167	Non-Syndromic Deafness, Recessive 35	608565	Communication Deficits	1	3	All Interventions	3	3	1	**11**
EYA1	601653	Branchiootorenal syndrome 1, with or w/o cataracts / Anterior segment anomalies with or w/o cataracts	113650	Communication Deficits or Renal anomalies, abnl function, electrolyte	1	3	Audiology / Nephrology eval - Test renal function --> Treat to prevent	2	3	2	**11**
EYA4	603550	Non-Syndromic Deafness, Dominant 10	601316	Communication Deficits	1	3	All Interventions	3	3	1	**11**
F5	612309	Thrombophilia due to activated protein C resistance	188055	Risk for thrombosis	2	2	Consider prophylaxis with surgery, avoidance of venous stasis	2	3	2	**11**
FGFR3	134934	CATSHL syndrome	610474	Communication deficits	1	3	Audiology --> Hearing Aids --> Cochlear Implant	3	3	1	**11**
FGFR3	134934	Hypochondroplasia	146000	Craniosynostosis --> increased intracranial pressure	1	3	Physical exam --> imaging to screen --> surgery if necessary	2	2	3	**11**
FOXE1	602617	Bamforth-Lazarus syndrome	241850	absent thyroid tissue, hypothyroidism	2	3	Thyroid Hormone Replacement	2	3	1	**11**

GLA	300644	Fabry disease - male hemizygous	301500	Heart / renal involvement	2	2	Enzyme replacement	2	2	3	**11**
GYS2	138571	Glycogen Storage Disease, type 0	240600	Hypoglycemia	1	3	Avoid fasting, frequent feeding, emergency letter	3	3	1	**11**
HBG2	142250	Cyanosis, transient neonatal	613977		0	3	Avoidance of Unnecessary Intervention	3	3	2	**11**
HFE - C282Y / H63D	613609	Hereditary hemochromatosis C282Y / H63D Compound Hets		Multiple system iron overload (heart, liver)	2	0	Yearly ferritin -> phlebotomy	3	3	3	**11**
HGF	142409	Deafness, autosomal recessive 39	608265	Communication Deficits	1	3	All Interventions	3	3	1	**11**
HMGCL	613898	HMG-CoA lyase deficiency	246450	Severe hypoglycemia, metabolic acidosis, coma, death	2	3	Avoid fasting, low protein diet, restrict leucine and supplement l-	2	2	2	**11**
HMGCS2	600234	HMG-CoA synthase-2 deficiency	605911	Hypoketotic hypoglycemic, sz and coma	2	2	Avoid fasting, carnitine supplementation	3	3	1	**11**
HSB11B2	614232	Apparent mineralocorticoid excess	218030	Hypertensive crisis (onset ranges from childhood to adult)	2	3	Monitoring, spironolactone	2	3	1	**11**
HSD11B2	614232	Apparent mineralocorticoid excess	218030	Hypertensive crisis (onset ranges from childhood to adult)	2	3	Monitoring, spironolactone	2	3	1	**11**
IDS	300823	Mucopolysaccharidosis Type II	309900	All Outcomes	2	3	Enzyme Replacement Therapy	2	2	2	**11**
IL2RA	147730	Interleukin-2 receptor, alpha chain, deficiency of	606367	T-cell immune deficiency; autoimmune disease; enteropathy;	2	3	'allogeneic bone marrow transplant following cytoreduction'	3	2	1	**11**
KARS	601421	Deafness, autosomal recessive 89	613916	Communication Deficits	1	3	All Interventions	3	3	1	**11**
KCNE2	603796	Long QT syndrome 6	613693	Sudden death due to arrhythmia	3	2	EKG screening / avoidance of triggers / ICD	2	3	1	**11**
KCNJ5	600734	Long QT syndrome 13	613485	Sudden death due to arrhythmia	3	2	EKG and avoidance of triggers	2	3	1	**11**
LHFPL5	609427	Deafness, autosomal recessive 67	610265	Communication Deficits	1	3	All Interventions	3	3	1	**11**
LMBRD1	612625	Methylmalonic aciduria and homocystinuria, cblF type	277380	All Outcomes	1	3	B12 replacement (Hydroxocobalamin)	2	3	2	**11**
LOXHD1	613072	Deafness, autosomal recessive 77	613079	Communication Deficits	1	3	All Interventions	3	3	1	**11**
MIR96	611606	Deafness, autosomal dominant 50	613074	Communication Deficits	1	3	All Interventions	3	3	1	**11**
MLYCD	606761	Malonyl-CoA Decarboxylase Deficiency	248360	Cardiomyopathy	2	2	High carb, low long chain fatty acid, and medium chain triglyceride and L-	2	3	2	**11**
MSRB3	613719	Deafness, autosomal recessive 74	613718	Communication Deficits	1	3	Audiology --> Hearing Aids	3	3	1	**11**
MTR	156570	Homocystinuria-megaloblastic anemia, cblG complementation type	250940	Severe failure to thrive, megaloblastic anemia, and neurologic manifestations	2	3	B12 replacement	2	3	1	**11**
MTRR	602568	Homocystinuria-megaloblastic anemia, cbl E type	236270	Severe failure to thrive, megaloblastic anemia, and neurologic manifestations	2	3	B12 replacement	2	3	1	**11**
MYH14	608568	Non-Syndromic Deafness, Dominant 4A	600652	Communication Deficits	1	3	Audiology --> Hearing Aids	3	3	1	**11**
MYH9	160775	Deafness, autosomal dominant 17	603622	Communication Deficits	1	3	Audiology --> Hearing Aids	3	3	1	**11**
MYO6	600970	Non-Syndromic Deafness, Recessive 37	607821	Communication Deficits	1	3	Audiology --> Hearing Aids	3	3	1	**11**
MYO7A	276903	Deafness, autosomal dominant 11	601317	Communication Deficits	1	3	Audiology --> Cochlear Implants	3	3	1	**11**
NF2	607379	Neurofibromatosis type 2	101000	Meningiomas; 2.5x increased risk mortality if present	1	3	Annual MRI starting @ 10 yr, avoid radiotherapy	2	3	2	**11**
NPC2	601015	Niemann-Pick Disease, type C2	607625	Mental deterioration --> developmental delay, seizures, psychiatric and	2	3	Miglustat for Stabilization	1	3	2	**11**
OAT	613349	Gyrate Atrophy of the Choroid and Retina	258870	Progressive Chorioretinal Degeneration	1	3	Dietary: restriction of arginine; some responsive to pyridoxal phosphate	2	2	3	**11**
OTOA	607038	Deafness, autosomal recessive 22	607039	Communication Deficits	1	3	All Interventions	3	3	1	**11**
OTOG	604487	AR Nonsyndromic Sensorineural Deafness Type DFNB	614945	Communication Deficits	1	3	Audiology --> Cochlear Implants	3	3	1	**11**
OTOGL	614925	Deafness, autosomal recessive 84B	614944	Communication Deficits	1	3	All Interventions	3	3	1	**11**
P2RX2	600844	Deafness, autosomal dominant 41	608224	Communication Deficits	1	3	All Interventions	3	3	1	**11**
PALB2	610355	{Breast cancer, susceptibility to}	114480	Breast cancer	2	2	Increased surveillance to include MRI	2	3	2	**11**
PAX3	606597	Waardenburg Syndrome, type 3	148820	Communication Deficits	1	2	Audiology --> Hearing Aid --> Cochlear Implant	3	3	2	**11**
PHKG2	172471	Liver Phosphorylase Kinase Deficiency	613027	Hypoglycemia / Hepatomegaly	1	3	Avoidance of fasting, corn starch, night time feedings	3	3	1	**11**
PKP2	602861	Arrhythmogenic right ventricular cardiomyopathy 9	609040	Sudden death due to arrhythmia	3	2	Echo screening/no sports/ICD	2	2	2	**11**
PNP	164050	Immunodeficiency due to purine nucleoside phosphorylase deficiency	613179	All Outcomes	3	3	HSCT	1	2	2	**11**
POLD1	174761	{Colorectal cancer, susceptibility to, 10}	612591	colon cancer	2	3	colonoscopy	3	2	1	**11**

PRKAG2	602743	Cardiomyopathy, Familial Hypertrophic, 6	600858	Sudden Death due to Arrhythmia	3	1	Echo screening / no sports / EKG / ICD	2	3	2	**11**
PRKAG2	602743	Glycogen storage disease of heart, lethal congenital	261740	Arrhythmia	3	1	No Effective Intervention	2	3	2	**11**
PRKAG2	602743	Wolff-Parkinson-White syndrome	194200	Arrhythmia	3	1	Echo screening / no sports / EKG / ICD/Ablation Therapy	2	3	2	**11**
PROC	612283	Thrombophilia due to protein C deficiency, autosomal dominant	176860	deep vein thrombosis with or without pulmonary embolism	2	2	anticoagulation, avoid immobility	2	3	2	**11**
PRPS1	311850	Deafness, X-linked 1	304500	Communication Deficits	1	3	All Interventions	3	3	1	**11**
RDX	179410	Deafness, autosomal recessive 24	611022	Communication Deficits	1	3	All Interventions	3	3	1	**11**
RIT1	609591	Noonan syndrome 8	615355	Hypertrophic cardiomyopathy	2	3	Echo, EKG	2	3	1	**11**
SCN4A	603967	Hyperkalemic periodic paralysis, type 2	170500	Episodic weakness, progressive myopathy	1	3	Dichlorphenamide	3	2	2	**11**
SCN4B	608256	Long QT syndrome-10	611819	Sudden death due to arrhythmia	3	2	EKG screening / avoidance of triggers / ICD	2	3	1	**11**
SERPINC1	107300	Thrombophilia due to antithrombin III deficiency	613118	Thrombosis	2	3	Avoidance of OCPs; immobility; prophylaxis with surgery	2	2	2	**11**
SLC26A5	604943	Deafness, autosomal recessive 61	613865	Communication Deficits	1	3	All Interventions	3	3	1	**11**
SLC2A1	138140	GLUT1 deficiency syndrome 1	606777	Infantile seizures, acquired microcephaly, and developmental delay	2	3	Ketogenic diet	2	2	2	**11**
SLC39A4	607059	Acrodermatitis enteropathica	201100	Dermatitis	0	3	zinc supplementation	3	3	2	**11**
SLC3A1	104614	Cystinuria	220100	Renal calculi and chronic renal failure	1	3	Urine screening, increased fluid, urinary alkalinization, other medical	2	3	2	**11**
SLC7A9	604144	Cystinuria	220100	Renal calculi and chronic renal failure	1	3	Urine screening, increased fluid, urinary alkalinization, other medical	2	3	2	**11**
SMAD4	600993	Juvenile polyposis/hereditary hemorrhagic telangiectasia syndrome	175050	Pulmonary AVM, GI bleeding, CVA from cerebral AVMs?	2	2	Contrast echo	2	3	2	**11**
SMPX	300226	Deafness, X-Linked 4	300066	Communication Deficits	1	3	All Interventions	3	3	1	**11**
SOX10	602229	Waardenburg Syndrome, type 2E	611584	Communication Deficits	1	3	Audiology --> Hearing Aids --> Cochlear Implant	2	3	2	**11**
STK11	602216	Peutz-Jeghers Syndrome	175200	Gastrointestinal Cancer	2	3	Colonoscopy / Upper Endoscopy	2	2	2	**11**
SYNE4	615535	Deafness, autosomal recessive 76	615540	Communication Deficits	1	3	All Interventions	3	3	1	**11**
TAT	613018	Tyrosinemia, type II	276600	All Outcomes	1	3	Dietary restriction	3	2	2	**11**
TBC1D24	613577	Deafness, autosomal recessive 86	614617	Communication Deficits	1	3	All Interventions	3	3	1	**11**
TBX19	604614	Adrenocorticotropic hormone deficiency	201400	Neonatal hypoglycemia, seizures	2	3	Glucocorticoid administration	2	2	2	**11**
TCN2	613441	Transcobalamin II deficiency	275350	FTT	1	2	Metabolic Evaluation --> Cobalamin Supplementation	3	3	2	**11**
TMC1	606706	Deafness, Autosomal Dominant 36	606705	Communication Deficits	1	3	All Interventions	3	3	1	**11**
TMEM43	612048	Arrhythmogenic right ventricular cardiomyopathy 5	604400	Sudden death due to arrhythmia	3	3	Echo screening/no sports/ICD	2	2	1	**11**
TNNT2	191045	Dilated cardiomyopathy 1D	601494	Death due to arrhythmia or heart failure	2	3	Echo screening/ICD	2	3	1	**11**
TP53	191170	Li-Fraumeni syndrome	151623	Multiple Cancers	2	3	Whole Body Imaging	1	3	2	**11**
TPM1	191010	Hypertrophic cardiomyopathy 3	115196	Arrhythmia	3	1	Echo Screening / no sports / ICD	3	3	1	**11**
TRH	613879	Thyrotropin-releasing hormone deficiency	275120	Central hypothyroidism	1	3	Thyroid hormone replacement	3	3	1	**11**
TSC1	605284	Tuberous Sclerosis Complex	191100	CNS tumors and renal lesions	2	3	Screening: Cranial and Renal imaging 1-3yrs, baseline Chest CT for women.	1	3	2	**11**
TSC2	191092	Tuberous Sclerosis Complex	613254	CNS tumors and renal lesions	2	3	Screening: Cranial and Renal imaging 1-3yrs. baseline Chest CT for women.	1	3	2	**11**
VWF	613160	von Willebrand disease, type 1	193400	Mild Mucocutaneous Bleeding	0	2	Hematology --> Desmopressin	3	3	3	**11**
WFS1	606201	Deafness, autosomal dominant 6/14/38	600965	Communication Deficits	0	3	All Interventions	3	3	2	**11**
WFS1	606201	Wolfram Syndrome	222300	All Outcomes	1	3	All Interventions	1	3	3	**11**
ABCD1	300371	Adrenoleukodystrophy	300100	Childhood onset cognitive decline	3	2	HSCT	1	2	2	**10**
ADCK3	606980	Coenzyme Q10 deficiency, primary, 4	612016	Cerebellar ataxia - slow, minimal progression	1	3	CoQ10 treatment	2	3	1	**10**
AHCY	180960	Hypermethioninemia with deficiency of S-Adenosylhomocysteine Hydrolase	613752	Mental and Motor Retardation / ID	2	3	Correction of Biochemical Abnormalities via Dietary Methionine	2	3	0	**10**
ALB	103600	familial dysalbuminemic hyperthyroxinemia	615999	Dysalbuminemic hyperthyroxinemia (typically benign)	1	1	education/avoidance of thyroidectomy	3	3	2	**10**

APTX	606350	Coenzyme Q10 deficiency, secondary	612016	Cerebellar ataxia - slow, minimal progression	1	3	CoQ10 treatment	2	3	1	**10**
ARG1	608313	Argininemia	207800	Spasticity	1	3	Diet, sodium phenylacetate and sodium benzoate	2	2	2	**10**
CABP2	607314	Deafness, autosomal recessive 93	614899	Communication Deficits	1	3	All Interventions	3	3	0	**10**
CDH1	192090	Hereditary diffuse gastric cancer	137215	Gastric Cancer	2	3	Gastrectomy	3	0	2	**10**
COL11A2	120290	Deafness, autosomal dominant 13 (DFNA13)	601868	Communication Deficits	0	3	Audiology --> Hearing Aids	3	3	1	**10**
COL1A1	120150	Osteogenesis Imperfecta, type I	166200	Fractures or Hearing Loss (Conductive --> Sensorineural)	1	2	Biophosphonates	2	2	3	**10**
COL1A2	120160	Osteogenesis imperfecta, type I	166200	Multiple Fractures	1	3	Bisphosphonates, Anticipatory management	1	2	3	**10**
COQ2	609825	Coenzyme Q10 deficiency, primary, 1	607426	Infantile or early childhood onset nephropathy, AND Infantile	2	3	CoQ10 treatment	1	3	1	**10**
COQ9	612837	Coenzyme Q10 deficiency, primary, 5	614654	Infantile multisystem dis w/ rapid progression and high mortality	2	3	CoQ10 treatment	1	3	1	**10**
CPT1A	600528	CPT deficiency, hepatic, type IA	255120	Hypoketotic hypoglycemia, liver failure	2	2	Diet, avoidance of fasting	2	2	2	**10**
CTSK	601105	Pycnodysostosis	265800	Short Stature	0	3	Odanacatib (Clinical Trial Underway)	2	3	2	**10**
DFNB31	607928	Usher Syndrome, type 2D	611383	Usher Phenotype (Communication Deficits + RP)	1	3	Audiology and Vision Services	2	3	1	**10**
DNAAF3	614566	Ciliary Dyskinesia, Primary, 2	606763	Chronic Sinopulmonary Disease	1	3	Management of symptoms: enhance mucous clearance similar to CF	2	3	1	**10**
EYA1	601653	?Otofaciocervical Syndrome 1	166780	Communication Deficits	1	3	Audiology --> Hearing Aids --> Cochlear Implant	3	3	0	**10**
F12	610619	Angioedema, hereditary, type III	610618	Swelling --> Respiratory Compromise	2	2	Tranexanic Acid (Prophylactically)	2	3	1	**10**
FGFR3	134934	Crouzon syndrome with acanthosis nigricans	612247	Craniosynostosis --> increased intracranial pressure	1	3	Physical exam --> imaging to screen --> surgery if necessary	3	1	2	**10**
FH (Dominant)	136850	Leiomyomatosis and renal cell cancer	150800	Renal Cancer	2	2	Abdominal imaging	2	3	1	**10**
FLCN	607273	Birt-Hogg-Dube syndrome	135150	Renal cancer	1	2	High Risk management / imaging, etc	2	3	2	**10**
FUCA1	612280	Fucosidosis	230000	All Outcomes	2	3	BMT / HSCT	2	1	2	**10**
GH1	139250	Growth hormone deficiency, isolated, type IA (recessive)	262400	Postnatal growth deficiency, hypoglycemia	2	2	Avoid fasting, GH replacement	2	2	2	**10**
GJB2	121011	Bart-Pumphrey Syndrome	149200	Communication Deficits	1	3	Audiology --> Hearing Aids --> Cochlear Implant	2	2	2	**10**
GRHL2	608576	Deafness, autosomal dominant 28	608641	Communication Deficits	0	3	All Interventions	3	3	1	**10**
GSS	601002	Glutathione Synthetase Deficiency	266130	Hemolytic Anemia + Metabolic Acidosis + CNS Dysfunction	2	2	Vitamin C and E	2	3	1	**10**
HARS	142810	Usher Syndrome, type 3B	614504	Usher Phenotype (Communication Deficits + RP)	1	3	Audiology and Vision Services	2	3	1	**10**
HARS2	600783	Perrault Syndrome 2	614926	Communication Deficits	1	3	Audiology --> Hearing Aids --> Cochlear Implant	3	3	0	**10**
HNF1A	142410	MODY, type III (MODY3)	600496	Type II diabetes and complications	1	3	Glucose monitoring and early treatment	1	3	2	**10**
HNF1B	189907	Renal cysts and diabetes syndrome (MODY5)	137920	Type II diabetes and complications	1	3	Glucose monitoring, early treatment	1	3	2	**10**
HNF4A	600281	MODY, type I (MODY1)	125850	Type II diabetes and complications	1	3	Glucose monitoring and early treatment	1	3	2	**10**
IDUA	252800	Mucopolysaccharidosis Ih	607014	Sudden Cardiac Death or Cognitive Disability	2	3	HSCT	2	1	2	**10**
IL21R	605383	Immunodeficiency, primary, autosomal recessive, IL21R-related	615207	chronic cholangitis and liver disease associated with cryptosporidial	2	3	HSCT	3	2	0	**10**
KCNJ5	600734	Hyperaldosteronism, familial, type III	613677	Hypertensive crisis (onset usually in childhood)	2	3	Adrenalectomy	3	1	1	**10**
LAMB1	150240	Lissencephaly 5	615191	psychomotor retardation/seizures	2	3	Antiepileptics	1	3	1	**10**
LARS2	604544	Perrault Syndrome 4	615300	Communication Deficits	1	3	Audiology --> Hearing Aids --> Cochlear Implant	3	3	0	**10**
LIPA	613497	Cholesteryl Ester Storage Disease (Lysosomal Acid Lipase) *OR* Wolman Disease	278000	CEPD: Late onset, slow progressing liver disease	1	3	Screening --> Liver Transplant; ERT w/ Sebelipase Alfa	2	2	2	**10**
LYST	606897	Chediak-Higashi syndrome	214500	Accelerated phase (multiorgan inflammation, lymphoproliferative	2	3	Monitoring of organomegaly and liver dysfunction. CBC for cytopenias -->	2	1	2	**10**
MMADHC	611935	Methylmalonic aciduria and homocystinuria, cblD type	277410	Metabolic decompensation	2	3	Diet, B-12, illness management	2	2	1	**10**
MYBPC3	600958	Hypertrophic cardiomyopathy 4	115197	Arrhythmia	3	1	Echo Screening / no sports / ICD	1	3	2	**10**
MYH9	160775	Macrothrombocytopenia and progressive sensorineural deafness	600208	Communication Deficits	1	3	Audiology --> Hearing Aids --> Cochlear Implant	2	2	2	**10**
NF1	613113	Neurofibromatosis, type 1	162200	Neurofibromatosis / MPNST	2	2	Annual exam, awareness of symptoms	1	3	2	**10**

PDSS1	607429	Coenzyme Q10 deficiency, primary, 2	614651	Infantile multisystem dis w/ rapid progression and high mortality	2	3	CoQ10 treatment	1	3	1	**10**
PDSS2	610564	Coenzyme Q10 deficiency, primary, 3	614652	Infantile multisystem dis w/ rapid progression and high mortality	2	3	CoQ10 treatment	1	3	1	**10**
PKP2	602861	Arrhythmogenic right ventricular cardiomyopathy 9	609040	Arrhythmia	3	2	Echo Screening / no sports / ICD	1	2	2	**10**
PNPT1	610316	Deafness, autosomal recessive 70	614934	Communication Deficits	1	3	All Interventions	3	3	0	**10**
PYGL	613741	Glycogen Storage Disease VI	232700	All Outcomes	0	3	Dietary increase in protein / corn starch 1-3 times daily	3	3	1	**10**
RAB23	606144	Carpenter Syndrome	201000	Increased Intracranial Pressure	2	2	Monitoring --> Surgery when indicated to correct skull sutures	3	1	2	**10**
SDHB	185470	Hereditary Paraganglioma-Pheochromocytoma Syndrome 4	115310	Nonmalignant PGL / PCC	2	2	Annual Biochemical Screening	1	3	2	**10**
SDHD	602690	Hereditary Paraganglioma-Pheochromocytoma Syndrome 1	168000	Nonmalignant PGL / PCC	1	3	Annual Biochemical Screening	1	3	2	**10**
SFTPB	178640	Surfactant metabolism dysfunction, pulmonary, 1	265120	All Outcomes	3	3	Lung Transplant	1	1	2	**10**
SFTPC	178620	Surfactant metabolism dysfunction, pulmonary, 2	610913	Respiratory distress / respiratory failure	1	3	anticipatory guidance/ hydroxychloroquine / systemic	2	2	2	**10**
SIX1	601205	Branchio-oto-renal related disorders	608389	Communication Deficits / Renal anomalies	1	3	Audiology / Nephrology evaluaton and management	2	3	1	**10**
SLC17A8	607557	Deafness, autosomal dominant 25	605583	Communication Deficits	0	3	Audiology --> Hearing Aids	3	3	1	**10**
SLC22A5	603377	Carnitine deficiency, systemic primary	212140	Metabolic decompensation, broad clinical spectrum	1	1	L-carnitine supplementation	3	3	2	**10**
SLC25A13	603859	Citrullinemia, type II, neonatal-onset	605814	Failure to thrive, cirrhosis. But most have resolution by 6 months	1	2	Dietary formula, vitamin D	2	3	2	**10**
SLC25A15	603861	Hyperornithinemia-Hyperammonemia-Homocitrullinemia Syndrome	238970	Neurocognitive Deficits	1	3	Dietary, Avoid high protein intake, Screening for Amonimia	2	2	2	**10**
SLC2A9	606142	Hypouricemia, renal, 2	612076	Hypouricemia -->exercise induced acute renal failure	1	2	Dialysis	3	2	2	**10**
SMAD3	603109	Loeys-Dietz Syndrome 3	613795	Aortic Dissection	3	1	Annual Echocardiogram	2	3	1	**10**
SNAI2	602150	Waardenburg Syndrome, type 2D	608890	Communication Deficits	1	3	Audiology --> Hearing Aid --> Cochlear Implant	3	3	0	**10**
SOX10	602229	PCWH Syndrome	609136	Neurologic Abnormalities (Developmental delay, mental	2	3	Audiology --> Hearing Aids --> Cochlear Implant	1	3	1	**10**
TBC1D24	613577	Deafness, autosomal dominant 65	616044	Communication Deficits	0	3	All Interventions	3	3	1	**10**
TSPEAR	612920	Deafness, autosomal recessive 98	614861	Communication Deficits	1	3	All Interventions	3	3	0	**10**
USH1C	605242	Deafness, autosomal recessive 18A	602092	Communication Deficits	1	3	Audiology --> Hearing Aids	3	3	0	**10**
WT1	607102	WT1-related Wilms	194070	Wilms Tumor	2	2	Abdominal US 3x/year --> surgical removal (if needed)	2	3	1	**10**
ACADSB	600301	2-Methylbutyrylglycinuria	610006	Hypoglycemia, acidosis, seizure, coma	2	0	Avoid Fasting	3	3	1	**9**
AGA	613228	Aspartylglucosaminuria	208400	Mental deterioration/Mental retardation --> seizures	2	3	BMT	1	1	2	**9**
ALDH4A1	606811	Hyperprolinemia, type II	239510	Epilepsy	1	3	Vitamin B6 Supplementation	1	3	1	**9**
AMT	238310	Glycine Encephalopathy	605899	Epileptic Encephalopathy or Seizures	2	3	Avoid valproate; NaBenzoate may have some effect or ketogenic diet	1	1	2	**9**
ANK2	106410	Long QT Syndrome 4	600919	Sudden Death due to Arrhythmia	3	0	EKG screening / avoidance of triggers / ICD	2	3	1	**9**
ARSA	607574	Metachromatic Leukodystrophy	250100	All Outcomes	2	3	All Interventions	1	1	2	**9**
BRIP1	605882	Breast Cancer, early-onset	114480	breast cancer	2	2	Early and increased screening (every 6 months) with breast MRI.	1	3	1	**9**
BRIP1	605882	Fanconi Anemia complementation group J	609054	Bone marrow failure, leukemia	2	3	Surveillance, HSCT	2	1	1	**9**
CLCNKB	602023	Bartter syndrome, type 3	607364	Renal failure due to salt-wasting	1	3	Sodium and potassium supplements and aldosterone antagonists and	1	3	1	**9**
CNNM2	607803	Hypomagnesemia 6, Renal	613882	Seizures	1	3	Magnesium Supplements --> Antiepileptics	1	3	1	**9**
COL1A1	120150	Osteogenesis Imperfecta Type IV	166220	Limb Deformity	1	3	Pamidronate Therapy	2	2	1	**9**
COL1A2	120160	Osteogenesis imperfecta 2	166210	Severe, congenital fractures	3	3	No Effective Intervention	0	0	3	**9**
COQ6	614647	Coenzyme Q10 deficiency, primary, 6	614650	Infant to juvenile onset nephropathy w/ deafness AND Infantile multisystem dis	1	3	CoQ10 Treatment	1	3	1	**9**
DMD	300377	Becker Muscular Dystrophy	300376	Heart Failure	2	3	cardiac evaluations --> ACE inhibitor / beta blockers	0	3	1	**9**
DMD	300377	Duchenne Muscular Dystrophy	310200	cardiopulmonary failure	2	3	cardiac evaluations --> ACE inhibitor / beta blockers	0	3	1	**9**
ETFA	608053	Glutaric acidemia IIA (severe forms)	231680	Neonatal acidosis and hypoglycemia	3	3	No Effective Intervention	0	0	3	**9**

ETFB	130410	Glutaric acidemia IIB (severe forms)	231680	Neonatal acidosis and hypoglycemia	3	3	No Effective Intervention	0	0	3	**9**
ETFDH	231680	Glutaric acidemia IIC (severe forms)	231680	Neonatal acidosis and hypoglycemia	3	3	No Effective Intervention	0	0	3	**9**
ETHE1	608451	Ethylmalonic Encephalopathy	602473	Death secondary to Neurodegeneration	3	3	Riboflavin and CoQ10 Supplementation	0	2	1	**9**
F11	264900	Factor XI deficiency, autosomal recessive	612416	Bleeding	0	2	Fresh frozen plasma with procedures / trauma or F11	3	2	2	**9**
FGFR3	134934	Thanatophoric dysplasia, type I	187600	Lethal Skeletal Dysplasia	3	3	No Effective Intervention	0	0	3	**9**
FGFR3	134934	Thanatophoric dysplasia, type II	187601	Lethal Skeletal Dysplasia	3	3	No Effective Intervention	0	0	3	**9**
FLCN	607273	Birt-Hogg-Dube syndrome	135150	Renal cancer	2	1	Abdominal imaging	2	3	1	**9**
FTCD	606806	Glutamate Formiminotransferase Deficiency	229100	Intellectual Disability	1	3		1	3	1	**9**
GALC	606890	Krabbe Disease	245200	Death by 2 years due to progressive neurologic deterioration	3	3	Supportive Management	0	0	3	**9**
GALNS	612222	Mucopolysaccharidosis IVA	253000	Skeletal	1	3	ERT	1	2	2	**9**
GAMT	601240	Cerebral creatine deficiency syndrome 2; Guanidinoacetate methyltransferase deficiency	612736	Autism, extrapyramidal symptoms	1	3	Creatine supplementation	1	3	1	**9**
GATM	602360	Cerebral creatine deficiency syndrome 3; L-arginine:glycine amidinotransferase deficiency	612718	Encephalopathy	1	3	Creatine supplementation	1	3	1	**9**
GBA	606463	Gaucher Disease, all other types (perinatal lethal, type II, type III, type IIIC)		Progressive Neurologic Deterioration	3	3	No Effective Intervention	0	0	3	**9**
GCK	138079	Diabetes mellitus, permanent neonatal	606176	Neonatal diabetes	3	0	Insulin	3	3	0	**9**
GJB6	604418	Non-Syndromic Deafness, Dominant 3B	612643	Communication Deficits	1	2	All Interventions	3	3	0	**9**
GRHL2	608576	Ectodermal dysplasia/short stature syndrome	616029	Communication Deficits	1	2	Audiology --> Hearing Aids --> Cochlear Implants	3	3	0	**9**
GUSB	611499	Mucopolysaccharidosis VII	253220	Moderate with some Organomegaly and Moderate Skeletal Abnormalities	2	3	Clinical Monitoring --> HSCT	2	1	1	**9**
HEXA	606869	Tay-Sachs Disease	272800	Psychomotor degeneration --> Hypotonia, seizures, dementia	3	3	No Effective Intervention	0	0	3	**9**
HEXB	268800	Sandhoff Disease	606873	All Outcomes	3	3	No Effective Intervention	0	0	3	**9**
HSD17B4	601860	Perrault Syndrome 1	233400	All Outcomes	1	3	All Interventions	2	3	0	**9**
HTT	613004	Huntington disease	143100	Neurodegeneration	3	3	No Effective Intervention	0	0	3	**9**
KMT2D	602113	Kabuki syndrome	147920	Intellectual disability	1	3	Early childhood intervention	1	3	1	**9**
LCK	153390	Immunodeficiency 22	615758	SCID	3	0	BMT	3	2	1	**9**
LIG4	601837	LIG4 Syndrome	606593	combined immune deficiency (not severe, infantile onset)	1	3	Hematologic evaluation --> HSCT	1	3	1	**9**
LIG4	601837	Severe Combined Immunodeficiency with Sensitivity to Ionizing Radiation	602450	SCID	3	0	Hematologic Evaluation --> HSCT	2	3	1	**9**
MAN2B1	609458	Mannosidosis, alpha-, types I and II	248500	Intellectual Disability/neurological motor problems	1	3	BMT	2	1	2	**9**
MAX	154950	pheochromocytoma susceptibility	171300	malignant pheochromocytoma/paraganglioma	2	2	Biochemical Screening	1	3	1	**9**
MYBPC3	600958	Cardiomyopathy, dilated, 1MM	615396	Arrhythmia or heart failure	3	0	Echo Screening / ICD	2	3	1	**9**
MYO3A	606808	Deafness, autosomal recessive 30	607101	Communication Deficits	0	3	Audiology --> Hearing Aids	3	3	0	**9**
MYOZ2	605602	Cardiomyopathy, Familial Hypertrophic, 16	613838	Sudden Death due to Arrhythmia	3	0	Echo Screening / No Sports / ICD	3	2	1	**9**
NDUFS4	602694	Leigh Syndrome	256000	Typical Leigh syndrome: neurodegeneration, lactic acidosis,	3	3	No Effective Intervention	0	0	3	**9**
NEUROD1	601724	MODY, type VI (MODY6)	606394	Type II diabetes and complications	1	3	Glucose monitoring, early treatment	1	3	1	**9**
NFKB2	164012	Immunodeficiency, common variable, 10	615577	immunodeficiency, common variable	1	2	Immune work-up -> antibiotics, immunoglobulin replacement	2	3	1	**9**
NHEJ1	611290	Severe combined immunodeficiency with microcephaly, growth retardation, and sensitivity to ionizing radiation	611291	Combined Immunodeficiency	3	0	Immunological Evaluation --> HSCT	2	3	1	**9**
NKX2-6	611770	Persistent truncus arteriosus / Conotruncal heart defects	217095	Cyanotic heart disease / heart failure	2	0	Echo evaluation --> Open heart surgery if needed	3	3	1	**9**
NODAL	601265	Heterotaxy, Visceral	270100	heart failure/biliary atresia - jaundice / splenic dysfunction - susceptibility to	2	2	Echo evaluation --> meds, surgery (heart transplant for most severe	1	3	1	**9**
PDX1	600733	MODY, type IV (MODY4)	606392	Type II diabetes and complications	1	3	Glucose monitoring, early treatment	1	3	1	**9**
PFKM	610681	Glycogen storage disease VII	232800	Exercise intolerance, muscle cramping, exertional myopathy, hemolytic anemia	1	2	Diet, avoid strenuous exercise	2	3	1	**9**
PGAM2	612931	Glycogen storage disease X	261670	Myoglobinuria, exercise intolerance, muscle cramps, rhabdomyolysis -->	1	2	Avoidance of Exercise	2	3	1	**9**

PPT1	600722	Ceroid Lipofuscinosis, neuronal, 1 (CLN1)	256730	Neural and retinal degeneration	3	3	No Effective Intervention	0	0	3	**9**
PTCH1	601309	Basal cell nevus syndrome	109400	Medulloblastoma	2	2	Neuro exam, FOC, ophtho (eval hydrocephalus)	1	3	1	**9**
PTS	612719	Hyperphenylalaninemia, BH4-deficient, A	261640	Cognitive impairment plus neurological features	1	3	Diet, BH4, L-DOPA, and 5-HTP	2	2	1	**9**
QDPR	612676	Hyperphenylalaninemia, BH4-deficient, C	261630	Cognitive impairment plus neurological features	1	3	Diet, BH4, L-DOPA, and 5-HTP	2	2	1	**9**
RSPO1	609595	Palmoplantar hyperkeratosis with squamous cell carcinoma of skin and sex reversal	610644	squamous cell carcinoma	2	2	avoidane of sun	1	3	1	**9**
SDHC	602413	Hereditary Paraganglioma-Pheochromocytoma Syndrome 3	605373	Nonmalignant PGL / PCC	1	3	Annual Biochemical Screening	0	3	2	**9**
SERPINB6	173321	?Deafness, Autosomal Recessive 91	613453	Communication Deficits	0	3	Audiology --> Hearing Aids	3	3	0	**9**
SLC26A2	606718	Multiple epiphyseal dysplasia	256050	Joint Pain	1	3	PT / OT	1	3	1	**9**
SLC2A1	138140	GLUT1 deficiency syndrome 2	612126	Paroxysmal exercise-induced dyskinesia	1	3	Ketogenic diet	2	2	1	**9**
SMPD1	607608	Niemann-Pick Disease, Type A	257200	Neurologic Degeneration	3	3	No Effective Intervention	0	0	3	**9**
SNTA1	601017	Long QT syndrome 12	612955	Sudden death due to arrhythmia	3	0	EKG screening / avoidance of triggers / ICD	2	3	1	**9**
TGFB3	190230	Arrhythmogenic right ventricular dysplasia 1	107970	Sudden death due to arrhythmia	3	0	Echo/MRI screening/no sports/ICD	2	3	1	**9**
TNNI3	191044	Hypertrophic cardiomyopathy 7	613690	Arrhythmia	3	1	Echo Screening / no sports / ICD	1	3	1	**9**
TPP1	607998	Ceroid lipofuscinosis, neuronal, 2 (CLN2)	204500	Neural and Retinal Degeneration	3	3	No Effective Intervention	0	0	3	**9**
TRHR	188545	Thyrotropin-releasing hormone resistance, generalized	188545	All Outcomes	0	3	Thyroxine Replacement Therapy	3	3	0	**9**
ZMYND10	607070	Ciliary Dyskinesia, Primary, 22	615444	Chronic Sinopulmonary Disease	1	2	Management of symptoms: enhance mucous clearance similar to CF	2	3	1	**9**
ACTC1	102540	Hypertrophic cardiomyopathy 11	612098	Arrhythmia	3	1	Echo Screening / no sports / ICD	1	3	0	**8**
AKAP9	604001	Long QT syndrome 11	611820	Sudden death due to arrhythmia	3	0	EKG screening / avoidance of triggers / ICD	2	3	0	**8**
ASAH1	613468	Farber Lipogranulomatosis	228000	All Outcomes	3	3	No Effective Intervention	0	0	2	**8**
CATSPER2	607249	Sensorineural Deafness and Male Infertility	611102	Male Infertility	0	3	ARTs such as Intracytoplasmic Sperm Injection (ICSI)	2	2	1	**8**
CAV3	601253	Long QT Syndrome 9	611818	Sudden Death due to Arrhythmia	3	0	EKG screening / avoidance of triggers / ICD	2	3	0	**8**
CD247	186780	Immunodeficiency due to defect in CD3-zeta	610163	SCID	2	0	BMT	3	2	1	**8**
CD3E	186830	Immunodeficiency 18 (Severe Combined Immunodeficiency)	615615	SCIDs	3	0	Immunoglobulin	3	2	0	**8**
CDKN1B	600778	Multiple endocrine neoplasia, type IV	610755	Multiple endocrine tumors	1	0	Biochemical/imaging screening	3	3	1	**8**
CLN3	607042	Ceroid lipofuscinosis, neuronal, 3	204200	Neural and retinal degeneration	2	3	Supportive / Palliative	0	0	3	**8**
DNAJC5	611203	Ceroid lipofuscinosis, neuronal, 4, Parry type (CLN4B)	162350	Neural and retinal degeneration	2	3	No Effective Intervention	0	0	3	**8**
DSC2	125645	Arrhythmogenic right ventricular cardiomyopathy 11	610476	Sudden death due to arrhythmia	3	0	Echo screening/no sports/ICD	2	2	1	**8**
FBXL4	605654	Mitochondrial DNA Depletion Syndrome 13 (Encephalomyopathic type)	615471	Lactic acidosis, encephalopathy leading to death	3	3	No Effective Intervention	0	0	2	**8**
GCK	138079	MODY, type II (MODY2)	125851	Type II diabetes and complications	1	1	Glucose monitoring and early treatment	1	3	2	**8**
GHRHR	139191	Growth hormone deficiency, isolated, type IB	612781	Pituitary dwarfism	1	3	GHRH, GH replacement	1	2	1	**8**
LAMC3	604349	Cortical malformations, occipital	614115	Epilepsy	2	3	Cortical resection	3	0	0	**8**
MCCC1	609010	3-Methylcrotonyl-CoA Carboxylase 1 Deficiency	210200	Mild Weakness	0	3	Leucine-restricted Diet, Carnitine Supplementation	1	3	1	**8**
MTHFR	607093	{Thromboembolism, susceptibility to}	188050	Risk for thrombosis	2	0	Folic acid	1	3	2	**8**
MYH11	160745	Familial Thoracic Aortic Aneurysms 4	132900	Aortic Dissection	3	0	Annual Echocardiogram	2	3	0	**8**
MYLK	600922	Familial Thoracic Aortic Aneurysms 7	613780	Aortic Dissection	3	0	Annual Echocardiogram	2	3	0	**8**
NAGLU	609701	Mucopolysaccharidosis type IIIB (Sanfilippo B)	252920	All Outcomes	3	3	No Effective Intervention	0	0	2	**8**
NDUFS3	603846	Leigh syndrome due to mitochondrial complex I deficiency	256000	encephalopathy, myopathy, developmental delay, lactic acidosis.	3	3	No Effective Intervention	0	0	2	**8**
NDUFS4	602694	Mitochondrial Complex I Deficiency	252010	Failure to thrive, hypotonia, cardiorespiratory failure with or w/o	3	3	No Effective Intervention	0	0	2	**8**
NKX2-5	600584	Hypothyroidism, congenital nongoitrous, 5	225250	Thyroid Dysgenesis	2	0	Thyroid Evaluation	3	3	0	**8**

NKX3-2	602183	Spondylo-megaepiphyseal-metaphyseal dysplasia	613330	C-spine instability	1	3	C-spine stabilization	2	1	1	**8**
PCSK9	607786	Familial hypercholesterolemia 3	603776	Hypercholesterolemia / Early MI	2	0	Cholesterol screening / Statins	2	3	1	**8**
PEX19	600279	Peroxisome biogenesis disorder 12A (Zellweger)	614886	death due to apnea or other respiratory compromise	3	3	No Effective Intervention	0	0	2	**8**
PTGER2	176804	Susceptibility to aspirin-induced asthma	208550	Aspirin-induced asthma	1	1	Avoidance of aspirin/NSAIDs	1	3	2	**8**
RAI1	607642	Smith-Magenis	182290	Intellectual disability and behavioral disturbance	2	3	No Effective Intervention	0	0	3	**8**
RNU4ATAC	601428	Microcephalic osteodysplastic primordial dwarfism, type I	210710	intrauterine growth retardation, abnormalities in multiple organs, and	3	3	No Effective Intervention	0	0	2	**8**
SECISBP2	607693	Thyroid hormone metabolism, abnormal	609698	ID	1	1	Selenium & L-T3 administration	2	3	1	**8**
SLC17A5	604322	Sialic acid storage disorder, infantile	269920	All Outcomes	3	3	No Effective Intervention	0	0	2	**8**
SMARCB1	601607	Schwannomatosis-1, susceptibility to	162091	Multiple cutaneous neurilemmomas and spinal schwannomas	1	2	MRI --> pain medicine / surgery	2	2	1	**8**
SMPD1	607608	Niemann-Pick Disease, Type B	607616	Hepatomegaly and Respiratory	2	3	No Effective Intervention	0	0	3	**8**
SURF1	185620	Leigh syndrome, due to COX deficiency	256000	All Outcomes	3	3	None	0	0	2	**8**
TPM1	191010	Cardiomyopathy, dilated, 1Y	611878	Arrhythmia or heart failure	3	0	Echo Screening / ICD	2	3	0	**8**
TSPAN12	613138	Familial exudative vitreoretinopathy (FEVR)	613310	vision loss due to retinal ischemia	0	2	ophthalmologic screening --> Prophylactic cryotherapy or argon	2	2	2	**8**
AGBL1	615496	Corneal Dystrophy, Fuchs endothelial, 8	615523	Marked Vision Loss	1	1	Surgery	2	1	2	**7**
AKT2	164731	Hypoinsulinemic Hypoglycemia with Hemihypertrophy	240900	Hypoglycemia	3	0	Oral Glucose Therapy	1	2	1	**7**
CAV3	601253	Familial HCM	192600	Sudden death due to arrhythmia	3	0	Echo screening/no sports/ICD	1	3	0	**7**
CEMIP	608366	?Deafness, Nonsyndromic		Communication Deficits	1	0	Audiology Screening --> Cochlear Implant	3	3	0	**7**
CHRNA1	100690	Congenital slow-channel myasthenic syndrome	601462	Congenital myasthenia	2	3	No Effective Intervention	0	0	2	**7**
CHRNA1	100690	Multiple pterygium syndrome, lethal type; Myasthenic syndrome, fast-channel congenital; Myasthenic		Fetal akinesia	3	3	No Effective Intervention	0	0	1	**7**
CLN5	608102	Ceroid lipofuscinosis, neuronal, 5 (CLN5)	256731	Neural and retinal degeneration	2	3	No Effective Intervention	0	0	2	**7**
CLN6	606725	Ceroid lipofuscinosis, neuronal, 6	601780	Neural and Retinal Degeneration	2	3	No Effective Intervention	0	0	2	**7**
CLN8	607837	Ceroid lipofuscinosis, neuronal, 8 (CLN8)	600143	Neural and Retinal Degeneration or Epileptic Seizures / ID (Northern	2	3	No Effective Intervention / Symptom Management	0	0	2	**7**
CLPP	601119	Deafness, autosomal recessive 81 (aka Perrault syndrome 3)	614129	Communication Deficits or Ovarian Dysfunction (Ovarian Dysgenesis -->	1	2	Audiology --> Hearing Aids --> Cochlear Implants or Oral or topical	2	2	0	**7**
COG6	606977	Congenital disorder of glycosylation, type III	614576	Encephalopathy, multisystem disease, inflammatory bowel	3	3	No Effective Intervention	0	0	1	**7**
COL6A3	120250	Ulrich congenital muscular dystrophy	254090	Childhood onset myopathy and respiratory failure	2	3	No Effective Intervention	0	0	2	**7**
CRYM	123740	Deafness, autosomal dominant 40		Communication Deficits	1	0	Audiology --> Hearing Aids	3	3	0	**7**
CTSD	116840	Ceroid lipofuscinosis, neuronal, 10 (CLN10)	610127	Neural and retinal degeneration	3	3	No Effective Intervention	0	0	1	**7**
DIABLO	605219	Deafness, autosomal dominant 64	614152	Communication Deficits	1	0	Audiology --> Hearing Aids	3	3	0	**7**
DLD	238331	Dihydrolipoamide dehydrogenase deficiency	246900	Mitochondrial encephalopathy / Leigh syndrome	3	3	Carnitine, CoQ10, mito cocktail	0	0	1	**7**
DMD	300377	Cardiomyopathy, Dilated, 3B	302045	Heart Failure	2	3	ECG--> anti-congestive medications/ cardiac transplantation	0	1	1	**7**
F11	264900	Factor XI deficiency, autosomal dominant	612416	Bleeding	0	1	Fresh frozen plasma with procedures / trauma or F11	3	2	1	**7**
FH (Recessive)	136850	Fumarase deficiency	606812	Encephalopathy	2	3	No Effective Intervention	0	0	2	**7**
GBE1	607839	Glycogen Storage Disease IV	232500	Progressive Neurodegerative, Fetal akinesia, Hypotonia --> Failure to thrive	3	2	No Effective Intervention or Liver Transplant	0	0	2	**7**
GJB3	603324	Non-Syndromic Deafness, Recessive		Communication Deficits	1	0	Audiology --> Hearing Aids	3	3	0	**7**
GLB1	611458	Beta-galactosidase-1 deficiency GLB1 deficiency		Skeletal Dysplasia	1	3	No Effective Intervention	0	0	3	**7**
GLIS3	610192	Diabetes mellitus, neonatal, with congenital hypothyroidism	610199	Diabetes	2	0	Insulin Therapy	3	1	1	**7**
GM2A	613109	GM2-gangliosidosis, AB variant	272750	Neural degeneration --> Psychomotor delay, seizures, paralysis, dementia,	3	3	No effective intervention / Symptom management	0	0	1	**7**
GNPTAB	607840	Mucolipidosis II (I-cell disease)	252500	"Syndromic"	2	3	No Effective Intervention	0	0	2	**7**

GNS	607664	Mucopolysaccharidosis type IIID	252940	Respiratory Distress	2	3	No Effective Intervention	0	0	2	**7**
HGD	607474	Alkaptonuria	203500	Arthritis	1	3	Oral Nitisinone	0	0	3	**7**
HGSNAT	610453	Mucopolysaccharidosis type IIIC	252930	CNS Degeneration --> behavioral problems, seizures, mental retardation,	2	3	No Effective Intervention	0	0	2	**7**
KIF1B	605995	Pheochromocytoma	171300	Pheochromocytoma	2	0	Biochemical screening, imaging	2	3	0	**7**
MCOLN1	605248	Mucolipidosis IV	252650	All Outcomes	2	3	No Effective Intervention	0	0	2	**7**
MECP2	300005	Rett syndrome	312750	Intellectual disability	2	3	No Effective Intervention	0	0	2	**7**
MFSD8	611124	Ceroid Lipofuscinosis, neuronal, 7 (CLN7)	610951	Neural and retinal degeneration	2	3	No Effective Intervention	0	0	2	**7**
MYL2	160781	Hypertrophic cardiomyopathy 10	608758	Arrhythmia	3	0	Echo Screening / no sports / ICD	1	3	0	**7**
MYL3	160790	Hypertrophic cardiomyopathy 8	608751	Arrhythmia	3	0	Echo Screening / no sports / ICD	1	3	0	**7**
NAGA	104170	Schindler disease, types I and III	609241	All Outcomes	3	3	No Effective Intervention	0	0	1	**7**
NEU1	608272	Sialidosis/ MUCOLIPIDOSIS I	256550	Type II Sialidosis	3	3	No Effective Intervention	0	0	1	**7**
NOTCH3	600276	CADASIL- Cerebral arteriopathy with subcortical infarcts and leukoencephalopathy	125310	cerebrovascular disease/TIAs	2	3	Antiplatelet Therapy	0	0	2	**7**
NTRK1	191315	Familial Medullary Thyroid Cancer (FMTC)	155240	Medullary thyroid cancer	2	0	Thyroidectomy	3	2	0	**7**
PLA2G6	603604	Neurodegeneration with brain iron accumulation	610217	progressive psychomotor decline	2	3	No Effective Intervention	0	0	2	**7**
PLA2G6	603604	Parkinson disease 14	612953	early onset parkinson's with death in young adulthood	2	3	No Effective Intervention	0	0	2	**7**
RP1	603937	Retinitis Pigmentosa	180100	Retinitis Pigmentosa (nonsyndromic, progressive blindness)	1	3	annual or biannual eye exam / vitamin A palmitate	0	0	3	**7**
RPS10	603632	Diamond-Blackfan anemia 9	613308	Anemia	1	0	hematological evaluation followed by coriticosteroid treatment	2	3	1	**7**
SDHAF2	613019	Hereditary Paraganglioma-Pheochromocytoma Syndrome 2	601650	Nonmalignant PGL / PCC	1	2	Annual Biochemical Screening	0	3	1	**7**
SGSH	605270	Mucopolysaccharidisis type IIIA (Sanfilippo A)	252900	CNS degeneration --> Severe behavioral problems, sleep disturbances,impaired	2	3	No Effective Intervention	0	0	2	**7**
SLC26A2	606718	Achondrogenesis Type 1B	600972	Neonatal Death	3	3	Palliative Care	0	0	1	**7**
SLC6A19	608893	Hartnup disorder	234500	Rash, photosensitivity, temporary ataxia, mood disturbance	0	1	Niacin, tryptophan supplementation	1	3	2	**7**
SPRY4	607984	Hypogonadotropic hypogonadism 17 with or without anosmia (Kallman syndrome)	615266	delayed or absent puberty	0	0	pulsatile GnRH or gonadotropin therapy, androgen therapy	3	3	1	**7**
SUMF1	607939	Multiple Sulfatase Deficiency	272200	All Outcomes	3	3	No Effective Intervention / Symptom Management	0	0	1	**7**
TNNT2	191045	Cardiomyopathy, familial hypertrophic, 2	115195	Arrhythmia	3	0	Echo Screening / no sports / ICD	1	3	0	**7**
TREH	275360	Trehalase deficiency	612119	Diarrhea, abdominal pain, increased flatulence	0	0	Avoidance of mushrooms	3	3	1	**7**
ABCA1	600046	Tangier, HDL deficiency, type 1	205400	Early onset coronary artery disease	2	2	lifestyle mods and monitoring, drugs	0	0	2	**6**
ACTG1	102560	Baraitser-Winter syndrome 2	614583	Intellectual disability / developmental delay	2	3	No Effective Intervention	0	0	1	**6**
ACTG2	102545	Visceral Myopathy	155310	Abnormal instestinal motility to functional gastrointestinal obstruction	2	2	No Effective Intervention	0	0	2	**6**
ALDH3A2	609523	Sjogren-Larsson Syndrome	270200	All Outcomes	1	3	All Interventions	0	0	2	**6**
APOA1	107680	Hypoalphalipoproteinemia	604091	Coronary artery disease	2	2	Lifestyle mods and monitoring, drugs	0	0	2	**6**
ASAH1	613468	Spinal Muscular Atrophy with Progressive Myoclonic Epilepsy	159950	Muscle weakness --> Respiratory insufficiency	2	3	No Effective Intervention	0	0	1	**6**
CFTR	602421	CAVD	277180	Azoospermia	0	3	No Effective Intervention	0	0	3	**6**
COL11A2	120290	Fibrochondrogenesis 2	614524	All Outcomes	2	3	No Effective Intervention	0	0	1	**6**
COL11A2	120290	Weissenbacher-Zweymuller syndrome	277610	rhizomelic chondrodysplasia with dumbbell-shaped femora and humeri	1	3	No Effective Intervention	0	0	2	**6**
COL1A1	120150	Osteogenesis Imperfecta Type II	166210	Respiratory Insufficiency	3	3	No Effective Intervention	0	0	0	**6**
CRADD	603454	AR mental retardation 34	614499	Severe cognitive impairment	2	3	No Effective Intervention	0	0	1	**6**
CTSA	613111	Galactosialidosis	256540	Cardiac Involvement	3	2	No Effective Intervention	0	0	1	**6**
DCHS1	603057	Van Maldergem syndrome 1	601390	Syndromic features	2	3	No Effective Intervention	0	0	1	**6**

DCXR	608347	Pentosuria	260800	Increased urinary excretion of L-xylulose	0	3	No Effective Intervention	0	0	3	**6**
DPM1	603503	Congenital Disorder of Glycosylation, Type Ie	608799	Intellectual Disability and Seizures	2	3	No Effective Intervention	0	0	1	**6**
EBP	300205	Chondrodysplasia Punctata, X-linked dominant	302960	Syndromic Features	1	3	No Effective Intervention	0	0	2	**6**
F9	300746	Thrombophilia, X-linked, due to factor IX defect	300807	DVT -> PE	2	0	Advance warning, avoidance of stasis	0	3	1	**6**
GALE	606953	Galactose epimerase deficiency	230350	Galactosemia / 'Intermediate'	1	0	Diet	2	2	1	**6**
GDF5	601146	Acromesomelic dysplasia, Grebe type		Skeletal dysplasia	1	3	No Effective Intervention	0	0	2	**6**
GIGYF2	612003	Parkinson disease 11	607688	Parkinsonism	1	3	No Effective Intervention	0	0	2	**6**
GJB3	603324	Non-Syndromic Deafness, Dominant 2B	612644	Communication Deficits	0	0	Audiology --> Hearing Aids	3	3	0	**6**
GNPTAB	607840	Mucolipidosis IIi alpha/beta, (pseudo-Hurler polydystrophy)		Syndromic features	1	3	No Effective Intervention	0	0	2	**6**
GNPTG	607838	Mucolipidosis III Gamma	252605	Skeletal abnormalities (Scoliosis, kyphosis, stiff joints, dysostosis	1	3	No Effective Intervention	0	0	2	**6**
GRM1	604473	Autosomal recessive SCA 13	614831	Cognitive impairment and movement disorder	2	3	No Effective Intervention	0	0	1	**6**
LAMB1	150240	Lissencephaly 5	615191	severe psychomotor retardation and seizures	2	3	No Effective Intervention	0	0	1	**6**
MANBA	609489	Beta Manosidosis	248510	Intellectual Disabilities and Seizures	1	3	No Effective Intervention	0	0	2	**6**
MMP2	120360	Multicentric osteolysis, nodulosis, and arthropathy	259600	Nodulosis, arthropathy, and osteolysis	1	3	No Effective Intervention	0	0	2	**6**
NAA10	300013	Ogden syndrome	300855	death due to cardiogenic shock and arrhythmia	3	3	N / A	0	0	0	**6**
NDUFS3	603846	Mitochondrial Complex I Deficiency	252010	Encephalopathy, myopathy, developmental delay, lactic acidosis	2	0		1	3	0	**6**
OPA3	606580	3-Methylglutaconic Aciduria, type III	258501	Neurologic Dysfunction	1	3	Metabolic Screening --> Symptom Support	0	0	2	**6**
PDZD7 / ADGRV1	612971	Usher Syndrome, type 2C, GPR98/PDZD7 Digenic	605472	Usher Phenotype (Communication Deficits + RP)	1	0	Audiology and Vision Services	2	3	0	**6**
PIEZO2	613329	Distal arthrogryposis 5	108145	arthrogryposis	1	3	none (no preventative therapy; PT, surg considered for symptomatic	0	0	2	**6**
PORCN	300651	Focal dermal hypoplasia (female carriers)	305600	Developmental delay, dysmorphology	1	3	No Effective Intervention	0	0	2	**6**
REEP1	609139	Hereditary spastic paraplegia 31	610250	Uncomplicated spastic paraplegia	1	3	No Effective Intervention	0	0	2	**6**
SLC6A8	300036	Cerebral creatine deficiency syndrome 1, X-linked	300352	Intellectual disability, seizures	1	3	Creatine supplementation	0	0	2	**6**
SNIP1	608241	Psychomotor retardation, epilepsy, and craniofacial dysmorphism	614501	Severe cognitive impairment, seizures	2	3	No Effective Intervention	0	0	1	**6**
TAS2R38	607751	Phenylthiocarbamide tasting	171200	bitter tasting (not a disease)	0	3	No Effective Intervention	0	0	3	**6**
TJP2		Deafness, autosomal dominant 51	613558	Communication Deficits	0	0	All Interventions	3	3	0	**6**
APOA1	107680	Amyloidosis, 3 or more types	105200	Visceral amyloidosis	2	2	monitor for signs of amylidosis with consideration of liver and renal	0	0	1	**5**
ARHGEF6	300267	Mental Retardation, X-linked 46	300436	Mental Retardation	1	3	Early Childhood Intervention Services	0	0	1	**5**
B4GALT7	604327	Ehlers-Danlos syndrome, progeroid type	130070	Syndromic features	1	3	No Effective Intervention	0	0	1	**5**
BLK	191305	MODY, type XI (MODY11)	613375	Type II diabetes and complications	1	0	Glucose monitoring, early treatment	1	3	0	**5**
CAV3	601253	Limb-girdle muscular dystrophy 1C	607801	Muscle weakness	1	2	No Effective Intervention	0	0	2	**5**
CEL	114840	MODY, type VIII (MODY8)	609812	Type II diabetes and complications	1	0	Glucose monitoring, early treatment	1	3	0	**5**
COG6	606977	Shaheen Syndrome	615328	Intellectual disability	1	3	No Effective Intervention	0	0	1	**5**
COL1A1	120150	Ehlers-Danlos Syndrome, type I	130000	Hypotonia	1	0	physiotherapy, anti-inflammatory drugs, non-weight-bearing exercise	0	3	1	**5**
DGAT1	604900	?Diarrhea 7	615863	Severe congenital diarrhea	2	3	No Effective Intervention	0	0	0	**5**
DNA2	601810	Progressive external ophthalmoplegia, with myopathy	615156	Mild, adult-onset myo-neuropathy	1	3	No Effective Intervention	0	0	1	**5**
DSPP	125485	Deafness, autosomal dominant 36, with dentinogenesis	605594	Dentinogenesis Imperfecta	1	2	No Effective Intervention	0	0	2	**5**
GRM6	604096	Night blindness, congenital stationary (complete), 1B, autosomal recessive	257270	Night blindness	0	3	No Effective Intervention	0	0	2	**5**
H6PD	138090	Cortisone reductase deficiency 1	604931	Hyperandrogenism; premature pseudopuberty (males); adult-onset	1	3	No Effective Intervention	0	0	1	**5**
HBG1	142200	Fetal hemoglobin quantitative trait locus 1	141749	Persistence of Fetal Hemoglobin	0	3	No Effective Intervention	0	0	2	**5**

HSD11B1	600713	Cortisone reductase deficiency 2	614662	Hyperandrogenism; premature pseudopuberty (males); adult-onset	1	3	No Effective Intervention	0	0	1	**5**
INS	176730	MODY, type X (MODY10)	613370	Type II diabetes and complications	1	0	Glucose monitoring, early treatment	1	3	0	**5**
KLF11	603301	MODY, type VII (MODY7)	610508	Type II diabetes and complications	1	0	Glucose monitoring, early treatment	1	3	0	**5**
LAMC3	604349	Cortical malformations, occipital	614115	Seizures / Epilepsy	2	3	No Effective Intervention	0	0	0	**5**
LDHA	150000	Glycogen storage disease XI (GSD11), or lactate dehydrogenase A deficiency	612933	Myopathy, muscle pain and stifness, exercise intolerance and myoglobinuria	1	3	No Effective Intervention	0	0	1	**5**
MAT1A	610550	Methionine Adenosyltransferase Deficiency, AR	250850	Benign Hypermethioninemia	0	3	Spectrum of Actions not Listed Here (No Effective Intervention)	0	0	2	**5**
MRE11A	600814	Ataxia-telangiectasia-like disorder	604391	Muscle wasting, contractures, movement disorder	1	3	No Effective Intervention	0	0	1	**5**
MTPAP	613669	Spastic ataxia 4	613672	Progressive ataxia, mild cognitive impairment	1	3	No Effective Intervention	0	0	1	**5**
NAGA	104170	Kanzaki disease (Schindler's Disease type II)	609242	All Outcomes	1	3	No Effective Intervention	0	0	1	**5**
NTRK1	191315	Congenital insensitivity to pain with anhidrosis	256800	Syndromic features	1	3	No Effective Intervention	0	0	1	**5**
PAX4	167413	MODY, type IX (MODY9)	612225	Type II diabetes and complications	1	0	Glucose monitoring, early treatment	1	3	0	**5**
POLD1	174761	Mandibular hypoplasia, deafness, progeroid features, and lipodystrophy syndrome	615381	progeroid disease	1	3	No Effective Intervention	0	0	1	**5**
SLC10A2	601295	Primary bile acid malabsorption	613291	Chronic diarrhea	1	3	No Effective Intervention	0	0	1	**5**
SLC26A2	606718	Multiple epiphyseal dysplasia 4	226900	Clubfoot and joint problems	1	3	No Effective Intervention	0	0	1	**5**
SLC2A1	138140	Dystonia 9	601042	Choreoathetosis, ataxia, and progressive spastic paraplegia	1	3	No Effective Intervention	0	0	1	**5**
SYP	313475	Mental retardation, X-linked 96	300802	Intellectual disability	1	3	No Effective Intervention	0	0	1	**5**
UBIAD1	611632	Corneal dystrophy, Schnyder type	121800	Visual morbidity, decreased daytime vision	0	3	None (Correction via surgery in advanced disease)	0	0	2	**5**
UPF3B	300298	Mental retardation, X-linked, syndromic 14	300676	Intellectual disability	1	3	No Effective Intervention	0	0	1	**5**
VAMP1	185880	Spastic ataxia 1	108600	Ataxia	1	3	No Known Intervention	0	0	1	**5**
ATP5E	606153	Mitochondrial Complex V (ATP synthase) Deficiency, Nuclear Type 3	614053	All Outcomes	1	0	Mitochondrial Cocktail	0	3	0	**4**
COL1A1	120150	Osteogenesis Imperfecta, type III	259420	All Outcomes	2	1	No Effective Intervention	0	0	1	**4**
DAG1	128239	Muscular dystrophy-dystroglycanopathy type C9	613818	Limb girdle muscular dystrophy and/or MR	1	3	No Effective Intervention	0	0	0	**4**
HYAL1	607071	Mucopolysaccharidosis type IX	601492	swollen joints, periarticular masses	0	3	No Effective Interventions	0	0	1	**4**
MCCC2	609014	3-Methylcrotonyl-CoA Carboxylase 2 Deficiency	210210	Encephalopathy	2	1	Mild Protein Restriction and Carnitine Supplementation	0	0	1	**4**
NRTN	602018	Hirschsprung disease		Colonic aganglionosis	1	0	Surgery	2	1	0	**4**
OPLAH	614243	5-oxoprolinase deficiency	260005	Biochemical Abnormality of High 5-oxoprolinuria	0	3	No Effective Intervention	0	0	1	**4**
PCBD1	126090	Hyperphenylalaninemia, BH4-deficient, D	264070	Hyperphenylalaninemia without cognitive impairment	0	3	No Effective Intervention	0	0	1	**4**
PLCG2	600220	Autoinflammation and PLCG2-associated antibody deficiency and immune dysregulation (APLAID)	614878	blistering skin lesions	1	0	IL-1 inhibitor	1	2	0	**4**
REEP1	609139	Distal hereditary motor neuropathy type Vb	614751	Muscle weakness, contractures	1	3	No Effective Intervention	0	0	0	**4**
ZNF644	614159	Myopia 21, autosomal dominant	614167	High grade myopia	0	3	No Effective Intervention	0	0	1	**4**
BLVRA	109750	Hyperbiliverdinemia	614156	Episodic hyperbiliverdinemia (green jaundice)	1	1	No Effective Intervention	0	0	1	**3**
COL1A1	120150	Ehlers-Danlos Syndrome, type VIIA	130060	Congenital Hip Dislocation	1	0	Open Reduction of Hip Dislocation	0	1	1	**3**
GNMT	606628	Glycine N-Methyltransferase Deficiency	606664	Hypermethioninemia --> Hepatomegaly	0	3	Dietary Methionine Restriction	0	0	0	**3**
GYS1	138570	Glycogen storage disease 0, muscle	611556	Left ventricular hypertrophy, risk of cardiac arrest --> Sudden death	3	0	No Effective Intervention	0	0	0	**3**
HPD	609695	Tyrosinemia, type III	276710	Intellectual disability, ataxia, seizures	1	0	Dietary restriction	0	2	0	**3**
IKBKG	300248	Immunodeficiency, isolated	300584	Recurrent infections	2	0	?immunoglobulin replacement; surveillance	1	0	0	**3**
PRKDC	600899	SCID		All Outcomes	3	0	No Effective Intervention	0	0	0	**3**
SLC25A13	603859	Citrullinemia, type II, adult-onset	603471	Liver Failure	2	0	No effective intervention	0	0	1	**3**
TRDN	603283	Ventricular tachycardia, catecholaminergic polymorphic, 2	615441	Sudden death due to arrhythmia	3	0	Echo screening/no sports/ICD	0	0	0	**3**

VANGL2	600533	Neural tube defects	182940	Neural tube defects	2	0	No Effective Intervention	0	0	1	**3**
ABCA1	600046	HDL deficiency, type 2	604091	Early onset coronary artery disease	2	0	lifestyle mods and monitoring, drugs	0	0	0	**2**
CARD11	607210	Persistent Polyclonal B-cell Lymphocytosis	606445	B-Cell Lymphoma	2	0	No Effective Intervention	0	0	0	**2**
EGLN1	606425	Erythrocytosis, familial, 3	609820	Paraganglioma	2	0	Biochemical screening, imaging	0	0	0	**2**
GSTZ1	603758	Tyrosinemia type 1b		Severe liver disease (cirrhosis or hepatocellular carcinoma)	2	0	No Effective Intervention	0	0	0	**2**
MAGT1	300715	X-linked immunodeficiency with magnesium defect, Epstein-Barr virus infection, and neoplasia (XMEN)	300853	Recurrent Infection including EBV	2	0		0	0	0	**2**
MCEE	608419	Methylmalonyl-CoA epimerase deficiency	251120	Metabolic decompensation	2	0	No Effective Intervention	0	0	0	**2**
TOP1	126420	DNA topoisomerase I, camptothecin-resistant		No inherited disorder associated as far as I can tell	0	0	N /A	0	0	2	**2**
DISP1	607502	Holoprosencephaly 10	612530	All Outcomes	1	0	No Effective Intervention	0	0	0	**1**
F12	610619	Factor XII deficiency	234000	Possible clotting	0	0	No Effective Intervention	0	0	1	**1**
SRPX2	300642	Rolandic epilepsy, mental retardation, and speech dyspraxia	300643	Intellectual disability, seizures	1	0	No Effective Intervention	0	0	0	**1**
SUGCT	609187	Glutaric aciduria III	231690	None	0	0	None	0	0	1	**1**

Gene	Gene MIM	Phenotype	Phenotype MIM	Outcome Considered	Severity	Likelihood	Intervention Considered	Efficacy	Acceptability	Knowledge	Total
ACADM	607008	Acyl-CoA Dehydrogenase, Medium Chain, Deficiency of	201450	Death from Hypoglycemic Crises	3	3	Avoid Fasting, frequent feeding, emergency letter	3	3	3	**15**
ACADVL	609575	VLCAD deficiency	201475	Hypoglycemic Crises	3	3	Prevention of fasting, dietary restriction of long chain fatty acids; carnitine supplement	3	3	3	**15**
HADHA	600890	LCHAD deficiency	609016	Hypoglycemic Crises	3	3	Prevention of fasting, dietary restriction of long chain fatty acids;	3	3	3	**15**
IL2RG	308380	Severe Combined Immunodeficiency, X-linked	300400	Immunodeficiency	3	3	Hematopoietic Stem Cell Transplantation (HSCT)	3	3	3	**15**
JAK3	600173	SCID, AR, T-negative/B-positive type	600802	Immunodeficiency	3	3	Transplantation of Hematopoietic Stem Cells	3	3	3	**15**
ALDOB	612724	Fructose intolerance	229600	All Outcomes	2	3	Strict Dietary Restriction	3	3	3	**14**
CTNS	606272	Cystinosis	219800	kidney failure (renal Fanconi Syndrome)	2	3	cysteamine, monitoring to determine if/when renal transplant indicated	3	3	3	**14**
CYP21A2	613815	Adrenal hyperplasia, congenital, due to 21-hydroxylase deficiency	201910	Salt-wasting crises	2	3	Glucocorticoid/mineralocorticoid administration	3	3	3	**14**
ELN	130160	Supravalvar aortic stenosis	185500	SVAS induced heart failure	2	3	Echocardiogram	3	3	3	**14**
GAA	606800	Glycogen storage disease II (GSD2)	232300	HCM, respiratory distress, hypotonia	3	3	ERT, indivualized care for cardiomyopathy	3	2	3	**14**
HFE2	608374	Hemochromatosis, type 2A	602390	Multiple system iron overload (heart, liver)	2	3	Yearly ferritin -> phlebotomy	3	3	3	**14**
HSD3B2	613890	3-beta-hydroxysteroid dehydrogenase, type II, deficiency	201810	Salt Wasting Crises	3	2	Endocrine eval, IV saline, glucocorticoid/mineralocorticoid if indicated	3	3	3	**14**
INS	176730	Diabetes mellitus, permanent neonatal	606176	Hyperglycemia, ketoacidosis	3	3	Insulin	3	3	2	**14**
KCNE1	176261	Jervell and Lange-Nielsen syndrome 2 (recessive)	612347	Sudden death due to arrhythmia	3	3	ICD	3	2	3	**14**
MEFV (heterozygous)	608107	Familial Mediterranean fever, AD, Classic mutations associated with renal failure	134610	Renal Failure	2	3	Colchisine	3	3	3	**14**
MEFV (homozygous)	608107	Familial Mediterranean fever, AR, Classic mutations associated with renal failure	249100	Renal Failure	2	3	Colchisine	3	3	3	**14**
NAGS	608300	N-acetylglutamate synthase deficiency	237310	Hyperammonemic crisis	3	3	All Interventions	3	2	3	**14**
OTC	300461	Ornithine transcarbamylase deficiency (Males)	311250	Hyperammonemic crisis	3	3	Diet, sodium phenylacetate and sodium benzoate, illness management	3	2	3	**14**
PTPN11	176876	Noonan syndrome 1	163950	Congenital Heart Defects	3	3	ECG / no sports / ICD (HCM), pulmonary balloon valvuloplasty or	3	2	3	**14**
RB1	614041	Retinoblastoma	180200	Retinoblastoma	2	3	Funduscopic Exam	3	3	3	**14**
STAR	600617	Lipoid adrenal hyperplasia	201710	salt-wasting, failure to thrive	3	3	Hormone Replacement	3	3	2	**14**
TG	188450	Thyroid dyshormonogenesis 3	274700	Brain, neuron damage, MR, FTT, jaundice, cretinism	2	3	Thyroid hormone replacement (L-thyroxine)	3	3	3	**14**
TPO	606765	Thyroid dyshormonogenesis 2A	274500	Brain, neuron damage, MR, FTT, jaundice, cretinism	2	3	Thyroid hormone replacement (L-thyroxine)	3	3	3	**14**
ZAP70	176947	Selective T-cell defect	269840	recurrent bacterial, viral, and opportunistic infections	3	3	HSCT	3	3	2	**14**
ACAT1	607809	Alpha-Methylacetoacetic Aciduria	203750	Severe Metabolic Acidosis	2	3	Dietary: Avoidance of Fasting, Low Protein	3	3	2	**13**
ALDH7A1	107323	Pyridoxine-dependent epilepsy	266100	Epileptic encephalopathy	1	3	B6 supplementation	3	3	3	**13**
APC	611731	Familial Adenomatous Polyposis	175100	Colorectal Cancer	2	3	Colonoscopy	3	2	3	**13**
ASS1	603570	Citrullinemia	215700	Hyperammonemic Crisis	2	3	Diet, sodium phenylacetate and sodium benzoate	3	2	3	**13**
BTD	609019	Biotinidase Deficiency	253260	Developmental Delay	1	3	Biotin	3	3	3	**13**
CACNA1C	114205	Timothy syndrome	601005	Sudden death due to arrhythmia	3	3	EKG screening / avoidance of triggers / ICD	2	3	2	**13**
CASQ2	114251	Ventricular tachycardia, catecholaminergic polymorphic, 2 (recessive)	611938	Sudden death due to arrhythmia	3	3	Stress testing/avoidance of triggers/beta-blockers/ICD	2	3	2	**13**

CBS	613381	Homocystinuria, B6-responsive and nonresponsive types	236200	Risk for Thrombosis	2	3	Diet +/- pyridoxine, cystadane	3	2	3	**13**
CDH23	605516	Usher Syndrome, type 1D	601067	Usher Phenotype (Communication Deficits + RP)	2	3	Audiology and Vision Services	2	3	3	**13**
CDKN2A	600160	Pancreatic cancer/melanoma syndrome	606719	Melanoma	2	2	Skin Exam	3	3	3	**13**
CIB2	605564	Deafness, autosomal recessive 48	609439	Communication Deficits	1	3	All Interventions	3	3	3	**13**
CPS1	608307	Carbamoylphosphate synthetase I deficiency	237300	Hyperammonemic Crisis	2	3	Diet, sodium phenylacetate and sodium benzoate	3	2	3	**13**
DCLRE1C	605988	Severe Combined Immunodeficiency, Athabascan Type	602450	Death Secondary to Immune Deficiency	3	3	HSCT (Transplant)	3	2	2	**13**
DUOX2	606759	Thyroid dyshormonogenesis 6	607200	Brain, neuron damage, MR, FTT, jaundice, cretinism	2	3	Thyroid hormone replacement (L-thyroxine)	3	3	2	**13**
DUOXA2	612772	Thyroid dyshormonogenesis 5	274900	Brain, neuron damage, MR, FTT, jaundice, cretinism	2	3	Thyroid hormone replacement (L-thyroxine)	3	3	2	**13**
EDN3	131242	Waardenburg syndrome, type 4B	613265	Communication Deficits or Hirschsprung Disease	1	3	Audiology --> Hearing Aids --> Cochlear Implant or Screening -->	3	3	3	**13**
F10	613872	Factor X deficiency	227600	Bleeding	1	3	Fresh-frozen plasma or plasma-derived Prothrombin Complex concentrates (PCCs) with procedures	3	3	3	**13**
F8	300841	Hemophilia A	306700	Bleeding --> possible exsanguination	2	3	Factor replacement	3	2	3	**13**
F9	300746	Hemophilia B	306900	Bleeding --> possible exsanguination	2	3	Factor replacement	3	2	3	**13**
FAH	613871	Tyrosinemia, type I	276700	Liver failure, hepatocellular carcinoma	2	3	NTBC, dietary intervention	3	2	3	**13**
FBN1	134797	Marfan Syndrome	154700	Aortic Dissection	3	2	Annual Echocardiogram	2	3	3	**13**
G6PC	613742	Glycogen Storage Disease 1a	232200	Severe Hypoglycemia	2	3	Dietary (Low sugar), avoid fasting, uncooked cornstarch	3	2	3	**13**
GALT	606999	Galactosemia	230400	Death from liver failure or E.coli sepsis	2	3	Dietary Restriction	3	2	3	**13**
GBA	606463	Gaucher Disease, Type I	230800	All Outcomes	1	3	Enzyme Replacement Therapy	3	3	3	**13**
GCDH	608801	Glutaricaciduria, type I	231670	Metabolic crisis	2	3	Diet, carnitine, Anticipatory emergent management	2	3	3	**13**
GCH1	600225	Dystonia, DOPA-responsive, with or without hyperphenylalaninemia	128230	Dystonia	1	3	Oral dopa/carbidopa	3	3	3	**13**
GIF	609342	Intrinsic factor deficiency	261000	Pernicious Anemia	2	3	B12 Injections	3	3	2	**13**
GJB2	121011	Deafness, autosomal recessive 1A (DFNB1A)	220290	Communication Deficits	1	3	Audiology --> Cochlear Implants	3	3	3	**13**
HADH	601609	3-Hydroxyacyl-CoA Dehydrogenase Deficiency	231530	Profound Hypoglycemia in Infancy	2	3	Matabolic eval; Adequate Carbohydrate Source; Diazoxide	3	3	2	**13**
HADHB	143450	Trifunctional protein deficiency	609015	Hypotonia, Respiratory Failure, Cardiomyopathy, SIDS-like	3	3	Prevention of fasting, dietary restriction of long chain fatty acids;	2	3	2	**13**
HAMP	606464	Hemochromatosis, type 2B	613313	Severe iron overload	2	3	Phlebotomy	3	3	2	**13**
HBB	141900	Thalassemias, beta- (Major, AR)	613985	life threatening anemia	2	3	transfusions, iron chelation (desferoxamine)	3	2	3	**13**
HFE2	608374	Hemochromatosis, type 2A	602390	Severe iron overload	2	3	Yearly ferritin -> phlebotomy	3	3	2	**13**
HLCS	609018	Holocarboxylase synthetase deficiency	253270	Seizures	2	3	Biotin	3	3	2	**13**
IL7R	146661	Severe Combined Immunodeficiency, T-cell Negative, B-cell / Natural Killer Cell-Positive Type	608971	Death	3	3	BMT	3	2	2	**13**
IVD	607036	Isovaleric acidemia	243500	Encephalopathy with metabolic decompensation	3	2	Diet, supplements	3	2	3	**13**
JUP	173325	Naxos disease (recessive)	601214	Sudden death due to arrhythmia	3	3	Echo/MRI screening/no sports/ICD	2	3	2	**13**
KCNH2	152427	Romano-Ward Long QT syndrome 2	613688	Arrhythmia	3	2	EKG screening / avoidance of triggers / ICD	2	3	3	**13**
KCNQ1	607542	Romano-Ward Long QT syndrome 1	192500	Arrhythmia	3	2	EKG screening / avoidance of triggers / ICD	2	3	3	**13**
LDLR	606945	Familial hypercholesterolemia	143890	Hypercholesterolemia / Early MI	2	3	Cholesterol screening / Statins	2	3	3	**13**
LHX3	600577	Pituitary hormone deficiency, combined, 3	221750	Combined pituitary hormone deficiency (CPHD)	2	3	Hormone Replacement Therapy	3	3	2	**13**
MEN1	613733	Multiple endocrine neoplasia 1	131100	Multiple Endocrine Tumors	1	3	Biochemical Screening / Imaging --> Surgery	3	3	3	**13**

MITF	156845	Waardenburg Syndrome, type 2A	193510	Communication Deficits	1	3	Audiology --> Hearing Aid --> Cochlear Implant	3	3	3	**13**
MLH1	120436	Lynch syndrome	609310	Colorectal Cancer	2	3	Colonoscopy	3	2	3	**13**
MMAA	607481	Methylmalonic aciduria, vitamin B12-responsive	251100	Metabolic decompensation	2	2	Diet, B-12, illness management	3	3	3	**13**
MMAB	607568	Methylmalonic aciduria, vitamin B12-responsive, due to defect insynthesis of adenosylcobalamin. cblB	251110	Metabolic decompensation	2	3	Diet, B-12, illness management	2	3	3	**13**
MMACHC	609831	Methylmalonic aciduria and homocystinuria, cblC type	277400	infantile presentation (failure to thrive, poor feeding, and hypotonia with an	2	3	Diet, B-12, illness management	2	3	3	**13**
MPI	154550	CDG1b	602579	All Outcomes	1	3	All Interventions	3	3	3	**13**
MSH2	609309	Lynch syndrome	120435	Colorectal Cancer	2	3	Colonoscopy	3	2	3	**13**
MTHFR	607093	Homocystinuria due to MTHFR deficiency	236250	Risk for thrombosis	2	3	Folate supplementation, B6, B12, Betaine	3	3	2	**13**
MUT	609058	Methylmalonic aciduria, mut(0) type	251000	Metabolic decompensation	3	3	Dietary restriction and emergency letter	2	2	3	**13**
MUTYH	604933	Attenuated FAP / MUTYH-associated polyposis	608456	Colorectal Cancer	2	3	Colonoscopy	3	2	3	**13**
MYO7A	276903	Deafness, autosomal recessive, 2	600060	Communication Deficits	1	3	Audiology --> Cochlear Implants	3	3	3	**13**
MYO7A	276903	Usher Syndrome, type 1B	276900	Usher Phenotype (Communication Deficits + RP)	2	3	Audiology and Vision Services	2	3	3	**13**
OTC	300461	Ornithine transcarbamylase deficiency (Females)	311250	Hyperammonemic crisis	2	2	Diet, sodium phenylacetate and sodium benzoate. illness	3	3	3	**13**
OTOF	603681	Deafness, autosomal recessive 9	601071	Communication Deficits and/or Auditory Neuropathy	1	3	All Interventions	3	3	3	**13**
PAH	612349	Phenylketonuria	261600	Severe intellectual disability	2	3	Dietary restriction	3	2	3	**13**
PAX3	606597	Waardenburg Syndrome, type 1	193500	Communication Deficits	1	3	Audiology --> Hearing Aid --> Cochlear Implant	3	3	3	**13**
PCCA	232000	Propionicacidemia	606054	Encephalopathy, coma, seizures, developmental regression and	3	3	Avoidance of catabolic stressors and immediate treatment of metabolic	1	3	3	**13**
PCCB	232050	Propionicacidemia	606054	Encephalopathy, coma, seizures, developmental regression and	3	3	Avoidance of catabolic stressors and immediate treatment of metabolic	1	3	3	**13**
PCDH15	605514	Usher Syndrome, type 1F	602083	Usher Phenotype (Communication Deficits + RP)	2	3	Audiology and Vision Services	2	3	3	**13**
PDX1	600733	Pancreatic agenesis 1	260370	Neonatal diabetes	2	3	Insulin	3	3	2	**13**
PROC	612283	Thrombophilia due to protein C deficiency, autosomal recessive	612304	Thrombosis, PE	2	3	Hematological evaluation --> protein C or plasma if biochemical evidence of protein C deficiency	3	3	2	**13**
PROP1	601538	Pituitary hormone deficiency, combined, 2	262600	Growth failure/failure to thrive	1	3	screening--> hormone replacement	3	3	3	**13**
PTPN11	176876	Metachondromatosis	156250	Exostoses and enchondromatosis	1	3	Bi-annual clinical review, imaging	3	3	3	**13**
RAG1	179615	Severe combined immunodeficiency, B cell-negative	601457	Immunodeficiency, overwhelming infections	3	3	Bone marrow transplant	2	2	3	**13**
RAG2	179616	Severe combined immunodeficiency, B cell-negative	601457	Immunodeficiency, overwhelming infections	3	3	Bone marrow transplant	2	2	3	**13**
RET	164761	Multiple endocrine neoplasia IIA	171400	Medullary thyroid cancer	2	3	Thyroidectomy	3	2	3	**13**
RET	164761	Multiple endocrine neoplasia IIB	162300	Medullary thyroid cancer	2	3	Thyroidectomy	3	2	3	**13**
RET	164761	Familial Medullary Thyroid Cancer (FMTC)	155240	Medullary thyroid cancer	2	3	Thyroidectomy	3	2	3	**13**
SCN5A	600163	Romano-Ward Long QT syndrome 3	603830	Arrhythmia	3	2	EKG screening / avoidance of triggers / ICD	2	3	3	**13**
SLC19A3	606152	Thiamine metabolism dysfunction syndrome 2 (biotin- or thiamine-responsive encephalopathy type 2)	607483	Recurrent subacute encephalopathy	2	3	Oral biotin and thiamine	3	3	2	**13**
SLC25A20	212138	Carnitine-Acylcarnitine Translocase Deficiency	212138	Hypoglycemia --> Neurological Disorder	2	3	Low fat diet, avoidance of fasting	3	3	2	**13**
SLC37A4	602671	Glycogen Storage Disease Ib/Ic		All Outcomes	2	3	All Interventions	3	2	3	**13**
SOX10	602229	Waardenburg syndrome, type 4C	613266	Communication Deficits	1	3	Audiology --> Hearing Aids --> Cochlear Implant	3	3	3	**13**
SRY	480000	46XY sex reversal 1	400044	gonadoblastoma	2	2	surgical removal of gonads	3	3	3	**13**
TECTA	602574	Deafness, autosomal dominant 8/12 (DFNA12)	601543	Communication Deficits	1	3	Audiology --> Cochlear Implants	3	3	3	**13**
TFR2	604720	Hemochromatosis, type 3	604250	Intermediate iron overload	2	3	Phlebotomy	3	3	2	**13**

TSHB	188540	Hypothyroidism, congenital, nongoitrous 4	275100	ID and growth retardation	2	3	T4 treatment	2	3	3	**13**
UNC13D	608897	Hemophagocytic lymphohistiocytosis, familial, 3	608898	Severe Inflammation / Immune Dysfunction	2	3	Chemotherapy and Immunotherapy --> HSCT	3	2	3	**13**
USH1C	605242	Usher Syndrome, type 1C	276904	Usher Phenotype (Communication Deficits + RP)	2	3	Audiology and Vision Services	2	3	3	**13**
VHL	608537	von Hippel-Lindau syndrome	193300	Renal cancer / CNS hemangioblastomas / Pheochromocytoma	2	3	Annual renal imaging / biochemical screening	2	3	3	**13**
VWF	613160	von Willibrand disease, type 3	277480	Severe Mucocutaneous and Musculoskeletal Bleeding	2	3	Prophylactic Infusions of VWF/FVIII Concentrates	3	3	2	**13**
ACTG1	102560	Deafness, autosomal dominant 20/26	604717	Communication Deficits	1	3	All Interventions	3	3	2	**12**
ACVRL1	601284	Telangiectasia, hereditary hemorrhagic, type 2	600376	GI bleeding, CVA from cerebral AVMs, infectious complications	2	2	annual CBC, O2 sats, contrast echo, one-time head MRI. Don't	2	3	3	**12**
ADGRV1	602851	Usher Syndrome, type 2C	605472	Usher Phenotype (Communication Deficits + RP)	1	3	Audiology and Vision Services	2	3	3	**12**
AGL	610860	Glycogen Storage Disease III	232400	All Outcomes	1	3	All Interventions	2	3	3	**12**
AMS1	606844	Alstrom Syndrome	203800	Alstrom Syndrome (Communication deficits. visual impairments.	2	3	Referral to audiology and vision services. surveillance and monitoring	1	3	3	**12**
APOB	107730	Familial hypercholesterolemia due to ligand-defective APOB	144010	Hypercholesterolemia / Early MI	2	3	Cholesterol screening / statins	2	3	2	**12**
ASL	608310	Argininosuccinic aciduria	207900	Hyperammonemic crisis, chronic liver disease	2	3	Diet (Normal diet with arginine supplement or a diet in which protein	2	2	3	**12**
ATP6V1B1	192132	Renal tubular acidosis with deafness	267300	Renal Tubular Acidosis	1	3	Urine and Blood Tests, Alkaline Treatment	3	3	2	**12**
ATP7A	300011	Menkes Disease	309400	Low serum copper (seizures, neurological deficits. failure to thrive)	3	3	Copper histidine or Copper chloride injections	1	3	2	**12**
ATP7B	606882	Wilson Disease	277900	Liver Cirrhosis	2	2	Monitoring, low copper diet, chelation (if Cu levels elevated)	2	3	3	**12**
BCHE	177400	Increased sensitivity to choline ester anesthesia		Avoiding prolonged apnea after use of choline ester anesthesia	2	1	Avoidance of suxamethonium (succinylcholine)	3	3	3	**12**
BCKDHA	608348	Maple syrup urine disease, type Ia, Ib, and type II	248600	MSUD	2	3	Diet	2	2	3	**12**
BCKDHB	248611	Maple syrup urine disease, type Ib	248600	MSUD	2	3	Diet	2	2	3	**12**
BMPR1A	601299	Polyposis, juvenile intestinal	174900	GI cancer	2	2	CBC, annual colonoscopy (scored on this). In severe cases. colectomy	3	2	3	**12**
BRCA1	113705	Hereditary Breast and Ovarian Cancer	604370	Breast Cancer / Ovarian Cancer	2	3	Prophylactic Mastectomy / BSO	3	1	3	**12**
BRCA2	600185	Hereditary Breast and Ovarian Cancer	612555	Breast Cancer / Ovarian Cancer	2	3	Prophylactic Mastectomy / BSO	3	1	3	**12**
CARD11	607210	Immunodeficiency 11	615206	All Outcomes or SCIDs or Profound Combined Immunodeficiency	3	3	BMT / HSCT	3	2	1	**12**
CD3D	186790	Immunodeficiency 19	615617	SCIDs	3	3	Bone Marrow Transplant	3	2	1	**12**
CDH23	605516	Deafness, autosomal recessive 12 (DFNB12)	601386	Communication Deficits	1	3	All Interventions	3	3	2	**12**
CFTR	602421	Cystic Fibrosis	219700	Pulmonary Disease	2	3	Antibiotics, bronchodilaters, chest PT	2	2	3	**12**
CIB2	605564	Usher Syndrome, type IJ	614869	Usher Phenotype (Communication Deficits + RP)	2	3	Audiology and Vision Services	2	3	2	**12**
CISD2	611507	Wolfram syndrome 2	604928	Diabetes Mellitus / Insipidus	2	3	Surveillance --> Treatment of Manifestations	3	2	2	**12**
CLDN14	605608	Deafness, autosomal recessive 29	614035	Communication Deficits	1	3	All Interventions	3	3	2	**12**
COCH	603196	Deafness, autosomal dominant 9 (Non-syndromic deafness. dominant)	601369	All Outcomes	1	3	All Interventions	3	3	2	**12**
COL11A2	120290	Otospondylomegaepiphyseal Dysplasia	215150	Communication Deficits	1	3	Audiology --> Hearing Aids --> Cochlear Implant	3	3	2	**12**
COL11A2	120290	Stickler Syndrome, Type III	184840	Communication Deficits	1	3	Audiology --> Hearing Aids --> Cochlear Implant	3	3	2	**12**
COL1A2	120160	Osteogenesis imperfecta 3	259420	Progressive fractures, intracranial bleed	2	3	Bisphosphates	1	3	3	**12**
CORO1A	605000	Immunodeficiency 8	615401	Recurrent Infections	2	3	Bone Marrow Transplant	3	2	2	**12**
CTP1A	600528	CPT deficiency, hepatic, type IA	255120	Hypoketotic hypoglycemia, liver failure	3	2	Diet, avoidance of fasting	2	3	2	**12**
CYP27B1	609506	Vitamin D-dependent rickets, type I	264700	Bone disease	1	2	Vitamin D	3	3	3	**12**
DBT	248610	Maple syrup urine disease, type II	248600	MSUD	2	3	Diet	2	2	3	**12**
DNMT3B	602900	Immunodeficiency-centromeric instability-facial anomalies syndrome 1	242860	Immunodeficiency	2	3	IVIG; allogeneic stem cell transplant	3	2	2	**12**
DSP	125647	Dilated cardiomyopathy with woolly hair and keratoderma	605676	Heart failure	2	3	Echocardiogram	2	3	2	**12**
ENG	131195	Telangiectasia, hereditary hemorrhagic, type 1	187300	GI bleeding, CVA from cerebral AVMs, infectious complications	2	2	Annual CBC, O2 sats, contrast echo, one-time head MRI. Don't	2	3	3	**12**

ETFA	608053	Glutaric acidemia IIA	231680	Metabolic crisis	2	2	Riboflavin, carnitine, Anticipatory emergent management	2	3	3	**12**
ETFB	130410	Glutaric acidemia IIB	231680	Metabolic crisis	2	2	Riboflavin, carnitine, Anticipatory emergent management	2	3	3	**12**
ETFDH	231675	Glutaric acidemia IIC	231680	Metabolic crisis	2	2	Riboflavin, carnitine, Anticipatory emergent management	2	3	3	**12**
F13A1	134570	Factor XIIIA deficiency	613225	Intracranial Hemorrhage	2	2	Hematology Consult --> FFP	3	3	2	**12**
F13B	134580	Factor XIIIB deficiency	613235	Intracranial Hemorrhage	2	2	Hematology consult, assess factor level; rfXIII if low or for acute	3	3	2	**12**
F2	176930	Prothrombin Deficiency, congenital (Dysprothrombinemia, Hypoprothrombinemia)	613679	Intracranial Hemorrhage	2	2	Hematology consult, Fresh Frozen Plasma with Procedures / Trauma.	3	3	2	**12**
F5	612309	Factor V deficiency	227400	Bleeding	1	3	Hematology Consult --> FFP	3	3	2	**12**
F7	613878	Factor VII deficiency	227500	Bleeding --> hemorrhagic stroke	2	2	Factor replacement	3	2	3	**12**
FBP1	611570	Fructose bisphosphatase deficiency	229700	Hypoglycemia and metabolic acidosis	2	2	avoidance of fasting, high carbohydrate diet and avoid fructose/	3	3	2	**12**
FGFR3	134934	Muenke syndrome	602849	Craniosynostosis --> increased intracranial pressure	1	3	Physical exam --> imaging to screen --> surgery if necessary	3	2	3	**12**
G6PD	305900	hemolytic anemia due to G6PD deficiency	300908	neurological damage	1	2	photo therapy or transfusion , avoidance of oxidative stress	3	3	3	**12**
GATA2	137295	Immunodeficiency 21	137295	susceptibility to infection and myeloid malignancies	2	3	HSCT	3	2	2	**12**
GGCX	137167	Vitamin K-dependent coagulation defect	277450	Bleeding (Severe)	2	3	Oral Vitamin K	3	3	1	**12**
GIPC3	608792	Deafness, Autosomal Recessive 15	601869	Communication Deficits	1	3	All Interventions	3	3	2	**12**
GJB2	121011	Deafness, autosomal dominant 3A (DFNA3A)	601544	Communication Deficits	1	3	Audiology --> Hearing Aids	3	3	2	**12**
GJB6	604418	Non-Syndromic Deafness, Recessive 1B	612645	Communication Deficits	1	3	All Interventions	3	3	2	**12**
GPSM2	609245	Chudley-McCullough Syndrome	604213	Communication Deficits	1	3	Audiology --> Hearing Aids --> Cochlear Implants	3	3	2	**12**
GRXCR1	613283	Deafness, autosomal recessive 25	613285	Communication Deficits, with or without vestibular dysfunction	1	3	Audiology --> Hearing Aids	3	3	2	**12**
HBB	141900	Sickle cell anemia	603903	All Outcomes	2	2	All Interventions	2	3	3	**12**
HFE	613609	Hereditary hemochromatosis	235200	Multiple system iron overload (heart, liver)	2	1	Yearly ferritin -> phlebotomy	3	3	3	**12**
HFE - C282Y homozygous	613609	Hereditary hemochromatosis	235200	Multiple system iron overload (heart, liver)	2	1	Yearly ferritin -> phlebotomy	3	3	3	**12**
ILDR1	609739	Deafness, autosomal recessive 42	609646	Communication Deficits	1	3	All Interventions	3	3	2	**12**
IYD	612025	Thyroid dyshormonogenesis 4	274800	Brain, neuron damage, MR, FTT, jaundice, cretinism	1	3	Thyroid hormone replacement (L-thyroxine)	2	3	3	**12**
KCNJ2	600681	Andersen-Tawil syndrome; LQT 7	170390	Sudden death due to arrhythmia	3	3	EKG screening / avoidance of triggers / ICD	2	2	2	**12**
KCNQ4	603537	Deafness, autosomal dominant 2A	600101	Communication Deficits	1	3	All Interventions	3	3	2	**12**
KRIT1	604214	Cerebral Cavernous Malformations - 1	116860	Symptomatic CCM	2	3	Monitoring --> Surgery as indicated to remove the cerebral cavernous	2	3	2	**12**
LAMP2	309060	Glycogen Storage Disease IIb/Danon disease	300257	Cardiovascular irregularties (i.e. hypertrophic cardiomyopathy, dilated	2	3	Cardiac screening --> management	2	3	2	**12**
LDLRAP1	605747	Hypercholesterolemia, familial, autosomal recessive	603813	Hypercholesterolemia, early MI	2	3	Lipid monitoring, statins	2	3	2	**12**
LMNA	150330	Dilated cardiomyopathy 1A	115200	Arrhythmia or heart failure	3	2	Echo Screening / ICD	2	3	2	**12**
LRTOMT	612414	Deafness, autosomal recessive 63	611451	Communication Deficits	1	3	All Interventions	3	3	2	**12**
MARVELD2	610572	Deafness, autosomal recessive 49	610153	Communication Deficits	1	3	Audiology --> Cochlear Implants	3	3	2	**12**
MSH6	600678	Lynch syndrome	120435	Colorectal Cancer	2	2	Colonoscopy	3	2	3	**12**
MYH7	160760	Hypertrophic cardiomyopathy 1	192600	Arrhythmia	3	1	Echo Screening / no sports / ICD	3	3	2	**12**
MYO15A	602666	Deafness, autosomal recessive 3	600316	Communication Deficits	1	3		3	3	2	**12**
MYO6	600970	Non-Syndromic Deafness, Dominant 22	606346	Communication Deficits	1	3	All Interventions	3	3	2	**12**
NKX2-1	600635	Choreoathetosis, hypothyroidism, and neonatal respiratory distress	610978	Hypothyroidism	1	3	Thyroid Hormone Replacement	3	3	2	**12**
NPC1	607623	Niemann-Pick Disease, types C1 and D	257220	Progressive neurological involvement / deterioration	2	3	Miglustat	1	3	3	**12**
PALB2	610355	Fanconi anemia, complementation group N	610832	Bone marrow failure	2	3	Blood counts, annual bone marrow aspirate, gCSF	2	2	3	**12**
PAX8	167415	Hypothyroidism, congenital, due to thyroid dysgenesis or hypoplasia	218700	Low T4, high TSH, MR if untreated	2	2	L-thyroxine replacement	3	3	2	**12**

PCDH15	605514	Deafness, autosomal recessive 23	609533	Communication Deficits	1	3	All Interventions	3	3	2	**12**
PHKA2	300798	Glycogen Storage Disease, type IXa1/IXa2	306000	Severe Hypoglycemic Episode	2	2	Dietary Management	3	3	2	**12**
PIK3CD	602839	Immunodeficiency 14	615513	primary immunodeficiency	2	3	Immune evaluation -> antibiotics/immunoglobulin	2	3	2	**12**
PMS2	600259	Lynch syndrome	120435	Colorectal Cancer	2	2	Colonoscopy	3	2	3	**12**
POU1F1	173110	Pituitary hormone deficiency, combined, 1	613038	FTT, short stature, cretinism, MR	2	3	Hormone replacement: Levothyroxine. rGH subQ to 17y	2	3	2	**12**
POU3F4	300039	Deafness, X-linked 2	304400	Communication Deficits	1	3	All Interventions	3	3	2	**12**
POU4F3	602460	Deafness, autosomal dominant 15	602459	Communication Deficits	1	3	All Interventions	3	3	2	**12**
PROS1	176880	Thrombophilia due to protein S deficiency (AR)	614514	Thrombosis, multiple sites	3	3	FFP replacement	2	2	2	**12**
PROS1	176880	Thrombophilia due to protein S deficiency (AD)	612336	Thrombosis, PE	2	3	Prophyaxis and avoidance of immobility	2	2	3	**12**
PTEN	601728	PTEN hamartoma tumor syndrome	158350	Breast / Uterine / Thyroid Cancer	2	3	Mammography / Thyroid Ultrasound	2	3	2	**12**
PTPN11	176876	LEOPARD syndrome 1	151100	Heart Defects	2	3	echo screening / no sports / ICD	1	3	3	**12**
PTPN11	176876	Leukemia, juvenile myelomonocytic	607785	Myelomonocytic leukemia	3	3	Monitoring for Noonan syndrome --> HSCT	1	2	3	**12**
PTPRC	151460	Severe Combined Immunodeficiency, T cell-negative, B-cell / Natural Killer-Cell Positive	608971	Chronic Generalized Infection leading to Death	3	3	HSCT / BMT	3	2	1	**12**
PTPRQ	603317	Deafness, autosomal recessive 84A	613391	Communication Deficits	1	3	Audiology --> Hearing Aids	3	3	2	**12**
PYGM	608455	McArdle Disease	232600	All Outcomes	1	3	Controlled physical training and programed glucose intake	2	3	3	**12**
RAF1	164760	LEOPARD syndrome 2	611554	Hypertrophic cardiomyopathy	2	3	Echo, EKG	2	3	2	**12**
RAF1	164760	Noonan syndrome 5	611553	Hypertrophic cardiomyopathy	2	3	Echo, EKG	2	3	2	**12**
RYR1	180901	Malignant hyperthermia susceptibility	145600	Anesthesia-induced malignant hyperthermia	2	1	Avoidance of certain anesthetics, extreme heat	3	3	3	**12**
RYR2	180902	Catecholaminergic polymorphic ventricular tachycardia	604772	Sudden death due to arrhythmia	3	3	Beta blockers, ICD	2	2	2	**12**
SCN5A	600163	Brugada Syndrome	601144	Sudden Death due to Arrhythmia	3	2	EKG screening / avoidance of triggers / ICD	2	3	2	**12**
SERPINA1	107400	Emphysema-cirrhosis, due to AAT deficiency	613490	COPD / Emphysema, liver cirrhosis	2	3	Smoking avoidance, periodic monitoring	1	3	3	**12**
SLC19A2	603941	Thiamine-responsive megaloblastic anemia syndrome	249270	Megablastic anemia, hearing loss, dm	2	3	Thiamine at pharmacologic doses	2	3	2	**12**
SLC40A1	604653	Hemochromatosis, type 4	606069	Multiple system iron overload (heart, liver)	2	2	Phlebotomy	3	3	2	**12**
SLC46A1	611672	Folate malabsorption, hereditary	229050	Anemia, hypogammaglobulinemia (like SCID)	2	3	Reduced folate supplementation (injection more common)	3	2	2	**12**
SLC5A5	601843	Thyroid dyshormonogenesis 1	274400	Brain, neuron damage, MR, FTT, jaundice. cretinism	1	3	Thyroid hormone replacement (L-thyroxine)	2	3	3	**12**
SLC7A7	603593	Lysinuric protein intolerance	222700	GI symptoms, FTT, vomiting, diarrhea	1	3	Protein restriction, citrulline, nitrogen-scavenging drugs, lysine, carnitine	2	3	3	**12**
SPR	182125	Dystonia, dopa-responsive, due to sepiapterin reductase deficiency	612716	Dystonia	1	3	Oral l-dopa and 5-hydroxytryptophan	3	3	2	**12**
STRC	606440	Deafness, autosomal recessive 16	603720	Communication Deficits	1	3	All Interventions	3	3	2	**12**
TAZ	300394	Barth Syndrome	302060	Cardiac disease (Dilated cardiomyopathy and sudden ventricular	2	3	Cardiac evaluation with consideration of medical therapy, heart transplant	2	3	2	**12**
TECTA	602574	Deafness, autosomal recessive 21 (DFNB21)	603629	Communication Deficits	1	3	All Interventions	3	3	2	**12**
TGFB2	190220	Loeys-Dietz Syndrome 4	614816	Aortic Dissection	3	3	Annual Echocardiogram	1	3	2	**12**
TGFBR1	190181	Loeys-Dietz Syndrome 1	609192	Aortic Dissection	3	3	Annual Echocardiogram	1	3	2	**12**
TGFBR2	190182	Loeys-Dietz Syndrome 2	610168	Aortic Dissection	3	3	Annual Echocardiogram	1	3	2	**12**
TMC1	606706	Deafness, Autosomal Recessive 7	606706	Communication Deficits	1	3	All Interventions	3	3	2	**12**
TMIE	607237	Deafness, autosomal recessive 6	600971	Communication Deficits	1	3	Audiology --> Cochlear Implants	3	3	2	**12**
TMPRSS3	605511	Deafness, autosomal recessive 8/10	601072	Communication Deficits	1	3	All Interventions	3	3	2	**12**
TPRN	613354	Deafness, autosomal recessive 79	613307	Communication Deficits	1	3	All Interventions	3	3	2	**12**
TRIOBP	609761	Deafness, autosomal recessive 28	609823	Communication Deficits	1	3	Audiology --> Hearing Aids	3	3	2	**12**
TSHR	603372	Hypothyroidism, congenital, nongoitrous, 1	275200	ID and growth retardation	2	2	T4 treatment	2	3	3	**12**

TTPA	600415	Ataxia with isolated vitamin E deficiency	277460	Ataxia, progressive	1	3	Vitamin E	3	3	2	**12**
USH1G	607696	Usher Syndrome, type 1G	606943	Usher Phenotype (Communication Deficits + RP)	2	3	Audiology and Vision Services	2	3	2	**12**
USH2A	608400	Usher Syndrome, type 2A	276901	Usher Phenotype (Communication Deficits + RP)	1	3	Audiology and Vision Services	2	3	3	**12**
VWF	613160	von Willebrand disease, types 2A, 2B, 2M, and 2N	613554	Mild to Moderate Mucocutaneous Bleeding	1	2	Hematology --> Desmopressin	3	3	3	**12**
ABCA3	601615	Surfactant metabolism dysfunction, pulmonary, 3	610921	Repiratory Distress	3	3	Surfactant Replacement	1	1	3	**11**
ABCD1	300371	Adrenoleukodystrophy	300100	Adult myeloneuropathy with childhood onset adrenal insufficiency	2	3	biochemical screening and corticosteroid replacement	2	3	1	**11**
ABCG5	605459	Sitosterolemia	210250	All Outcomes	2	3	All Interventions	2	2	2	**11**
ACTA2	102620	Familial Thoracic Aortic Aneurysms	611788	Aortic Dissection	3	2	Annual Echocardiogram	2	3	1	**11**
AK2	103020	Reticular Dysgenesis	267500	SCID	3	3	HSCT	1	2	2	**11**
ARSB	611542	Mucopolysaccharidosis type VI (Maroteaux-Lamy)	253200		2	3	HSCT or ERT	2	2	2	**11**
BLM	604610	Bloom syndrome	210900	Colorectal cancer	2	2	Colonoscopy	3	2	2	**11**
CACNA1D	114206	Sinoatrial Node Dysfunction and Deafness	614896	Communication Deficits	1	3	Audiology --> Hearing Aids --> Cochlear Implant	3	3	1	**11**
CACNA1S	114208	Malignant hyperthermia susceptibility 5	601887	Anesthesia-induced malignant hyperthermia	2	1	Avoidance of Certain Anesthetics	3	3	2	**11**
CCDC50	611051	Deafness, autosomal dominant 44	607453	Communication Deficits	1	3	All Interventions	3	3	1	**11**
CD40LG	300386	Immunodeficiency, X-linked, with hyper-IgM	308230	Primary Immunodeficiency	2	3	BMT	2	2	2	**11**
CDC73	607393	Hyperparathyroidism, familial primary	145000	Parathyroid carcinoma	2	2	Biochemical screening	3	3	1	**11**
CDH23 / PCDH15	605514	Usher Syndrome, type ID / F Digenic	601067	Usher Phenotype (Communication Deficits + RP)	2	3	Audiology and Vision Services	2	3	1	**11**
CEACAM16	614591	?Deafness, autosomal dominant 4B	614614	Communication Deficits	1	3	Audiology --> Hearing Aids --> Cochlear Implant	3	3	1	**11**
CLRN1	606397	Usher Syndrome, type 3A	276902	Usher Phenotype (Communication Deficits + RP)	1	3	Audiology and Vision Services	2	3	2	**11**
COL11A2	120290	Deafness, autosomal recessive 53	609706	Communication Deficits	1	3	Audiology --> Hearing Aids	3	3	1	**11**
COL1A1	120150	Caffey Disease	114000	Hyperostosis and pain	1	3	Monitor for pain, early pain management with symptoms	2	3	2	**11**
COL3A1	120180	Ehlers-Danlos Syndrome - Vascular Type	130050	Arterial Dissection / Organ Rupture	3	3	Echocardiogram / MRA	1	3	1	**11**
CYP11B1	610613	Adrenal hyperplasia, congenital, due to 11-beta-hydroxylase deficiency	202010	Androgen excess, virilization, and hypertension	2	2	Glucocorticoid Administration	2	3	2	**11**
DFNA5	608798	Deafness, autosomal dominant 5	600994	Communication Deficits	0	3	All Interventions	3	3	2	**11**
DFNB59	610219	Non-Syndromic Deafness, Recessive	610220	Communication Deficits, with or without auditory neuropathy spectrum	1	3	All Interventions	3	3	1	**11**
DHCR7	602858	Smith-Lemli-Opitz syndrome	270400	Microcephaly, developmental delay, behavioral issues, congenital anomalies	2	3	Cholesterol supplementation	1	3	2	**11**
DIAPH1	602121	Deafness, autosomal dominant 1	124900	Communication Deficits	1	3	All Interventions	3	3	1	**11**
DOCK8	611432	Hyper-IgE recurrent infection syndrome, autosomal recessive	243700	Combined Immunodeficiency	2	2	HSCT	3	2	2	**11**
DSG2	125671	Arrhythmogenic right ventricular cardiomyopathy 10	610193	Sudden death due to arrhythmia	3	2	Echo screening/no sports/ICD	2	2	2	**11**
DSP	125647	Arrhythmogenic right ventricular cardiomyopathy 8	607450	Sudden death due to arrhythmia	3	2	Echo screening/no sports/ICD	2	2	2	**11**
EDNRB	131244	Waardenburg syndrome, type 4A	277580	Communication Deficits or Hirschsprung Disease	1	2	Audiology --> Hearing Aids --> Cochlear Implant or Screening -->	3	3	2	**11**
ESPN	606351	Deafness, autosomal recessive 36	609006	Communication Deficits with or without Vestibular Involvement	1	3	Audiology --> Hearing Aids	3	3	1	**11**
ESRRB	602167	Non-Syndromic Deafness, Recessive 35	608565	Communication Deficits	1	3	All Interventions	3	3	1	**11**
EYA1	601653	Branchiootorenal syndrome 1, with or w/o cataracts / Anterior segment anomalies with or w/o cataracts	113650	Communication Deficits or Renal anomalies, abnl function, electrolyte	1	3	Audiology / Nephrology eval - Test renal function --> Treat to prevent	2	3	2	**11**
EYA4	603550	Non-Syndromic Deafness, Dominant 10	601316	Communication Deficits	1	3	All Interventions	3	3	1	**11**
F5	612309	Thrombophilia due to activated protein C resistance	188055	Risk for thrombosis	2	2	Consider prophylaxis with surgery, avoidance of venous stasis	2	3	2	**11**
FGFR3	134934	CATSHL syndrome	610474	Communication deficits	1	3	Audiology --> Hearing Aids --> Cochlear Implant	3	3	1	**11**
FGFR3	134934	Hypochondroplasia	146000	Craniosynostosis --> increased intracranial pressure	1	3	Physical exam --> imaging to screen --> surgery if necessary	2	2	3	**11**
FOXE1	602617	Bamforth-Lazarus syndrome	241850	absent thyroid tissue, hypothyroidism	2	3	Thyroid Hormone Replacement	2	3	1	**11**

GLA	300644	Fabry disease - male hemizygous	301500	Heart / renal involvement	2	2	Enzyme replacement	2	2	3	**11**
GYS2	138571	Glycogen Storage Disease, type 0	240600	Hypoglycemia	1	3	Avoid fasting, frequent feeding, emergency letter	3	3	1	**11**
HBG2	142250	Cyanosis, transient neonatal	613977		0	3	Avoidance of Unnecessary Intervention	3	3	2	**11**
HFE - C282Y / H63D	613609	Hereditary hemochromatosis C282Y / H63D Compound Hets		Multiple system iron overload (heart, liver)	2	0	Yearly ferritin -> phlebotomy	3	3	3	**11**
HGF	142409	Deafness, autosomal recessive 39	608265	Communication Deficits	1	3	All Interventions	3	3	1	**11**
HMGCL	613898	HMG-CoA lyase deficiency	246450	Severe hypoglycemia, metabolic acidosis, coma, death	2	3	Avoid fasting, low protein diet, restrict leucine and supplement l-	2	2	2	**11**
HMGCS2	600234	HMG-CoA synthase-2 deficiency	605911	Hypoketotic hypoglycemic, sz and coma	2	2	Avoid fasting, carnitine supplementation	3	3	1	**11**
HSB11B2	614232	Apparent mineralocorticoid excess	218030	Hypertensive crisis (onset ranges from childhood to adult)	2	3	Monitoring, spironolactone	2	3	1	**11**
HSD11B2	614232	Apparent mineralocorticoid excess	218030	Hypertensive crisis (onset ranges from childhood to adult)	2	3	Monitoring, spironolactone	2	3	1	**11**
IDS	300823	Mucopolysaccharidosis Type II	309900	All Outcomes	2	3	Enzyme Replacement Therapy	2	2	2	**11**
IL2RA	147730	Interleukin-2 receptor, alpha chain, deficiency of	606367	T-cell immune deficiency; autoimmune disease; enteropathy;	2	3	'allogeneic bone marrow transplant following cytoreduction'	3	2	1	**11**
KARS	601421	Deafness, autosomal recessive 89	613916	Communication Deficits	1	3	All Interventions	3	3	1	**11**
KCNE2	603796	Long QT syndrome 6	613693	Sudden death due to arrhythmia	3	2	EKG screening / avoidance of triggers / ICD	2	3	1	**11**
KCNJ5	600734	Long QT syndrome 13	613485	Sudden death due to arrhythmia	3	2	EKG and avoidance of triggers	2	3	1	**11**
LHFPL5	609427	Deafness, autosomal recessive 67	610265	Communication Deficits	1	3	All Interventions	3	3	1	**11**
LMBRD1	612625	Methylmalonic aciduria and homocystinuria, cblF type	277380	All Outcomes	1	3	B12 replacement (Hydroxocobalamin)	2	3	2	**11**
LOXHD1	613072	Deafness, autosomal recessive 77	613079	Communication Deficits	1	3	All Interventions	3	3	1	**11**
MIR96	611606	Deafness, autosomal dominant 50	613074	Communication Deficits	1	3	All Interventions	3	3	1	**11**
MLYCD	606761	Malonyl-CoA Decarboxylase Deficiency	248360	Cardiomyopathy	2	2	High carb, low long chain fatty acid, and medium chain triglyceride and L-	2	3	2	**11**
MSRB3	613719	Deafness, autosomal recessive 74	613718	Communication Deficits	1	3	Audiology --> Hearing Aids	3	3	1	**11**
MTR	156570	Homocystinuria-megaloblastic anemia, cblG complementation type	250940	Severe failure to thrive, megaloblastic anemia, and neurologic manifestations	2	3	B12 replacement	2	3	1	**11**
MTRR	602568	Homocystinuria-megaloblastic anemia, cbl E type	236270	Severe failure to thrive, megaloblastic anemia, and neurologic manifestations	2	3	B12 replacement	2	3	1	**11**
MYH14	608568	Non-Syndromic Deafness, Dominant 4A	600652	Communication Deficits	1	3	Audiology --> Hearing Aids	3	3	1	**11**
MYH9	160775	Deafness, autosomal dominant 17	603622	Communication Deficits	1	3	Audiology --> Hearing Aids	3	3	1	**11**
MYO6	600970	Non-Syndromic Deafness, Recessive 37	607821	Communication Deficits	1	3	Audiology --> Hearing Aids	3	3	1	**11**
MYO7A	276903	Deafness, autosomal dominant 11	601317	Communication Deficits	1	3	Audiology --> Cochlear Implants	3	3	1	**11**
NF2	607379	Neurofibromatosis type 2	101000	Meningiomas; 2.5x increased risk mortality if present	1	3	Annual MRI starting @ 10 yr, avoid radiotherapy	2	3	2	**11**
NPC2	601015	Niemann-Pick Disease, type C2	607625	Mental deterioration --> developmental delay, seizures, psychiatric and	2	3	Miglustat for Stabilization	1	3	2	**11**
OAT	613349	Gyrate Atrophy of the Choroid and Retina	258870	Progressive Chorioretinal Degeneration	1	3	Dietary: restriction of arginine; some responsive to pyridoxal phosphate	2	2	3	**11**
OTOA	607038	Deafness, autosomal recessive 22	607039	Communication Deficits	1	3	All Interventions	3	3	1	**11**
OTOG	604487	AR Nonsyndromic Sensorineural Deafness Type DFNB	614945	Communication Deficits	1	3	Audiology --> Cochlear Implants	3	3	1	**11**
OTOGL	614925	Deafness, autosomal recessive 84B	614944	Communication Deficits	1	3	All Interventions	3	3	1	**11**
P2RX2	600844	Deafness, autosomal dominant 41	608224	Communication Deficits	1	3	All Interventions	3	3	1	**11**
PALB2	610355	{Breast cancer, susceptibility to}	114480	Breast cancer	2	2	Increased surveillance to include MRI	2	3	2	**11**
PAX3	606597	Waardenburg Syndrome, type 3	148820	Communication Deficits	1	2	Audiology --> Hearing Aid --> Cochlear Implant	3	3	2	**11**
PHKG2	172471	Liver Phosphorylase Kinase Deficiency	613027	Hypoglycemia / Hepatomegaly	1	3	Avoidance of fasting, corn starch, night time feedings	3	3	1	**11**
PKP2	602861	Arrhythmogenic right ventricular cardiomyopathy 9	609040	Sudden death due to arrhythmia	3	2	Echo screening/no sports/ICD	2	2	2	**11**
PNP	164050	Immunodeficiency due to purine nucleoside phosphorylase deficiency	613179	All Outcomes	3	3	HSCT	1	2	2	**11**
POLD1	174761	{Colorectal cancer, susceptibility to, 10}	612591	colon cancer	2	3	colonoscopy	3	2	1	**11**

PRKAG2	602743	Cardiomyopathy, Familial Hypertrophic, 6	600858	Sudden Death due to Arrhythmia	3	1	Echo screening / no sports / EKG / ICD	2	3	2	**11**
PRKAG2	602743	Glycogen storage disease of heart, lethal congenital	261740	Arrhythmia	3	1	No Effective Intervention	2	3	2	**11**
PRKAG2	602743	Wolff-Parkinson-White syndrome	194200	Arrhythmia	3	1	Echo screening / no sports / EKG / ICD/Ablation Therapy	2	3	2	**11**
PROC	612283	Thrombophilia due to protein C deficiency, autosomal dominant	176860	deep vein thrombosis with or without pulmonary embolism	2	2	anticoagulation, avoid immobility	2	3	2	**11**
PRPS1	311850	Deafness, X-linked 1	304500	Communication Deficits	1	3	All Interventions	3	3	1	**11**
RDX	179410	Deafness, autosomal recessive 24	611022	Communication Deficits	1	3	All Interventions	3	3	1	**11**
RIT1	609591	Noonan syndrome 8	615355	Hypertrophic cardiomyopathy	2	3	Echo, EKG	2	3	1	**11**
SCN4A	603967	Hyperkalemic periodic paralysis, type 2	170500	Episodic weakness, progressive myopathy	1	3	Dichlorphenamide	3	2	2	**11**
SCN4B	608256	Long QT syndrome-10	611819	Sudden death due to arrhythmia	3	2	EKG screening / avoidance of triggers / ICD	2	3	1	**11**
SERPINC1	107300	Thrombophilia due to antithrombin III deficiency	613118	Thrombosis	2	3	Avoidance of OCPs; immobility; prophylaxis with surgery	2	2	2	**11**
SLC26A5	604943	Deafness, autosomal recessive 61	613865	Communication Deficits	1	3	All Interventions	3	3	1	**11**
SLC2A1	138140	GLUT1 deficiency syndrome 1	606777	Infantile seizures, acquired microcephaly, and developmental delay	2	3	Ketogenic diet	2	2	2	**11**
SLC39A4	607059	Acrodermatitis enteropathica	201100	Dermatitis	0	3	zinc supplementation	3	3	2	**11**
SLC3A1	104614	Cystinuria	220100	Renal calculi and chronic renal failure	1	3	Urine screening, increased fluid, urinary alkalinization, other medical	2	3	2	**11**
SLC7A9	604144	Cystinuria	220100	Renal calculi and chronic renal failure	1	3	Urine screening, increased fluid, urinary alkalinization, other medical	2	3	2	**11**
SMAD4	600993	Juvenile polyposis/hereditary hemorrhagic telangiectasia syndrome	175050	Pulmonary AVM, GI bleeding, CVA from cerebral AVMs?	2	2	Contrast echo	2	3	2	**11**
SMPX	300226	Deafness, X-Linked 4	300066	Communication Deficits	1	3	All Interventions	3	3	1	**11**
SOX10	602229	Waardenburg Syndrome, type 2E	611584	Communication Deficits	1	3	Audiology --> Hearing Aids --> Cochlear Implant	2	3	2	**11**
STK11	602216	Peutz-Jeghers Syndrome	175200	Gastrointestinal Cancer	2	3	Colonoscopy / Upper Endoscopy	2	2	2	**11**
SYNE4	615535	Deafness, autosomal recessive 76	615540	Communication Deficits	1	3	All Interventions	3	3	1	**11**
TAT	613018	Tyrosinemia, type II	276600	All Outcomes	1	3	Dietary restriction	3	2	2	**11**
TBC1D24	613577	Deafness, autosomal recessive 86	614617	Communication Deficits	1	3	All Interventions	3	3	1	**11**
TBX19	604614	Adrenocorticotropic hormone deficiency	201400	Neonatal hypoglycemia, seizures	2	3	Glucocorticoid administration	2	2	2	**11**
TCN2	613441	Transcobalamin II deficiency	275350	FTT	1	2	Metabolic Evaluation --> Cobalamin Supplementation	3	3	2	**11**
TMC1	606706	Deafness, Autosomal Dominant 36	606705	Communication Deficits	1	3	All Interventions	3	3	1	**11**
TMEM43	612048	Arrhythmogenic right ventricular cardiomyopathy 5	604400	Sudden death due to arrhythmia	3	3	Echo screening/no sports/ICD	2	2	1	**11**
TNNT2	191045	Dilated cardiomyopathy 1D	601494	Death due to arrhythmia or heart failure	2	3	Echo screening/ICD	2	3	1	**11**
TP53	191170	Li-Fraumeni syndrome	151623	Multiple Cancers	2	3	Whole Body Imaging	1	3	2	**11**
TPM1	191010	Hypertrophic cardiomyopathy 3	115196	Arrhythmia	3	1	Echo Screening / no sports / ICD	3	3	1	**11**
TRH	613879	Thyrotropin-releasing hormone deficiency	275120	Central hypothyroidism	1	3	Thyroid hormone replacement	3	3	1	**11**
TSC1	605284	Tuberous Sclerosis Complex	191100	CNS tumors and renal lesions	2	3	Screening: Cranial and Renal imaging 1-3yrs, baseline Chest CT for women.	1	3	2	**11**
TSC2	191092	Tuberous Sclerosis Complex	613254	CNS tumors and renal lesions	2	3	Screening: Cranial and Renal imaging 1-3yrs. baseline Chest CT for women.	1	3	2	**11**
VWF	613160	von Willebrand disease, type 1	193400	Mild Mucocutaneous Bleeding	0	2	Hematology --> Desmopressin	3	3	3	**11**
WFS1	606201	Deafness, autosomal dominant 6/14/38	600965	Communication Deficits	0	3	All Interventions	3	3	2	**11**
WFS1	606201	Wolfram Syndrome	222300	All Outcomes	1	3	All Interventions	1	3	3	**11**
ABCD1	300371	Adrenoleukodystrophy	300100	Childhood onset cognitive decline	3	2	HSCT	1	2	2	**10**
ADCK3	606980	Coenzyme Q10 deficiency, primary, 4	612016	Cerebellar ataxia - slow, minimal progression	1	3	CoQ10 treatment	2	3	1	**10**
AHCY	180960	Hypermethioninemia with deficiency of S-Adenosylhomocysteine Hydrolase	613752	Mental and Motor Retardation / ID	2	3	Correction of Biochemical Abnormalities via Dietary Methionine	2	3	0	**10**
ALB	103600	familial dysalbuminemic hyperthyroxinemia	615999	Dysalbuminemic hyperthyroxinemia (typically benign)	1	1	education/avoidance of thyroidectomy	3	3	2	**10**

APTX	606350	Coenzyme Q10 deficiency, secondary	612016	Cerebellar ataxia - slow, minimal progression	1	3	CoQ10 treatment	2	3	1	**10**
ARG1	608313	Argininemia	207800	Spasticity	1	3	Diet, sodium phenylacetate and sodium benzoate	2	2	2	**10**
CABP2	607314	Deafness, autosomal recessive 93	614899	Communication Deficits	1	3	All Interventions	3	3	0	**10**
CDH1	192090	Hereditary diffuse gastric cancer	137215	Gastric Cancer	2	3	Gastrectomy	3	0	2	**10**
COL11A2	120290	Deafness, autosomal dominant 13 (DFNA13)	601868	Communication Deficits	0	3	Audiology --> Hearing Aids	3	3	1	**10**
COL1A1	120150	Osteogenesis Imperfecta, type I	166200	Fractures or Hearing Loss (Conductive --> Sensorineural)	1	2	Biophosphonates	2	2	3	**10**
COL1A2	120160	Osteogenesis imperfecta, type I	166200	Multiple Fractures	1	3	Bisphosphonates, Anticipatory management	1	2	3	**10**
COQ2	609825	Coenzyme Q10 deficiency, primary, 1	607426	Infantile or early childhood onset nephropathy, AND Infantile	2	3	CoQ10 treatment	1	3	1	**10**
COQ9	612837	Coenzyme Q10 deficiency, primary, 5	614654	Infantile multisystem dis w/ rapid progression and high mortality	2	3	CoQ10 treatment	1	3	1	**10**
CPT1A	600528	CPT deficiency, hepatic, type IA	255120	Hypoketotic hypoglycemia, liver failure	2	2	Diet, avoidance of fasting	2	2	2	**10**
CTSK	601105	Pycnodysostosis	265800	Short Stature	0	3	Odanacatib (Clinical Trial Underway)	2	3	2	**10**
DFNB31	607928	Usher Syndrome, type 2D	611383	Usher Phenotype (Communication Deficits + RP)	1	3	Audiology and Vision Services	2	3	1	**10**
DNAAF3	614566	Ciliary Dyskinesia, Primary, 2	606763	Chronic Sinopulmonary Disease	1	3	Management of symptoms: enhance mucous clearance similar to CF	2	3	1	**10**
EYA1	601653	?Otofaciocervical Syndrome 1	166780	Communication Deficits	1	3	Audiology --> Hearing Aids --> Cochlear Implant	3	3	0	**10**
F12	610619	Angioedema, hereditary, type III	610618	Swelling --> Respiratory Compromise	2	2	Tranexanic Acid (Prophylactically)	2	3	1	**10**
FGFR3	134934	Crouzon syndrome with acanthosis nigricans	612247	Craniosynostosis --> increased intracranial pressure	1	3	Physical exam --> imaging to screen --> surgery if necessary	3	1	2	**10**
FH (Dominant)	136850	Leiomyomatosis and renal cell cancer	150800	Renal Cancer	2	2	Abdominal imaging	2	3	1	**10**
FLCN	607273	Birt-Hogg-Dube syndrome	135150	Renal cancer	1	2	High Risk management / imaging, etc	2	3	2	**10**
FUCA1	612280	Fucosidosis	230000	All Outcomes	2	3	BMT / HSCT	2	1	2	**10**
GH1	139250	Growth hormone deficiency, isolated, type IA (recessive)	262400	Postnatal growth deficiency, hypoglycemia	2	2	Avoid fasting, GH replacement	2	2	2	**10**
GJB2	121011	Bart-Pumphrey Syndrome	149200	Communication Deficits	1	3	Audiology --> Hearing Aids --> Cochlear Implant	2	2	2	**10**
GRHL2	608576	Deafness, autosomal dominant 28	608641	Communication Deficits	0	3	All Interventions	3	3	1	**10**
GSS	601002	Glutathione Synthetase Deficiency	266130	Hemolytic Anemia + Metabolic Acidosis + CNS Dysfunction	2	2	Vitamin C and E	2	3	1	**10**
HARS	142810	Usher Syndrome, type 3B	614504	Usher Phenotype (Communication Deficits + RP)	1	3	Audiology and Vision Services	2	3	1	**10**
HARS2	600783	Perrault Syndrome 2	614926	Communication Deficits	1	3	Audiology --> Hearing Aids --> Cochlear Implant	3	3	0	**10**
HNF1A	142410	MODY, type III (MODY3)	600496	Type II diabetes and complications	1	3	Glucose monitoring and early treatment	1	3	2	**10**
HNF1B	189907	Renal cysts and diabetes syndrome (MODY5)	137920	Type II diabetes and complications	1	3	Glucose monitoring, early treatment	1	3	2	**10**
HNF4A	600281	MODY, type I (MODY1)	125850	Type II diabetes and complications	1	3	Glucose monitoring and early treatment	1	3	2	**10**
IDUA	252800	Mucopolysaccharidosis Ih	607014	Sudden Cardiac Death or Cognitive Disability	2	3	HSCT	2	1	2	**10**
IL21R	605383	Immunodeficiency, primary, autosomal recessive, IL21R-related	615207	chronic cholangitis and liver disease associated with cryptosporidial	2	3	HSCT	3	2	0	**10**
KCNJ5	600734	Hyperaldosteronism, familial, type III	613677	Hypertensive crisis (onset usually in childhood)	2	3	Adrenalectomy	3	1	1	**10**
LAMB1	150240	Lissencephaly 5	615191	psychomotor retardation/seizures	2	3	Antiepileptics	1	3	1	**10**
LARS2	604544	Perrault Syndrome 4	615300	Communication Deficits	1	3	Audiology --> Hearing Aids --> Cochlear Implant	3	3	0	**10**
LIPA	613497	Cholesteryl Ester Storage Disease (Lysosomal Acid Lipase) *OR* Wolman Disease	278000	CEPD: Late onset, slow progressing liver disease	1	3	Screening --> Liver Transplant; ERT w/ Sebelipase Alfa	2	2	2	**10**
LYST	606897	Chediak-Higashi syndrome	214500	Accelerated phase (multiorgan inflammation, lymphoproliferative	2	3	Monitoring of organomegaly and liver dysfunction. CBC for cytopenias -->	2	1	2	**10**
MMADHC	611935	Methylmalonic aciduria and homocystinuria, cblD type	277410	Metabolic decompensation	2	3	Diet, B-12, illness management	2	2	1	**10**
MYBPC3	600958	Hypertrophic cardiomyopathy 4	115197	Arrhythmia	3	1	Echo Screening / no sports / ICD	1	3	2	**10**
MYH9	160775	Macrothrombocytopenia and progressive sensorineural deafness	600208	Communication Deficits	1	3	Audiology --> Hearing Aids --> Cochlear Implant	2	2	2	**10**
NF1	613113	Neurofibromatosis, type 1	162200	Neurofibromatosis / MPNST	2	2	Annual exam, awareness of symptoms	1	3	2	**10**

PDSS1	607429	Coenzyme Q10 deficiency, primary, 2	614651	Infantile multisystem dis w/ rapid progression and high mortality	2	3	CoQ10 treatment	1	3	1	**10**
PDSS2	610564	Coenzyme Q10 deficiency, primary, 3	614652	Infantile multisystem dis w/ rapid progression and high mortality	2	3	CoQ10 treatment	1	3	1	**10**
PKP2	602861	Arrhythmogenic right ventricular cardiomyopathy 9	609040	Arrhythmia	3	2	Echo Screening / no sports / ICD	1	2	2	**10**
PNPT1	610316	Deafness, autosomal recessive 70	614934	Communication Deficits	1	3	All Interventions	3	3	0	**10**
PYGL	613741	Glycogen Storage Disease VI	232700	All Outcomes	0	3	Dietary increase in protein / corn starch 1-3 times daily	3	3	1	**10**
RAB23	606144	Carpenter Syndrome	201000	Increased Intracranial Pressure	2	2	Monitoring --> Surgery when indicated to correct skull sutures	3	1	2	**10**
SDHB	185470	Hereditary Paraganglioma-Pheochromocytoma Syndrome 4	115310	Nonmalignant PGL / PCC	2	2	Annual Biochemical Screening	1	3	2	**10**
SDHD	602690	Hereditary Paraganglioma-Pheochromocytoma Syndrome 1	168000	Nonmalignant PGL / PCC	1	3	Annual Biochemical Screening	1	3	2	**10**
SFTPB	178640	Surfactant metabolism dysfunction, pulmonary, 1	265120	All Outcomes	3	3	Lung Transplant	1	1	2	**10**
SFTPC	178620	Surfactant metabolism dysfunction, pulmonary, 2	610913	Respiratory distress / respiratory failure	1	3	anticipatory guidance/ hydroxychloroquine / systemic	2	2	2	**10**
SIX1	601205	Branchio-oto-renal related disorders	608389	Communication Deficits / Renal anomalies	1	3	Audiology / Nephrology evaluaton and management	2	3	1	**10**
SLC17A8	607557	Deafness, autosomal dominant 25	605583	Communication Deficits	0	3	Audiology --> Hearing Aids	3	3	1	**10**
SLC22A5	603377	Carnitine deficiency, systemic primary	212140	Metabolic decompensation, broad clinical spectrum	1	1	L-carnitine supplementation	3	3	2	**10**
SLC25A13	603859	Citrullinemia, type II, neonatal-onset	605814	Failure to thrive, cirrhosis. But most have resolution by 6 months	1	2	Dietary formula, vitamin D	2	3	2	**10**
SLC25A15	603861	Hyperornithinemia-Hyperammonemia-Homocitrullinemia Syndrome	238970	Neurocognitive Deficits	1	3	Dietary, Avoid high protein intake, Screening for Amonimia	2	2	2	**10**
SLC2A9	606142	Hypouricemia, renal, 2	612076	Hypouricemia -->exercise induced acute renal failure	1	2	Dialysis	3	2	2	**10**
SMAD3	603109	Loeys-Dietz Syndrome 3	613795	Aortic Dissection	3	1	Annual Echocardiogram	2	3	1	**10**
SNAI2	602150	Waardenburg Syndrome, type 2D	608890	Communication Deficits	1	3	Audiology --> Hearing Aid --> Cochlear Implant	3	3	0	**10**
SOX10	602229	PCWH Syndrome	609136	Neurologic Abnormalities (Developmental delay, mental	2	3	Audiology --> Hearing Aids --> Cochlear Implant	1	3	1	**10**
TBC1D24	613577	Deafness, autosomal dominant 65	616044	Communication Deficits	0	3	All Interventions	3	3	1	**10**
TSPEAR	612920	Deafness, autosomal recessive 98	614861	Communication Deficits	1	3	All Interventions	3	3	0	**10**
USH1C	605242	Deafness, autosomal recessive 18A	602092	Communication Deficits	1	3	Audiology --> Hearing Aids	3	3	0	**10**
WT1	607102	WT1-related Wilms	194070	Wilms Tumor	2	2	Abdominal US 3x/year --> surgical removal (if needed)	2	3	1	**10**
ACADSB	600301	2-Methylbutyrylglycinuria	610006	Hypoglycemia, acidosis, seizure, coma	2	0	Avoid Fasting	3	3	1	**9**
AGA	613228	Aspartylglucosaminuria	208400	Mental deterioration/Mental retardation --> seizures	2	3	BMT	1	1	2	**9**
ALDH4A1	606811	Hyperprolinemia, type II	239510	Epilepsy	1	3	Vitamin B6 Supplementation	1	3	1	**9**
AMT	238310	Glycine Encephalopathy	605899	Epileptic Encephalopathy or Seizures	2	3	Avoid valproate; NaBenzoate may have some effect or ketogenic diet	1	1	2	**9**
ANK2	106410	Long QT Syndrome 4	600919	Sudden Death due to Arrhythmia	3	0	EKG screening / avoidance of triggers / ICD	2	3	1	**9**
ARSA	607574	Metachromatic Leukodystrophy	250100	All Outcomes	2	3	All Interventions	1	1	2	**9**
BRIP1	605882	Breast Cancer, early-onset	114480	breast cancer	2	2	Early and increased screening (every 6 months) with breast MRI.	1	3	1	**9**
BRIP1	605882	Fanconi Anemia complementation group J	609054	Bone marrow failure, leukemia	2	3	Surveillance, HSCT	2	1	1	**9**
CLCNKB	602023	Bartter syndrome, type 3	607364	Renal failure due to salt-wasting	1	3	Sodium and potassium supplements and aldosterone antagonists and	1	3	1	**9**
CNNM2	607803	Hypomagnesemia 6, Renal	613882	Seizures	1	3	Magnesium Supplements --> Antiepileptics	1	3	1	**9**
COL1A1	120150	Osteogenesis Imperfecta Type IV	166220	Limb Deformity	1	3	Pamidronate Therapy	2	2	1	**9**
COL1A2	120160	Osteogenesis imperfecta 2	166210	Severe, congenital fractures	3	3	No Effective Intervention	0	0	3	**9**
COQ6	614647	Coenzyme Q10 deficiency, primary, 6	614650	Infant to juvenile onset nephropathy w/ deafness AND Infantile multisystem dis	1	3	CoQ10 Treatment	1	3	1	**9**
DMD	300377	Becker Muscular Dystrophy	300376	Heart Failure	2	3	cardiac evaluations --> ACE inhibitor / beta blockers	0	3	1	**9**
DMD	300377	Duchenne Muscular Dystrophy	310200	cardiopulmonary failure	2	3	cardiac evaluations --> ACE inhibitor / beta blockers	0	3	1	**9**
ETFA	608053	Glutaric acidemia IIA (severe forms)	231680	Neonatal acidosis and hypoglycemia	3	3	No Effective Intervention	0	0	3	**9**

ETFB	130410	Glutaric acidemia IIB (severe forms)	231680	Neonatal acidosis and hypoglycemia	3	3	No Effective Intervention	0	0	3	**9**
ETFDH	231680	Glutaric acidemia IIC (severe forms)	231680	Neonatal acidosis and hypoglycemia	3	3	No Effective Intervention	0	0	3	**9**
ETHE1	608451	Ethylmalonic Encephalopathy	602473	Death secondary to Neurodegeneration	3	3	Riboflavin and CoQ10 Supplementation	0	2	1	**9**
F11	264900	Factor XI deficiency, autosomal recessive	612416	Bleeding	0	2	Fresh frozen plasma with procedures / trauma or F11	3	2	2	**9**
FGFR3	134934	Thanatophoric dysplasia, type I	187600	Lethal Skeletal Dysplasia	3	3	No Effective Intervention	0	0	3	**9**
FGFR3	134934	Thanatophoric dysplasia, type II	187601	Lethal Skeletal Dysplasia	3	3	No Effective Intervention	0	0	3	**9**
FLCN	607273	Birt-Hogg-Dube syndrome	135150	Renal cancer	2	1	Abdominal imaging	2	3	1	**9**
FTCD	606806	Glutamate Formiminotransferase Deficiency	229100	Intellectual Disability	1	3		1	3	1	**9**
GALC	606890	Krabbe Disease	245200	Death by 2 years due to progressive neurologic deterioration	3	3	Supportive Management	0	0	3	**9**
GALNS	612222	Mucopolysaccharidosis IVA	253000	Skeletal	1	3	ERT	1	2	2	**9**
GAMT	601240	Cerebral creatine deficiency syndrome 2; Guanidinoacetate methyltransferase deficiency	612736	Autism, extrapyramidal symptoms	1	3	Creatine supplementation	1	3	1	**9**
GATM	602360	Cerebral creatine deficiency syndrome 3; L-arginine:glycine amidinotransferase deficiency	612718	Encephalopathy	1	3	Creatine supplementation	1	3	1	**9**
GBA	606463	Gaucher Disease, all other types (perinatal lethal, type II, type III, type IIIC)		Progressive Neurologic Deterioration	3	3	No Effective Intervention	0	0	3	**9**
GCK	138079	Diabetes mellitus, permanent neonatal	606176	Neonatal diabetes	3	0	Insulin	3	3	0	**9**
GJB6	604418	Non-Syndromic Deafness, Dominant 3B	612643	Communication Deficits	1	2	All Interventions	3	3	0	**9**
GRHL2	608576	Ectodermal dysplasia/short stature syndrome	616029	Communication Deficits	1	2	Audiology --> Hearing Aids --> Cochlear Implants	3	3	0	**9**
GUSB	611499	Mucopolysaccharidosis VII	253220	Moderate with some Organomegaly and Moderate Skeletal Abnormalities	2	3	Clinical Monitoring --> HSCT	2	1	1	**9**
HEXA	606869	Tay-Sachs Disease	272800	Psychomotor degeneration --> Hypotonia, seizures, dementia	3	3	No Effective Intervention	0	0	3	**9**
HEXB	268800	Sandhoff Disease	606873	All Outcomes	3	3	No Effective Intervention	0	0	3	**9**
HSD17B4	601860	Perrault Syndrome 1	233400	All Outcomes	1	3	All Interventions	2	3	0	**9**
HTT	613004	Huntington disease	143100	Neurodegeneration	3	3	No Effective Intervention	0	0	3	**9**
KMT2D	602113	Kabuki syndrome	147920	Intellectual disability	1	3	Early childhood intervention	1	3	1	**9**
LCK	153390	Immunodeficiency 22	615758	SCID	3	0	BMT	3	2	1	**9**
LIG4	601837	LIG4 Syndrome	606593	combined immune deficiency (not severe, infantile onset)	1	3	Hematologic evaluation --> HSCT	1	3	1	**9**
LIG4	601837	Severe Combined Immunodeficiency with Sensitivity to Ionizing Radiation	602450	SCID	3	0	Hematologic Evaluation --> HSCT	2	3	1	**9**
MAN2B1	609458	Mannosidosis, alpha-, types I and II	248500	Intellectual Disability/neurological motor problems	1	3	BMT	2	1	2	**9**
MAX	154950	pheochromocytoma susceptibility	171300	malignant pheochromocytoma/paraganglioma	2	2	Biochemical Screening	1	3	1	**9**
MYBPC3	600958	Cardiomyopathy, dilated, 1MM	615396	Arrhythmia or heart failure	3	0	Echo Screening / ICD	2	3	1	**9**
MYO3A	606808	Deafness, autosomal recessive 30	607101	Communication Deficits	0	3	Audiology --> Hearing Aids	3	3	0	**9**
MYOZ2	605602	Cardiomyopathy, Familial Hypertrophic, 16	613838	Sudden Death due to Arrhythmia	3	0	Echo Screening / No Sports / ICD	3	2	1	**9**
NDUFS4	602694	Leigh Syndrome	256000	Typical Leigh syndrome: neurodegeneration, lactic acidosis,	3	3	No Effective Intervention	0	0	3	**9**
NEUROD1	601724	MODY, type VI (MODY6)	606394	Type II diabetes and complications	1	3	Glucose monitoring, early treatment	1	3	1	**9**
NFKB2	164012	Immunodeficiency, common variable, 10	615577	immunodeficiency, common variable	1	2	Immune work-up -> antibiotics, immunoglobulin replacement	2	3	1	**9**
NHEJ1	611290	Severe combined immunodeficiency with microcephaly, growth retardation, and sensitivity to ionizing radiation	611291	Combined Immunodeficiency	3	0	Immunological Evaluation --> HSCT	2	3	1	**9**
NKX2-6	611770	Persistent truncus arteriosus / Conotruncal heart defects	217095	Cyanotic heart disease / heart failure	2	0	Echo evaluation --> Open heart surgery if needed	3	3	1	**9**
NODAL	601265	Heterotaxy, Visceral	270100	heart failure/biliary atresia - jaundice / splenic dysfunction - susceptibility to	2	2	Echo evaluation --> meds, surgery (heart transplant for most severe	1	3	1	**9**
PDX1	600733	MODY, type IV (MODY4)	606392	Type II diabetes and complications	1	3	Glucose monitoring, early treatment	1	3	1	**9**
PFKM	610681	Glycogen storage disease VII	232800	Exercise intolerance, muscle cramping, exertional myopathy, hemolytic anemia	1	2	Diet, avoid strenuous exercise	2	3	1	**9**
PGAM2	612931	Glycogen storage disease X	261670	Myoglobinuria, exercise intolerance, muscle cramps, rhabdomyolysis -->	1	2	Avoidance of Exercise	2	3	1	**9**

PPT1	600722	Ceroid Lipofuscinosis, neuronal, 1 (CLN1)	256730	Neural and retinal degeneration	3	3	No Effective Intervention	0	0	3	**9**
PTCH1	601309	Basal cell nevus syndrome	109400	Medulloblastoma	2	2	Neuro exam, FOC, ophtho (eval hydrocephalus)	1	3	1	**9**
PTS	612719	Hyperphenylalaninemia, BH4-deficient, A	261640	Cognitive impairment plus neurological features	1	3	Diet, BH4, L-DOPA, and 5-HTP	2	2	1	**9**
QDPR	612676	Hyperphenylalaninemia, BH4-deficient, C	261630	Cognitive impairment plus neurological features	1	3	Diet, BH4, L-DOPA, and 5-HTP	2	2	1	**9**
RSPO1	609595	Palmoplantar hyperkeratosis with squamous cell carcinoma of skin and sex reversal	610644	squamous cell carcinoma	2	2	avoidane of sun	1	3	1	**9**
SDHC	602413	Hereditary Paraganglioma-Pheochromocytoma Syndrome 3	605373	Nonmalignant PGL / PCC	1	3	Annual Biochemical Screening	0	3	2	**9**
SERPINB6	173321	?Deafness, Autosomal Recessive 91	613453	Communication Deficits	0	3	Audiology --> Hearing Aids	3	3	0	**9**
SLC26A2	606718	Multiple epiphyseal dysplasia	256050	Joint Pain	1	3	PT / OT	1	3	1	**9**
SLC2A1	138140	GLUT1 deficiency syndrome 2	612126	Paroxysmal exercise-induced dyskinesia	1	3	Ketogenic diet	2	2	1	**9**
SMPD1	607608	Niemann-Pick Disease, Type A	257200	Neurologic Degeneration	3	3	No Effective Intervention	0	0	3	**9**
SNTA1	601017	Long QT syndrome 12	612955	Sudden death due to arrhythmia	3	0	EKG screening / avoidance of triggers / ICD	2	3	1	**9**
TGFB3	190230	Arrhythmogenic right ventricular dysplasia 1	107970	Sudden death due to arrhythmia	3	0	Echo/MRI screening/no sports/ICD	2	3	1	**9**
TNNI3	191044	Hypertrophic cardiomyopathy 7	613690	Arrhythmia	3	1	Echo Screening / no sports / ICD	1	3	1	**9**
TPP1	607998	Ceroid lipofuscinosis, neuronal, 2 (CLN2)	204500	Neural and Retinal Degeneration	3	3	No Effective Intervention	0	0	3	**9**
TRHR	188545	Thyrotropin-releasing hormone resistance, generalized	188545	All Outcomes	0	3	Thyroxine Replacement Therapy	3	3	0	**9**
ZMYND10	607070	Ciliary Dyskinesia, Primary, 22	615444	Chronic Sinopulmonary Disease	1	2	Management of symptoms: enhance mucous clearance similar to CF	2	3	1	**9**
ACTC1	102540	Hypertrophic cardiomyopathy 11	612098	Arrhythmia	3	1	Echo Screening / no sports / ICD	1	3	0	**8**
AKAP9	604001	Long QT syndrome 11	611820	Sudden death due to arrhythmia	3	0	EKG screening / avoidance of triggers / ICD	2	3	0	**8**
ASAH1	613468	Farber Lipogranulomatosis	228000	All Outcomes	3	3	No Effective Intervention	0	0	2	**8**
CATSPER2	607249	Sensorineural Deafness and Male Infertility	611102	Male Infertility	0	3	ARTs such as Intracytoplasmic Sperm Injection (ICSI)	2	2	1	**8**
CAV3	601253	Long QT Syndrome 9	611818	Sudden Death due to Arrhythmia	3	0	EKG screening / avoidance of triggers / ICD	2	3	0	**8**
CD247	186780	Immunodeficiency due to defect in CD3-zeta	610163	SCID	2	0	BMT	3	2	1	**8**
CD3E	186830	Immunodeficiency 18 (Severe Combined Immunodeficiency)	615615	SCIDs	3	0	Immunoglobulin	3	2	0	**8**
CDKN1B	600778	Multiple endocrine neoplasia, type IV	610755	Multiple endocrine tumors	1	0	Biochemical/imaging screening	3	3	1	**8**
CLN3	607042	Ceroid lipofuscinosis, neuronal, 3	204200	Neural and retinal degeneration	2	3	Supportive / Palliative	0	0	3	**8**
DNAJC5	611203	Ceroid lipofuscinosis, neuronal, 4, Parry type (CLN4B)	162350	Neural and retinal degeneration	2	3	No Effective Intervention	0	0	3	**8**
DSC2	125645	Arrhythmogenic right ventricular cardiomyopathy 11	610476	Sudden death due to arrhythmia	3	0	Echo screening/no sports/ICD	2	2	1	**8**
FBXL4	605654	Mitochondrial DNA Depletion Syndrome 13 (Encephalomyopathic type)	615471	Lactic acidosis, encephalopathy leading to death	3	3	No Effective Intervention	0	0	2	**8**
GCK	138079	MODY, type II (MODY2)	125851	Type II diabetes and complications	1	1	Glucose monitoring and early treatment	1	3	2	**8**
GHRHR	139191	Growth hormone deficiency, isolated, type IB	612781	Pituitary dwarfism	1	3	GHRH, GH replacement	1	2	1	**8**
LAMC3	604349	Cortical malformations, occipital	614115	Epilepsy	2	3	Cortical resection	3	0	0	**8**
MCCC1	609010	3-Methylcrotonyl-CoA Carboxylase 1 Deficiency	210200	Mild Weakness	0	3	Leucine-restricted Diet, Carnitine Supplementation	1	3	1	**8**
MTHFR	607093	{Thromboembolism, susceptibility to}	188050	Risk for thrombosis	2	0	Folic acid	1	3	2	**8**
MYH11	160745	Familial Thoracic Aortic Aneurysms 4	132900	Aortic Dissection	3	0	Annual Echocardiogram	2	3	0	**8**
MYLK	600922	Familial Thoracic Aortic Aneurysms 7	613780	Aortic Dissection	3	0	Annual Echocardiogram	2	3	0	**8**
NAGLU	609701	Mucopolysaccharidosis type IIIB (Sanfilippo B)	252920	All Outcomes	3	3	No Effective Intervention	0	0	2	**8**
NDUFS3	603846	Leigh syndrome due to mitochondrial complex I deficiency	256000	encephalopathy, myopathy, developmental delay, lactic acidosis.	3	3	No Effective Intervention	0	0	2	**8**
NDUFS4	602694	Mitochondrial Complex I Deficiency	252010	Failure to thrive, hypotonia, cardiorespiratory failure with or w/o	3	3	No Effective Intervention	0	0	2	**8**
NKX2-5	600584	Hypothyroidism, congenital nongoitrous, 5	225250	Thyroid Dysgenesis	2	0	Thyroid Evaluation	3	3	0	**8**

NKX3-2	602183	Spondylo-megaepiphyseal-metaphyseal dysplasia	613330	C-spine instability	1	3	C-spine stabilization	2	1	1	**8**
PCSK9	607786	Familial hypercholesterolemia 3	603776	Hypercholesterolemia / Early MI	2	0	Cholesterol screening / Statins	2	3	1	**8**
PEX19	600279	Peroxisome biogenesis disorder 12A (Zellweger)	614886	death due to apnea or other respiratory compromise	3	3	No Effective Intervention	0	0	2	**8**
PTGER2	176804	Susceptibility to aspirin-induced asthma	208550	Aspirin-induced asthma	1	1	Avoidance of aspirin/NSAIDs	1	3	2	**8**
RAI1	607642	Smith-Magenis	182290	Intellectual disability and behavioral disturbance	2	3	No Effective Intervention	0	0	3	**8**
RNU4ATAC	601428	Microcephalic osteodysplastic primordial dwarfism, type I	210710	intrauterine growth retardation, abnormalities in multiple organs, and	3	3	No Effective Intervention	0	0	2	**8**
SECISBP2	607693	Thyroid hormone metabolism, abnormal	609698	ID	1	1	Selenium & L-T3 administration	2	3	1	**8**
SLC17A5	604322	Sialic acid storage disorder, infantile	269920	All Outcomes	3	3	No Effective Intervention	0	0	2	**8**
SMARCB1	601607	Schwannomatosis-1, susceptibility to	162091	Multiple cutaneous neurilemmomas and spinal schwannomas	1	2	MRI --> pain medicine / surgery	2	2	1	**8**
SMPD1	607608	Niemann-Pick Disease, Type B	607616	Hepatomegaly and Respiratory	2	3	No Effective Intervention	0	0	3	**8**
SURF1	185620	Leigh syndrome, due to COX deficiency	256000	All Outcomes	3	3	None	0	0	2	**8**
TPM1	191010	Cardiomyopathy, dilated, 1Y	611878	Arrhythmia or heart failure	3	0	Echo Screening / ICD	2	3	0	**8**
TSPAN12	613138	Familial exudative vitreoretinopathy (FEVR)	613310	vision loss due to retinal ischemia	0	2	ophthalmologic screening --> Prophylactic cryotherapy or argon	2	2	2	**8**
AGBL1	615496	Corneal Dystrophy, Fuchs endothelial, 8	615523	Marked Vision Loss	1	1	Surgery	2	1	2	**7**
AKT2	164731	Hypoinsulinemic Hypoglycemia with Hemihypertrophy	240900	Hypoglycemia	3	0	Oral Glucose Therapy	1	2	1	**7**
CAV3	601253	Familial HCM	192600	Sudden death due to arrhythmia	3	0	Echo screening/no sports/ICD	1	3	0	**7**
CEMIP	608366	?Deafness, Nonsyndromic		Communication Deficits	1	0	Audiology Screening --> Cochlear Implant	3	3	0	**7**
CHRNA1	100690	Congenital slow-channel myasthenic syndrome	601462	Congenital myasthenia	2	3	No Effective Intervention	0	0	2	**7**
CHRNA1	100690	Multiple pterygium syndrome, lethal type; Myasthenic syndrome, fast-channel congenital; Myasthenic		Fetal akinesia	3	3	No Effective Intervention	0	0	1	**7**
CLN5	608102	Ceroid lipofuscinosis, neuronal, 5 (CLN5)	256731	Neural and retinal degeneration	2	3	No Effective Intervention	0	0	2	**7**
CLN6	606725	Ceroid lipofuscinosis, neuronal, 6	601780	Neural and Retinal Degeneration	2	3	No Effective Intervention	0	0	2	**7**
CLN8	607837	Ceroid lipofuscinosis, neuronal, 8 (CLN8)	600143	Neural and Retinal Degeneration or Epileptic Seizures / ID (Northern	2	3	No Effective Intervention / Symptom Management	0	0	2	**7**
CLPP	601119	Deafness, autosomal recessive 81 (aka Perrault syndrome 3)	614129	Communication Deficits or Ovarian Dysfunction (Ovarian Dysgenesis -->	1	2	Audiology --> Hearing Aids --> Cochlear Implants or Oral or topical	2	2	0	**7**
COG6	606977	Congenital disorder of glycosylation, type III	614576	Encephalopathy, multisystem disease, inflammatory bowel	3	3	No Effective Intervention	0	0	1	**7**
COL6A3	120250	Ulrich congenital muscular dystrophy	254090	Childhood onset myopathy and respiratory failure	2	3	No Effective Intervention	0	0	2	**7**
CRYM	123740	Deafness, autosomal dominant 40		Communication Deficits	1	0	Audiology --> Hearing Aids	3	3	0	**7**
CTSD	116840	Ceroid lipofuscinosis, neuronal, 10 (CLN10)	610127	Neural and retinal degeneration	3	3	No Effective Intervention	0	0	1	**7**
DIABLO	605219	Deafness, autosomal dominant 64	614152	Communication Deficits	1	0	Audiology --> Hearing Aids	3	3	0	**7**
DLD	238331	Dihydrolipoamide dehydrogenase deficiency	246900	Mitochondrial encephalopathy / Leigh syndrome	3	3	Carnitine, CoQ10, mito cocktail	0	0	1	**7**
DMD	300377	Cardiomyopathy, Dilated, 3B	302045	Heart Failure	2	3	ECG--> anti-congestive medications/ cardiac transplantation	0	1	1	**7**
F11	264900	Factor XI deficiency, autosomal dominant	612416	Bleeding	0	1	Fresh frozen plasma with procedures / trauma or F11	3	2	1	**7**
FH (Recessive)	136850	Fumarase deficiency	606812	Encephalopathy	2	3	No Effective Intervention	0	0	2	**7**
GBE1	607839	Glycogen Storage Disease IV	232500	Progressive Neurodegerative, Fetal akinesia, Hypotonia --> Failure to thrive	3	2	No Effective Intervention or Liver Transplant	0	0	2	**7**
GJB3	603324	Non-Syndromic Deafness, Recessive		Communication Deficits	1	0	Audiology --> Hearing Aids	3	3	0	**7**
GLB1	611458	Beta-galactosidase-1 deficiency GLB1 deficiency		Skeletal Dysplasia	1	3	No Effective Intervention	0	0	3	**7**
GLIS3	610192	Diabetes mellitus, neonatal, with congenital hypothyroidism	610199	Diabetes	2	0	Insulin Therapy	3	1	1	**7**
GM2A	613109	GM2-gangliosidosis, AB variant	272750	Neural degeneration --> Psychomotor delay, seizures, paralysis, dementia,	3	3	No effective intervention / Symptom management	0	0	1	**7**
GNPTAB	607840	Mucolipidosis II (I-cell disease)	252500	"Syndromic"	2	3	No Effective Intervention	0	0	2	**7**

GNS	607664	Mucopolysaccharidosis type IIID	252940	Respiratory Distress	2	3	No Effective Intervention	0	0	2	**7**
HGD	607474	Alkaptonuria	203500	Arthritis	1	3	Oral Nitisinone	0	0	3	**7**
HGSNAT	610453	Mucopolysaccharidosis type IIIC	252930	CNS Degeneration --> behavioral problems, seizures, mental retardation,	2	3	No Effective Intervention	0	0	2	**7**
KIF1B	605995	Pheochromocytoma	171300	Pheochromocytoma	2	0	Biochemical screening, imaging	2	3	0	**7**
MCOLN1	605248	Mucolipidosis IV	252650	All Outcomes	2	3	No Effective Intervention	0	0	2	**7**
MECP2	300005	Rett syndrome	312750	Intellectual disability	2	3	No Effective Intervention	0	0	2	**7**
MFSD8	611124	Ceroid Lipofuscinosis, neuronal, 7 (CLN7)	610951	Neural and retinal degeneration	2	3	No Effective Intervention	0	0	2	**7**
MYL2	160781	Hypertrophic cardiomyopathy 10	608758	Arrhythmia	3	0	Echo Screening / no sports / ICD	1	3	0	**7**
MYL3	160790	Hypertrophic cardiomyopathy 8	608751	Arrhythmia	3	0	Echo Screening / no sports / ICD	1	3	0	**7**
NAGA	104170	Schindler disease, types I and III	609241	All Outcomes	3	3	No Effective Intervention	0	0	1	**7**
NEU1	608272	Sialidosis/ MUCOLIPIDOSIS I	256550	Type II Sialidosis	3	3	No Effective Intervention	0	0	1	**7**
NOTCH3	600276	CADASIL- Cerebral arteriopathy with subcortical infarcts and leukoencephalopathy	125310	cerebrovascular disease/TIAs	2	3	Antiplatelet Therapy	0	0	2	**7**
NTRK1	191315	Familial Medullary Thyroid Cancer (FMTC)	155240	Medullary thyroid cancer	2	0	Thyroidectomy	3	2	0	**7**
PLA2G6	603604	Neurodegeneration with brain iron accumulation	610217	progressive psychomotor decline	2	3	No Effective Intervention	0	0	2	**7**
PLA2G6	603604	Parkinson disease 14	612953	early onset parkinson's with death in young adulthood	2	3	No Effective Intervention	0	0	2	**7**
RP1	603937	Retinitis Pigmentosa	180100	Retinitis Pigmentosa (nonsyndromic, progressive blindness)	1	3	annual or biannual eye exam / vitamin A palmitate	0	0	3	**7**
RPS10	603632	Diamond-Blackfan anemia 9	613308	Anemia	1	0	hematological evaluation followed by coriticosteroid treatment	2	3	1	**7**
SDHAF2	613019	Hereditary Paraganglioma-Pheochromocytoma Syndrome 2	601650	Nonmalignant PGL / PCC	1	2	Annual Biochemical Screening	0	3	1	**7**
SGSH	605270	Mucopolysaccharidisis type IIIA (Sanfilippo A)	252900	CNS degeneration --> Severe behavioral problems, sleep disturbances,impaired	2	3	No Effective Intervention	0	0	2	**7**
SLC26A2	606718	Achondrogenesis Type 1B	600972	Neonatal Death	3	3	Palliative Care	0	0	1	**7**
SLC6A19	608893	Hartnup disorder	234500	Rash, photosensitivity, temporary ataxia. mood disturbance	0	1	Niacin, tryptophan supplementation	1	3	2	**7**
SPRY4	607984	Hypogonadotropic hypogonadism 17 with or without anosmia (Kallman syndrome)	615266	delayed or absent puberty	0	0	pulsatile GnRH or gonadotropin therapy, androgen therapy	3	3	1	**7**
SUMF1	607939	Multiple Sulfatase Deficiency	272200	All Outcomes	3	3	No Effective Intervention / Symptom Management	0	0	1	**7**
TNNT2	191045	Cardiomyopathy, familial hypertrophic, 2	115195	Arrhythmia	3	0	Echo Screening / no sports / ICD	1	3	0	**7**
TREH	275360	Trehalase deficiency	612119	Diarrhea, abdominal pain, increased flatulence	0	0	Avoidance of mushrooms	3	3	1	**7**
ABCA1	600046	Tangier, HDL deficiency, type 1	205400	Early onset coronary artery disease	2	2	lifestyle mods and monitoring, drugs	0	0	2	**6**
ACTG1	102560	Baraitser-Winter syndrome 2	614583	Intellectual disability / developmental delay	2	3	No Effective Intervention	0	0	1	**6**
ACTG2	102545	Visceral Myopathy	155310	Abnormal instestinal motility to functional gastrointestinal obstruction	2	2	No Effective Intervention	0	0	2	**6**
ALDH3A2	609523	Sjogren-Larsson Syndrome	270200	All Outcomes	1	3	All Interventions	0	0	2	**6**
APOA1	107680	Hypoalphalipoproteinemia	604091	Coronary artery disease	2	2	Lifestyle mods and monitoring, drugs	0	0	2	**6**
ASAH1	613468	Spinal Muscular Atrophy with Progressive Myoclonic Epilepsy	159950	Muscle weakness --> Respiratory insufficiency	2	3	No Effective Intervention	0	0	1	**6**
CFTR	602421	CAVD	277180	Azoospermia	0	3	No Effective Intervention	0	0	3	**6**
COL11A2	120290	Fibrochondrogenesis 2	614524	All Outcomes	2	3	No Effective Intervention	0	0	1	**6**
COL11A2	120290	Weissenbacher-Zweymuller syndrome	277610	rhizomelic chondrodysplasia with dumbbell-shaped femora and humeri	1	3	No Effective Intervention	0	0	2	**6**
COL1A1	120150	Osteogenesis Imperfecta Type II	166210	Respiratory Insufficiency	3	3	No Effective Intervention	0	0	0	**6**
CRADD	603454	AR mental retardation 34	614499	Severe cognitive impairment	2	3	No Effective Intervention	0	0	1	**6**
CTSA	613111	Galactosialidosis	256540	Cardiac Involvement	3	2	No Effective Intervention	0	0	1	**6**
DCHS1	603057	Van Maldergem syndrome 1	601390	Syndromic features	2	3	No Effective Intervention	0	0	1	**6**

DCXR	608347	Pentosuria	260800	Increased urinary excretion of L-xylulose	0	3	No Effective Intervention	0	0	3	**6**
DPM1	603503	Congenital Disorder of Glycosylation, Type Ie	608799	Intellectual Disability and Seizures	2	3	No Effective Intervention	0	0	1	**6**
EBP	300205	Chondrodysplasia Punctata, X-linked dominant	302960	Syndromic Features	1	3	No Effective Intervention	0	0	2	**6**
F9	300746	Thrombophilia, X-linked, due to factor IX defect	300807	DVT -> PE	2	0	Advance warning, avoidance of stasis	0	3	1	**6**
GALE	606953	Galactose epimerase deficiency	230350	Galactosemia / 'Intermediate'	1	0	Diet	2	2	1	**6**
GDF5	601146	Acromesomelic dysplasia, Grebe type		Skeletal dysplasia	1	3	No Effective Intervention	0	0	2	**6**
GIGYF2	612003	Parkinson disease 11	607688	Parkinsonism	1	3	No Effective Intervention	0	0	2	**6**
GJB3	603324	Non-Syndromic Deafness, Dominant 2B	612644	Communication Deficits	0	0	Audiology --> Hearing Aids	3	3	0	**6**
GNPTAB	607840	Mucolipidosis IIi alpha/beta, (pseudo-Hurler polydystrophy)		Syndromic features	1	3	No Effective Intervention	0	0	2	**6**
GNPTG	607838	Mucolipidosis III Gamma	252605	Skeletal abnormalities (Scoliosis, kyphosis, stiff joints, dysostosis	1	3	No Effective Intervention	0	0	2	**6**
GRM1	604473	Autosomal recessive SCA 13	614831	Cognitive impairment and movement disorder	2	3	No Effective Intervention	0	0	1	**6**
LAMB1	150240	Lissencephaly 5	615191	severe psychomotor retardation and seizures	2	3	No Effective Intervention	0	0	1	**6**
MANBA	609489	Beta Manosidosis	248510	Intellectual Disabilities and Seizures	1	3	No Effective Intervention	0	0	2	**6**
MMP2	120360	Multicentric osteolysis, nodulosis, and arthropathy	259600	Nodulosis, arthropathy, and osteolysis	1	3	No Effective Intervention	0	0	2	**6**
NAA10	300013	Ogden syndrome	300855	death due to cardiogenic shock and arrhythmia	3	3	N / A	0	0	0	**6**
NDUFS3	603846	Mitochondrial Complex I Deficiency	252010	Encephalopathy, myopathy, developmental delay, lactic acidosis	2	0		1	3	0	**6**
OPA3	606580	3-Methylglutaconic Aciduria, type III	258501	Neurologic Dysfunction	1	3	Metabolic Screening --> Symptom Support	0	0	2	**6**
PDZD7 / ADGRV1	612971	Usher Syndrome, type 2C, GPR98/PDZD7 Digenic	605472	Usher Phenotype (Communication Deficits + RP)	1	0	Audiology and Vision Services	2	3	0	**6**
PIEZO2	613329	Distal arthrogryposis 5	108145	arthrogryposis	1	3	none (no preventative therapy; PT, surg considered for symptomatic	0	0	2	**6**
PORCN	300651	Focal dermal hypoplasia (female carriers)	305600	Developmental delay, dysmorphology	1	3	No Effective Intervention	0	0	2	**6**
REEP1	609139	Hereditary spastic paraplegia 31	610250	Uncomplicated spastic paraplegia	1	3	No Effective Intervention	0	0	2	**6**
SLC6A8	300036	Cerebral creatine deficiency syndrome 1, X-linked	300352	Intellectual disability, seizures	1	3	Creatine supplementation	0	0	2	**6**
SNIP1	608241	Psychomotor retardation, epilepsy, and craniofacial dysmorphism	614501	Severe cognitive impairment, seizures	2	3	No Effective Intervention	0	0	1	**6**
TAS2R38	607751	Phenylthiocarbamide tasting	171200	bitter tasting (not a disease)	0	3	No Effective Intervention	0	0	3	**6**
TJP2		Deafness, autosomal dominant 51	613558	Communication Deficits	0	0	All Interventions	3	3	0	**6**
APOA1	107680	Amyloidosis, 3 or more types	105200	Visceral amyloidosis	2	2	monitor for signs of amylidosis with consideration of liver and renal	0	0	1	**5**
ARHGEF6	300267	Mental Retardation, X-linked 46	300436	Mental Retardation	1	3	Early Childhood Intervention Services	0	0	1	**5**
B4GALT7	604327	Ehlers-Danlos syndrome, progeroid type	130070	Syndromic features	1	3	No Effective Intervention	0	0	1	**5**
BLK	191305	MODY, type XI (MODY11)	613375	Type II diabetes and complications	1	0	Glucose monitoring, early treatment	1	3	0	**5**
CAV3	601253	Limb-girdle muscular dystrophy 1C	607801	Muscle weakness	1	2	No Effective Intervention	0	0	2	**5**
CEL	114840	MODY, type VIII (MODY8)	609812	Type II diabetes and complications	1	0	Glucose monitoring, early treatment	1	3	0	**5**
COG6	606977	Shaheen Syndrome	615328	Intellectual disability	1	3	No Effective Intervention	0	0	1	**5**
COL1A1	120150	Ehlers-Danlos Syndrome, type I	130000	Hypotonia	1	0	physiotherapy, anti-inflammatory drugs, non-weight-bearing exercise	0	3	1	**5**
DGAT1	604900	?Diarrhea 7	615863	Severe congenital diarrhea	2	3	No Effective Intervention	0	0	0	**5**
DNA2	601810	Progressive external ophthalmoplegia, with myopathy	615156	Mild, adult-onset myo-neuropathy	1	3	No Effective Intervention	0	0	1	**5**
DSPP	125485	Deafness, autosomal dominant 36, with dentinogenesis	605594	Dentinogenesis Imperfecta	1	2	No Effective Intervention	0	0	2	**5**
GRM6	604096	Night blindness, congenital stationary (complete), 1B, autosomal recessive	257270	Night blindness	0	3	No Effective Intervention	0	0	2	**5**
H6PD	138090	Cortisone reductase deficiency 1	604931	Hyperandrogenism; premature pseudopuberty (males); adult-onset	1	3	No Effective Intervention	0	0	1	**5**
HBG1	142200	Fetal hemoglobin quantitative trait locus 1	141749	Persistence of Fetal Hemoglobin	0	3	No Effective Intervention	0	0	2	**5**

HSD11B1	600713	Cortisone reductase deficiency 2	614662	Hyperandrogenism; premature pseudopuberty (males); adult-onset	1	3	No Effective Intervention	0	0	1	**5**
INS	176730	MODY, type X (MODY10)	613370	Type II diabetes and complications	1	0	Glucose monitoring, early treatment	1	3	0	**5**
KLF11	603301	MODY, type VII (MODY7)	610508	Type II diabetes and complications	1	0	Glucose monitoring, early treatment	1	3	0	**5**
LAMC3	604349	Cortical malformations, occipital	614115	Seizures / Epilepsy	2	3	No Effective Intervention	0	0	0	**5**
LDHA	150000	Glycogen storage disease XI (GSD11), or lactate dehydrogenase A deficiency	612933	Myopathy, muscle pain and stifness, exercise intolerance and myoglobinuria	1	3	No Effective Intervention	0	0	1	**5**
MAT1A	610550	Methionine Adenosyltransferase Deficiency, AR	250850	Benign Hypermethioninemia	0	3	Spectrum of Actions not Listed Here (No Effective Intervention)	0	0	2	**5**
MRE11A	600814	Ataxia-telangiectasia-like disorder	604391	Muscle wasting, contractures, movement disorder	1	3	No Effective Intervention	0	0	1	**5**
MTPAP	613669	Spastic ataxia 4	613672	Progressive ataxia, mild cognitive impairment	1	3	No Effective Intervention	0	0	1	**5**
NAGA	104170	Kanzaki disease (Schindler's Disease type II)	609242	All Outcomes	1	3	No Effective Intervention	0	0	1	**5**
NTRK1	191315	Congenital insensitivity to pain with anhidrosis	256800	Syndromic features	1	3	No Effective Intervention	0	0	1	**5**
PAX4	167413	MODY, type IX (MODY9)	612225	Type II diabetes and complications	1	0	Glucose monitoring, early treatment	1	3	0	**5**
POLD1	174761	Mandibular hypoplasia, deafness, progeroid features, and lipodystrophy syndrome	615381	progeroid disease	1	3	No Effective Intervention	0	0	1	**5**
SLC10A2	601295	Primary bile acid malabsorption	613291	Chronic diarrhea	1	3	No Effective Intervention	0	0	1	**5**
SLC26A2	606718	Multiple epiphyseal dysplasia 4	226900	Clubfoot and joint problems	1	3	No Effective Intervention	0	0	1	**5**
SLC2A1	138140	Dystonia 9	601042	Choreoathetosis, ataxia, and progressive spastic paraplegia	1	3	No Effective Intervention	0	0	1	**5**
SYP	313475	Mental retardation, X-linked 96	300802	Intellectual disability	1	3	No Effective Intervention	0	0	1	**5**
UBIAD1	611632	Corneal dystrophy, Schnyder type	121800	Visual morbidity, decreased daytime vision	0	3	None (Correction via surgery in advanced disease)	0	0	2	**5**
UPF3B	300298	Mental retardation, X-linked, syndromic 14	300676	Intellectual disability	1	3	No Effective Intervention	0	0	1	**5**
VAMP1	185880	Spastic ataxia 1	108600	Ataxia	1	3	No Known Intervention	0	0	1	**5**
ATP5E	606153	Mitochondrial Complex V (ATP synthase) Deficiency, Nuclear Type 3	614053	All Outcomes	1	0	Mitochondrial Cocktail	0	3	0	**4**
COL1A1	120150	Osteogenesis Imperfecta, type III	259420	All Outcomes	2	1	No Effective Intervention	0	0	1	**4**
DAG1	128239	Muscular dystrophy-dystroglycanopathy type C9	613818	Limb girdle muscular dystrophy and/or MR	1	3	No Effective Intervention	0	0	0	**4**
HYAL1	607071	Mucopolysaccharidosis type IX	601492	swollen joints, periarticular masses	0	3	No Effective Interventions	0	0	1	**4**
MCCC2	609014	3-Methylcrotonyl-CoA Carboxylase 2 Deficiency	210210	Encephalopathy	2	1	Mild Protein Restriction and Carnitine Supplementation	0	0	1	**4**
NRTN	602018	Hirschsprung disease		Colonic aganglionosis	1	0	Surgery	2	1	0	**4**
OPLAH	614243	5-oxoprolinase deficiency	260005	Biochemical Abnormality of High 5-oxoprolinuria	0	3	No Effective Intervention	0	0	1	**4**
PCBD1	126090	Hyperphenylalaninemia, BH4-deficient, D	264070	Hyperphenylalaninemia without cognitive impairment	0	3	No Effective Intervention	0	0	1	**4**
PLCG2	600220	Autoinflammation and PLCG2-associated antibody deficiency and immune dysregulation (APLAID)	614878	blistering skin lesions	1	0	IL-1 inhibitor	1	2	0	**4**
REEP1	609139	Distal hereditary motor neuropathy type Vb	614751	Muscle weakness, contractures	1	3	No Effective Intervention	0	0	0	**4**
ZNF644	614159	Myopia 21, autosomal dominant	614167	High grade myopia	0	3	No Effective Intervention	0	0	1	**4**
BLVRA	109750	Hyperbiliverdinemia	614156	Episodic hyperbiliverdinemia (green jaundice)	1	1	No Effective Intervention	0	0	1	**3**
COL1A1	120150	Ehlers-Danlos Syndrome, type VIIA	130060	Congenital Hip Dislocation	1	0	Open Reduction of Hip Dislocation	0	1	1	**3**
GNMT	606628	Glycine N-Methyltransferase Deficiency	606664	Hypermethioninemia --> Hepatomegaly	0	3	Dietary Methionine Restriction	0	0	0	**3**
GYS1	138570	Glycogen storage disease 0, muscle	611556	Left ventricular hypertrophy, risk of cardiac arrest --> Sudden death	3	0	No Effective Intervention	0	0	0	**3**
HPD	609695	Tyrosinemia, type III	276710	Intellectual disability, ataxia, seizures	1	0	Dietary restriction	0	2	0	**3**
IKBKG	300248	Immunodeficiency, isolated	300584	Recurrent infections	2	0	?immunoglobulin replacement; surveillance	1	0	0	**3**
PRKDC	600899	SCID		All Outcomes	3	0	No Effective Intervention	0	0	0	**3**
SLC25A13	603859	Citrullinemia, type II, adult-onset	603471	Liver Failure	2	0	No effective intervention	0	0	1	**3**
TRDN	603283	Ventricular tachycardia, catecholaminergic polymorphic, 2	615441	Sudden death due to arrhythmia	3	0	Echo screening/no sports/ICD	0	0	0	**3**

VANGL2	600533	Neural tube defects	182940	Neural tube defects	2	0	No Effective Intervention	0	0	1	**3**
ABCA1	600046	HDL deficiency, type 2	604091	Early onset coronary artery disease	2	0	lifestyle mods and monitoring, drugs	0	0	0	**2**
CARD11	607210	Persistent Polyclonal B-cell Lymphocytosis	606445	B-Cell Lymphoma	2	0	No Effective Intervention	0	0	0	**2**
EGLN1	606425	Erythrocytosis, familial, 3	609820	Paraganglioma	2	0	Biochemical screening, imaging	0	0	0	**2**
GSTZ1	603758	Tyrosinemia type 1b		Severe liver disease (cirrhosis or hepatocellular carcinoma)	2	0	No Effective Intervention	0	0	0	**2**
MAGT1	300715	X-linked immunodeficiency with magnesium defect, Epstein-Barr virus infection, and neoplasia (XMEN)	300853	Recurrent Infection including EBV	2	0		0	0	0	**2**
MCEE	608419	Methylmalonyl-CoA epimerase deficiency	251120	Metabolic decompensation	2	0	No Effective Intervention	0	0	0	**2**
TOP1	126420	DNA topoisomerase I, camptothecin-resistant		No inherited disorder associated as far as I can tell	0	0	N /A	0	0	2	**2**
DISP1	607502	Holoprosencephaly 10	612530	All Outcomes	1	0	No Effective Intervention	0	0	0	**1**
F12	610619	Factor XII deficiency	234000	Possible clotting	0	0	No Effective Intervention	0	0	1	**1**
SRPX2	300642	Rolandic epilepsy, mental retardation, and speech dyspraxia	300643	Intellectual disability, seizures	1	0	No Effective Intervention	0	0	0	**1**
SUGCT	609187	Glutaric aciduria III	231690	None	0	0	None	0	0	1	**1**

The NC NEXUS Study

The North Carolina Newborn Exome Sequencing for Universal Screening Study

Making the Right Decision for You and Your Family

NC Nexus

North Carolina Newborn Exome Sequencing for Universal Screening

You are being invited to take part in a research study called NC NEXUS.

This brochure will help you learn more about the study, including:

The purpose of the study

Genomic sequencing

What you will be asked to do if you join

The NC NEXUS Study

What is the purpose of this study?

- The NC NEXUS study is using a technique called "genomic sequencing" to see how well it can find children with genetic conditions like the ones found with newborn screening.
- All babies in the United States are tested for at least 30 conditions by newborn screening.
- Doctors do newborn screening to find children with these rare conditions so they can treat them early.
- Early treatment helps to prevent serious health problems.
- The conditions screened for in North Carolina are listed here: http://www.babysfirsttest.org/newborn-screening/states/north-carolina
- The NC NEXUS study wants to find out if genomic sequencing finds these same conditions plus hundreds of others like them.

The study also hopes to learn:

- What parents think about when deciding if they want to have genomic sequencing for their child
- The types of things that parents want to learn from genomic sequencing
- If parents find it helpful to learn their child's genomic sequencing results
- If a decision guide is useful to parents making these decisions

What is genomic sequencing?

- Genomic sequencing is a way to study a person's genetic makeup, or DNA.
- Sequencing looks for differences in a person's DNA that could cause genetic conditions.
- Because it looks at thousands of genes, genomic sequencing can find much more information than the current newborn screening test.
- NC NEXUS researchers are using genomic sequencing to find children with genetic conditions like those found with newborn screening.

What happens if you decide to join the study?

- You will read an information sheet that describes what you will be asked to do.
- We will ask for your phone number so we can contact you.
- You will give your consent to join the study.
- You will get a link to an online decision guide that explains genomic sequencing and the kinds of results that you can learn.
- You will get a link to an online survey. You will receive $20 for completing it.
- At the end of the decision guide, you will be asked if you want to schedule a study visit at UNC Hospitals.
- You can decide not to schedule a visit. After you complete an online survey, you will stop participation in the study.
- If you think you might want your child to have sequencing or if you are not sure, you can schedule a study visit to learn more.

- At the visit, you will meet with a genetic counselor who will answer questions and discuss your decision.
- Parents who come to the visit can decide to accept or decline sequencing.

What happens if you decide to have genomic sequencing for your child?

- You will come to the UNC Hospitals with your child at a time convenient for you.
- We will obtain a sample of saliva (spit) for testing by lightly rubbing the inside of your child's mouth with a small sponge.

- We will call you to schedule a second study visit to discuss the results.
- All parents will learn the results for conditions found by newborn screening and other conditions like them.
- You will complete an online survey.
- All parents in the study will be placed into one of two groups. These groups will be decided by a random drawing.
- One group will complete two more on-line surveys and then stop their participation in the study.

- The other group will use a second on-line decision guide to make decisions about whether or not to request any additional information from their child's sequencing.
- Parents in this group will be able to discuss their decisions with a genetic counselor.
- We currently don't know if learning this additional information will be helpful or harmful to parents and families.
- No matter which group you are in, if genomic sequencing finds that your child has a genetic cause for a condition:
  - The results will be confirmed with another test.
  - A genetic counselor and doctor will meet with you to discuss the results and help you plan the next step.

Important things to know about your study participation.

- In two parent families, both parents need to agree to join the study. Please discuss this with a member of our study team if you have questions.
- You can stop taking part in the study at any point if you do not want to continue. Your child will still receive care from doctors as he or she usually would.
- You will not be charged for the study visits or the testing done during the time you are in the study.
- Each parent will get a $20 Visa card after each online survey is completed. You will also get parking vouchers for the study visits.

Is Joining the NC NEXUS Study the Right Decision for You and Your Family?

There are lots of things to think about when deciding to join a research study. Right now we are asking you to decide if we can contact you to learn more about the study. After learning more, you can decide whether or not to join the study. You can join the study and decide not to have genomic sequencing for your child. The decision is up to you. Whatever you decide will help us learn more about how parents make these decisions. On this page and the next are some ways other parents thought about this decision.

"It's important for me to learn more about genomic sequencing. I'm the type of person that just wants all the information"

Some reasons why you might want to join the NC NEXUS study

- You think that genomic sequencing might help doctors better understand your child's condition.
- You would like to learn more about genomic sequencing for your child so you can make the right decision for you and your family.
- You want to have the option of having genomic sequencing for your child.
- You are curious about using an online decision guide that will help you learn more.
- You have already decided you want to join the study.

If these reasons are important to you, then you may want to learn more about the NC NEXUS study and decide to take part in it.

"I don't want to learn anything more about the study. I don't think participating will be helpful or is right for my family right now"

Reasons why you might NOT want to join the NC NEXUS study

- You are satisfied with the information you currently have about your child's condition.
- You do not want to learn more about genetics or genomic sequencing.
- You have already decided you do not want to take part in the study.
- You do not have time to participate in the study activities.
- You are not interested in using an online decision guide to help you learn more.

*If these reasons are important to you, then you may decide you do **not** want to learn more about the NC NEXUS study.*

Should My Family Learn More about the NC NEXUS Study?

Make the decision that is best for you and your family.

Here are some questions to help you decide.

Yes	No	
☐	☐	Do you want to learn more about genetics and genomic sequencing?
☐	☐	Do you want to learn more about the genetic conditions that genomic sequencing may find?
☐	☐	Do you want the option of having genomic sequencing for your child?
☐	☐	Are you willing to use an online decision guide to learn more?
☐	☐	Do you have time to participate in the study activities

If you have more ***Yes*** answers than ***No*** answers, you and your family may be ready to learn more so that you can decide if you want to join the study.

If you have more ***No*** answers than ***Yes*** answers, taking part in this study may not be right for you and your family.

Please contact a member of our study team about these and any other questions you may have.

Phone: ###-###-####

Email: NC_Nexus@unc.edu.

This brochure was developed with support from the National Institutes of Health's Eunice Kennedy Shriver National Institute of Child Health and Human Development and the National Human Genome Research Institute.

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

RTI INTERNATIONAL

The NC NEXUS Study

The North Carolina Newborn Exome Sequencing for Universal Screening Study

Making the Right Decision for You and Your Family

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What is the purpose of this study?

- The NC NEXUS study is using a technique called "genomic sequencing" to see how well it can find children with genetic conditions like the ones found with newborn screening.
- All babies in the United States are tested for at least 30 conditions by newborn screening.
- Doctors do newborn screening to find children with these rare conditions so they can treat them early.
- Early treatment helps to prevent serious health problems.
- The conditions screened for in North Carolina are listed here: http://www.babysfirsttest.org/newborn-screening/states/north-carolina
- The NC NEXUS study wants to find out if genomic sequencing finds these same conditions plus hundreds of others like them.
- The genomic sequencing done in NC NEXUS will not replace the newborn screening your child has at birth.

The study also hopes to learn:

- What parents think about when deciding if they want to have genomic sequencing for their child
- The types of things that parents want to learn from genomic sequencing
- If parents find it helpful to learn their child's genomic sequencing results
- If a decision guide is useful to parents making these decisions

What is genomic sequencing?

- Genomic sequencing is a way to study a person's genetic makeup, or DNA.
- Sequencing looks for differences in a person's DNA that could cause genetic conditions.
- Because it looks at thousands of genes, genomic sequencing can find much more information than the current newborn screening test.
- NC NEXUS researchers are using genomic sequencing to find children with genetic conditions like those found with newborn screening.

What happens if you decide to join the study?

- You will read an information sheet that describes what you will be asked to do.
- We will ask for your phone number so we can contact you.
- You will give your consent to join the study.
- You will get a link to an online decision guide that explains genomic sequencing and the kinds of results that you can learn.
- You will get a link to an online survey. You will receive $20 for completing it.
- At the end of the decision guide, you will be asked if you want to schedule a study visit at UNC Hospitals.
- You can decide not to schedule a visit. After you complete an online survey, you will stop participation in the study.
- If you think you might want your child to have sequencing or if you are not sure, you can schedule a study visit to learn more.

- At the visit, you will meet with a genetic counselor who will answer questions and discuss your decision.
- Parents who come to the visit can decide to accept or decline sequencing.

What happens if you decide to have genomic sequencing for your child?

- After your baby is born, you will come to the UNC Hospitals with your child at a convenient time for you.
- We will obtain a sample of saliva (spit) for testing by lightly rubbing the inside of your child's mouth with a small sponge.

- We will call you to schedule a second study visit to discuss the results.
- All parents will learn the results for conditions found by newborn screening and other conditions like them.
- You will complete an online survey.
- All parents in the study will be placed into one of two groups. These groups will be decided by a random drawing.
- One group will complete two more on-line surveys and then stop their participation in the study.
- The other group will use a second on-line decision guide to make decisions about whether or not to request any additional information from their child's sequencing.

- Parents in this group will be able to discuss their decisions with a genetic counselor.
- We currently don't know if learning this additional information will be helpful or harmful to parents and families.
- No matter which group you are in, if genomic sequencing finds that your child has a genetic cause for a condition:
  - The results will be confirmed with another test.
  - A genetic counselor and doctor will meet with you to discuss the results and help you plan the next step.

Important things to know about your study participation.

- In two parent families, both parents need to agree to join the study. Please discuss this with a member of our study team if you have questions.
- You can stop taking part in the study at any point if you do not want to continue. Your child will still receive care from doctors as he or she usually would.
- You will not be charged for the study visits or the testing done during the time you are in the study.
- Each parent will get a $20 Visa card after each online survey is completed. You will also get parking vouchers for the study visits.

Is Joining the NC NEXUS Study the Right Decision for You and Your Family?

There are lots of things to think about when deciding to join a research study. Right now we are asking you to decide if we can contact you to learn more about the study. After learning more, you can decide whether or not to join the study. You can join the study and decide not to have genomic sequencing for your child. The decision is up to you. Whatever you decide will help us learn more about how parents make these decisions. On this page and the next are some ways other parents thought about this decision.

"It's important for me to learn more about genomic sequencing. I'm the type of person that just wants all the information"

Some reasons why you might want to join the NC NEXUS study

- You are interested in learning about the conditions like those found by newborn screening which may be found by genomic sequencing.
- You would like to learn more about genomic sequencing for your child so you can make the right decision for you and your family.
- You want to have the option of having genomic sequencing for your child.
- You are curious about using an online decision guide that will help you learn more.
- You have already decided you want to join the study.

If these reasons are important to you, then you may want to learn more about the NC NEXUS study and decide to take part in it.

"I don't want to learn anything more about the study. I don't think participating will be helpful or is right for my family right now"

Reasons why you might NOT want to join the NC NEXUS study

- You are satisfied with knowing your child will have current newborn screening.
- You do not want to learn more about genetics or genomic sequencing.
- You have already decided you do not want to take part in the study.
- You do not have time to participate in the study activities.
- You are not interested in using an online decision guide to help you learn more.

*If these reasons are important to you, then you may decide you do **not** want to learn more about the NC NEXUS study.*

Should My Family Learn More about the NC NEXUS Study?

Make the decision that is best for you and your family.

Here are some questions to help you decide.

Yes	No	
☐	☐	Do you want to learn more about genetics and genomic sequencing?
☐	☐	Do you want to learn more about the genetic conditions that genomic sequencing may find?
☐	☐	Do you want the option of having genomic sequencing for your child?
☐	☐	Are you willing to use an online decision guide to learn more?
☐	☐	Do you have time to participate in the study activities

If you have more ***Yes*** answers than ***No*** answers, you and your family may be ready to learn more so that you can decide if you want to join the study.

If you have more ***No*** answers than ***Yes*** answers, taking part in this study may not be right for you and your family.

Please contact a member of our study team about these and any other questions you may have.

Phone: ###-###-####

Email: NC_Nexus@unc.edu.

This brochure was developed with support from the National Institutes of Health's Eunice Kennedy Shriver National Institute of Child Health and Human Development and the National Human Genome Research Institute.

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

RTI INTERNATIONAL

University of North Carolina at Chapel Hill
Information Sheet: Phase I of NCNEXUS
Adult Participants, "Diagnosed" Cohort
Biomedical Form

Information Sheet: 5/15/2015
Title of Study: North **C**arolina **N**ewborn **Ex**ome **S**equencing as **U**niversal **S**creening **(NC NEXUS)**
Principal Investigators: Cynthia Powell, M.D. and Jonathan S. Berg, MD, PhD
UNC-Chapel Hill Department: Genetics
UNC-Chapel Hill Phone number: 919-966-7043
Email Address: powellcm@med.unc.edu; jsberg@med.unc.edu
Co-Investigators: Donald Bailey, Karen Weck, Kirk Wilhelmsen
Funding Source: National Human Genome Research Institute (National Institutes of Health)
Study Contact:
Study Contact telephone number:
Study Contact email:

This information sheet is for couples thinking about joining Phase I of NCNEXUS.

What are some general things you should know about research?
Research studies are done to learn information that may help others in the future. You and your child may *not* get any direct benefits from joining and there may be risks. Joining a study is up to you.

It is important that you understand the information on this sheet so that you can make an informed choice about whether or not to join. You have the right to ask, and have answered, any questions you have about this study by contacting the researchers listed at the top of this form.

What is the purpose of the NCNEXUS study?
The purpose of this study is to learn whether a new kind of testing, called "genomic sequencing" can help identify children who have or are likely to develop some kinds of genetic conditions.

Newborn screening is done to look for conditions that can be successfully treated when they are found early. Screening can identify some, but not all, genetic conditions.

Genomic sequencing looks for **genetic** differences, called "variants," that cause conditions like the ones identified by newborn screening and many more. The technology that allows many genes to be studied at once is called "Next-generation sequencing." It is a new way to test for these conditions.

We are interested in knowing how parents decide whether or not to have sequencing of their child and, if so, how they understand and respond to the different kinds of information they can learn from testing.

NCNEXUS study has two phases; Phase I and Phase II.
In Phase I, we want to find out what information parents need to help them decide whether or not to have genomic sequencing of their child to find conditions like those identified by newborn screening.

At the end of Phase I, parents will be asked if they want their child to have sequencing.
Parents who consent to sequencing will enter Phase II.
Parents who decline will complete a questionnaire and then end their participation in the study. Phase II is described on a separate consent form.
If you join Phase I, you do not have to join Phase II.

You have agreed that we can contact you by phone to ask whether or not you want to join **Phase I.** To help you decide before we call, we have provided the following materials:

- A **brochure** that tells about the study, how genomic sequencing is done, the kinds of genetic conditions that might be found, and information to help you decide whether or not to join.

- This **information form**. Please read it before we call you.

When we call to ask if you want to join **Phase I**, you can tell us your answer over the phone**.** If both parents are involved in the child's life, they **both** have to agree to join but each member of the couple will complete the questionnaires on his or her own. If only one parent has custody of the child, he or she can join by him or herself.

What happens if you do not want to join Phase I?
We will ask you some questions about yourself and your reasons for declining. After you answer the questions, your part in the study will end and we will shred your identifying information.

The rest of this information sheet is about what happens if you decide to join Phase I.

How many people will take part in the study?
We expect to have about 400 children and their parents complete the whole study.

How long will your part in Phase I last?
Phase I lasts until both parents either agree to sequencing or decline and complete the questionnaire.

What will happen after you join Phase I?

You will complete an **intake form.**

If you have access to an Internet-enabled computer:
1) We will give you a link to complete a questionnaire.
2) We will then give you a link to an online electronic decision guide. The guide has information about sequencing, describes the types of results you might learn, the risks and benefits of testing and helps you think about if sequencing for your child would be the right decision for you and your family.

At the end of the decision guide, you will be asked to pick one of the following 3 options:

(1) We **do not** want our child to have genomic sequencing for conditions like those found in newborn screening and **do not** want to schedule a study visit;
(2) We **are** interested in genomic sequencing for our child and **want** to schedule a study visit; or,
(3) We **are undecided** about genomic sequencing and **want** to schedule a study visit to learn more.

If you come to the study visit, it does <u>not</u> mean that you have to consent to sequencing.

If you do not have an Internet-enabled computer
We will send you a questionnaire to complete and return it in the pre-paid envelope. If you are interested in scheduling a visit to view the decision guide and learn more, please let us know.

What happens next?
If you decide to schedule a study visit, you will meet with a genetic counselor to discuss why you may or may not want to have sequencing of your child. This visit will last about 1 hour but may last longer if you have more questions.

You will then be asked if you want to consent to having genomic sequencing of your child.

If both parents consent, you will both sign the consent form and a form so we can obtain your child's health records.

Parents who consent to sequencing will enter into Phase II. They will be randomized to 1 of 2 groups. One group will be asked to decide if they want to request additional genetic information about conditions that are not related to those found with newborn screening. The other group will not be asked to make these decisions.

If you decide not to consent, you will answer a questionnaire and then your part in the study will end.

What are the possible benefits to you of participating in Phase I?
There is little chance that you will benefit, but it will help us learn how parents make these decisions.

What are the possible risks or discomforts to you by participating in Phase I of this study?
You may be uncomfortable answering some questions on the forms. You can refuse to answer a question or stop completing the forms but not completing them means you can't continue in the study.

You will *not* be charged for any of the activities in NC NEXUS.
You will be paid with a $20 VISA card for completing each questionnaire.

We will give you any new information that might affect your willingness to continue participation. You can stop participating at any time, without penalty, by contacting the researchers on the first page.

Who is sponsoring this research?
This research is funded by a grant from the National Human Genome Research Institute and the National Institutes of Child Health and Development at the National Institutes of Health. The research team is paid to carry out the study but they do ***not*** have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about your rights as a research participant?
The Institutional Review Board (IRB) reviews all research on human volunteers in order to protect their rights and welfare. If you have questions or concerns about you and your child's rights as research participants, you may contact the IRB at 919-966-3113 or IRB_subjects@unc.edu. You do not have to use your name.

You will be asked to give your verbal consent to join Phase I over the phone.

University of North Carolina at Chapel Hill
Information Sheet: Phase I of NCNEXUS
Adult Participants, "Well-Child" Cohort
Biomedical Form

Information Sheet: 5/15/2015
Title of Study: North **C**arolina **N**ewborn **Ex**ome **S**equencing as **U**niversal **S**creening **(NC NEXUS)**
Principal Investigators: Cynthia Powell, M.D. and Jonathan S. Berg, MD, PhD
UNC-Chapel Hill Department: Genetics
UNC-Chapel Hill Phone number: 919-966-7043
Email Address: powellcm@med.unc.edu; jsberg@med.unc.edu
Co-Investigators: Donald Bailey, Karen Weck, Kirk Wilhelmsen
Funding Source: National Human Genome Research Institute (National Institutes of Health)
Study Contact:
Study Contact telephone number:
Study Contact email:

This information sheet is for couples thinking about joining Phase I of NCNEXUS.

What are some general things you should know about research?
Research studies are done to learn information that may help others in the future. You may *not* get any direct benefits from joining and there may also be risks. Joining a research study is up to you.

It is important that you understand the information on this sheet so that you can make an informed choice about whether or not to join. You have the right to ask, and have answered, any questions you have about this study by contacting the researchers listed at the top of this form.

What is the purpose of the NCNEXUS study?
The purpose of this study is to learn whether a new kind of testing, called "genomic sequencing" can help identify children who have or are likely to develop some kinds of genetic conditions.

After a baby is born, newborn screening is done to look for conditions that can be successfully treated when they are found early. Screening can identify some, but not all, genetic conditions.

Genomic sequencing looks for **genetic** differences, called "variants," that cause conditions like the ones identified by newborn screening and many more. The technology that allows many genes to be studied at once is called "Next-generation sequencing." It is a new way to test for these conditions.

We are interested in knowing how parents decide whether or not to have sequencing of their child and, if so, how they understand and respond to the different kinds of information they can learn from testing.

NCNEXUS study has two phases; Phase I and Phase II.
In Phase I, we want to find out what information parents need to help them decide whether or not to have genomic sequencing of their child to find conditions like those identified by newborn screening.

At the end of Phase I, parents will be asked if they want their child to have sequencing.
Parents who consent will enter Phase II.
Parents who decline will complete a questionnaire and then end their participation in the study.
Phase II is described on a separate consent form. If you join Phase I, you do not have to join Phase II.

You have agreed that we can contact you by phone to ask whether or not you want to join **Phase I.** To help you decide before we call, we have provided the following materials:

- A **brochure** that tells about the study, how genomic sequencing is done, the kinds of genetic conditions might be found, and information to help you decide whether or not to join.

- This **information form**. Please read it before we call you.

When we call to ask if you want to join **Phase I**, you can tell us your answer over the phone**.** If both parents will be involved in the child's life, they **both** have to agree to join but each member of the couple will complete the questionnaires on his or her own. If only one parent will have custody of the child, he or she can join by him or herself.

What happens if you do not want to join Phase I?
We will ask you some questions about yourself and your reasons for declining. After you answer the questions, your part in the study will end and we will shred your identifying information.

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<u>How many people will take part in the study?</u>
We expect to have about 400 children and their parents complete the whole study.

<u>How long will your part in Phase I last?</u>
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If you have access to an Internet-enabled computer:
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At the end of the decision guide, you will be asked to pick one of the following 3 options:

(1) We **do not** want our child to have genomic sequencing for conditions like those found in newborn screening and **do not** want to schedule a study visit;
(2) We **are** interested in genomic sequencing for our child and **want** to schedule a study visit; or,
(3) We **are undecided** about genomic sequencing and **want** to schedule a study visit to learn more.

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We will send you a questionnaire to complete and return it in the pre-paid envelope. If you are interested in scheduling a visit to view the decision guide and learn more, please let us know.

What happens next?
If you decide to schedule a study visit, you will meet with a genetic counselor to discuss why you may or may not want to have sequencing of your child after birth. This visit will last about 1 hour but may last longer if you have more questions.

You will then be asked if you want to consent to having genomic sequencing of your child.

If both parents consent, you will both sign the consent form and a form so we can obtain your child's health records after birth.

Parents who consent to sequencing will enter into Phase II. They will be randomized to 1 of 2 groups. One group will be asked to decide if they want to request additional genetic information about other conditions that are not related to those found with newborn screening. The other group will not be asked to make these decisions.

If you decide not to consent, you will answer a questionnaire and then your part in the study will end.

What are the possible benefits to you of participating in Phase I?
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The Institutional Review Board (IRB) reviews all research on human volunteers in order to protect their rights and welfare. If you have questions or concerns about your rights as a research participant, you may contact the IRB at 919-966-3113 or IRB_subjects@unc.edu. You do not have to use your name.

You will be asked to give your verbal consent to join Phase I over the phone.

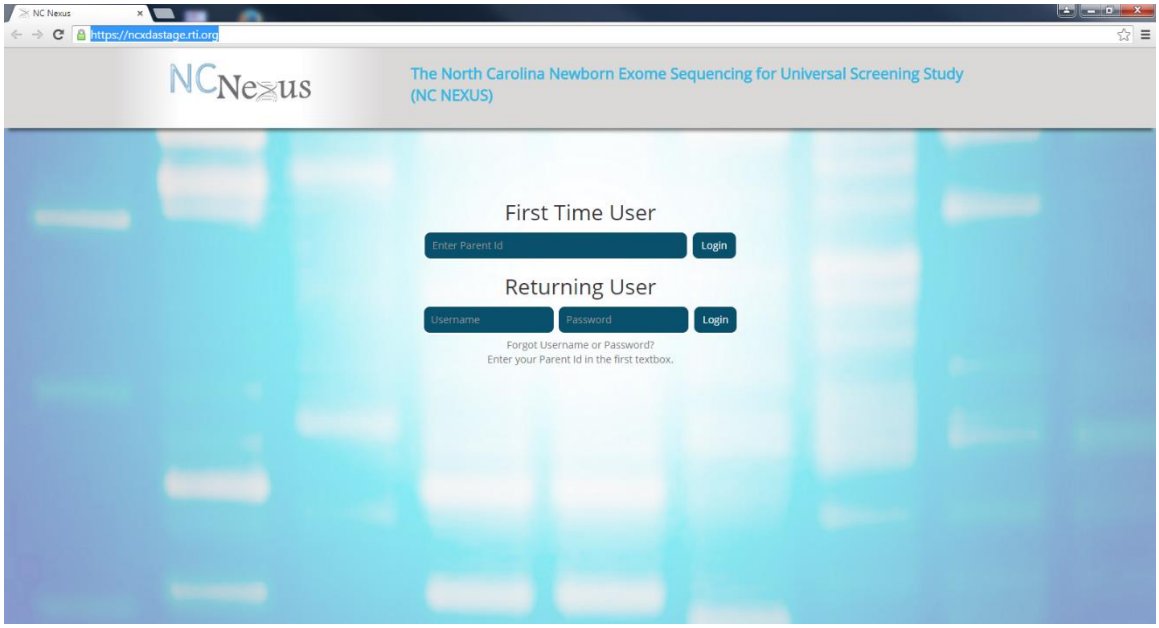
List of Appendices

Volume 3

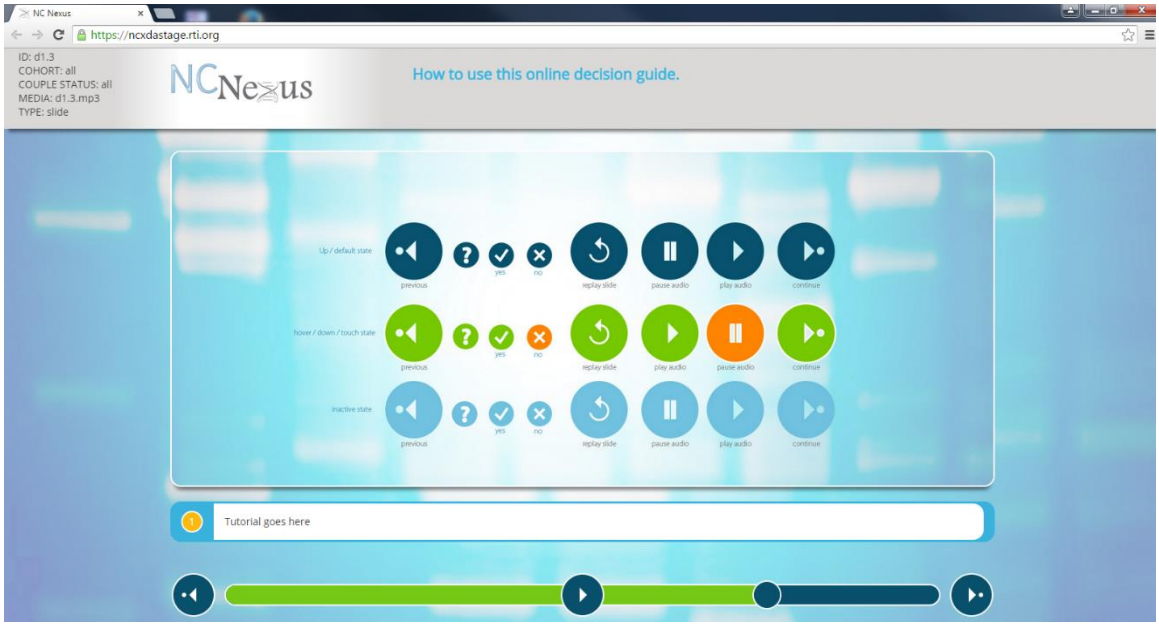
Screen shots of the NC NEXUS draft decision aids

Decision Aid 1

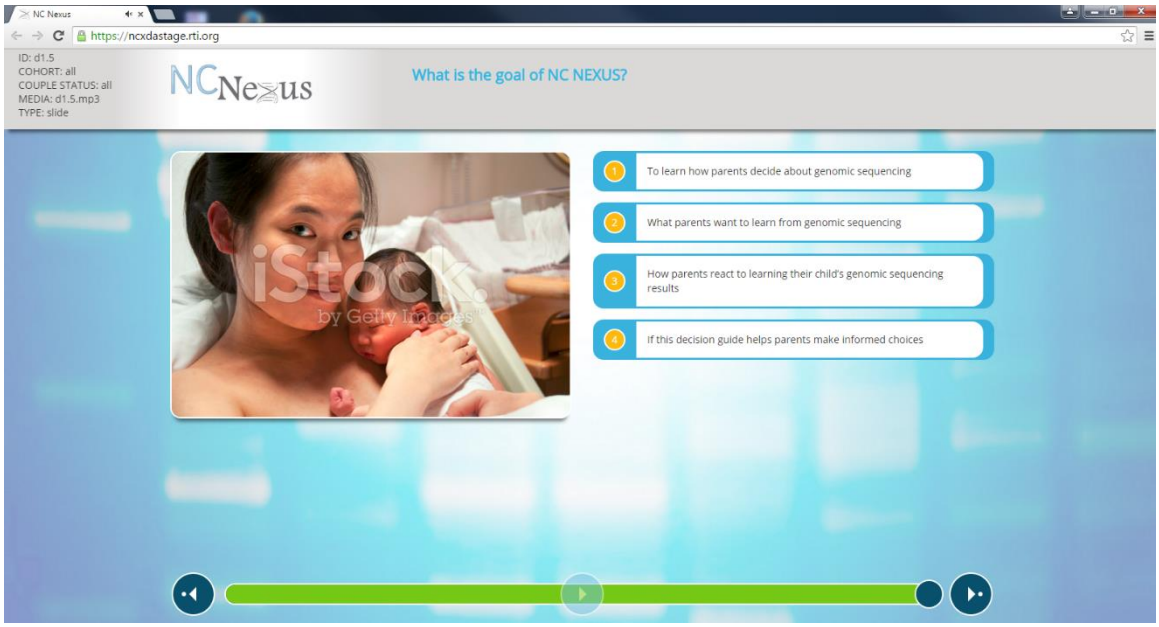
1. ***Login screen*** **–** Users will enter a unique user ID and password to securely access the NC NEXUS decision aid.



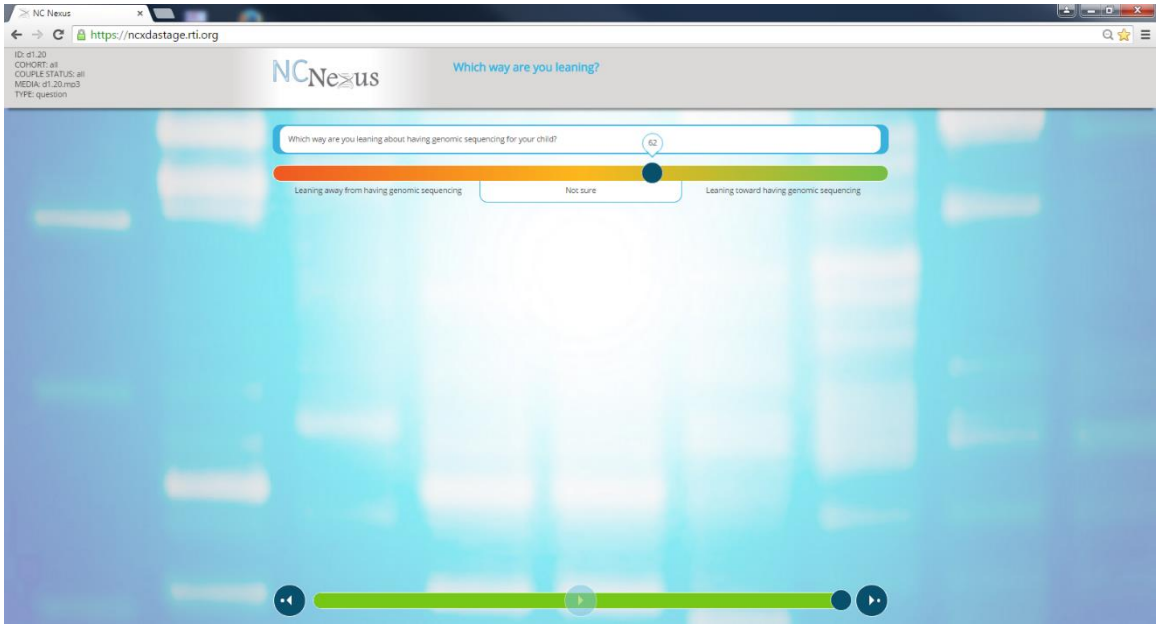
2. ***Navigation tutorial*** **–** A set of screens in Decision Aid 1 (DA1) will provide an audiovisual look at the navigation controls that users will use to move through the guide. The tutorial will also explain additional interactive features, like slider scales and sorting tasks.



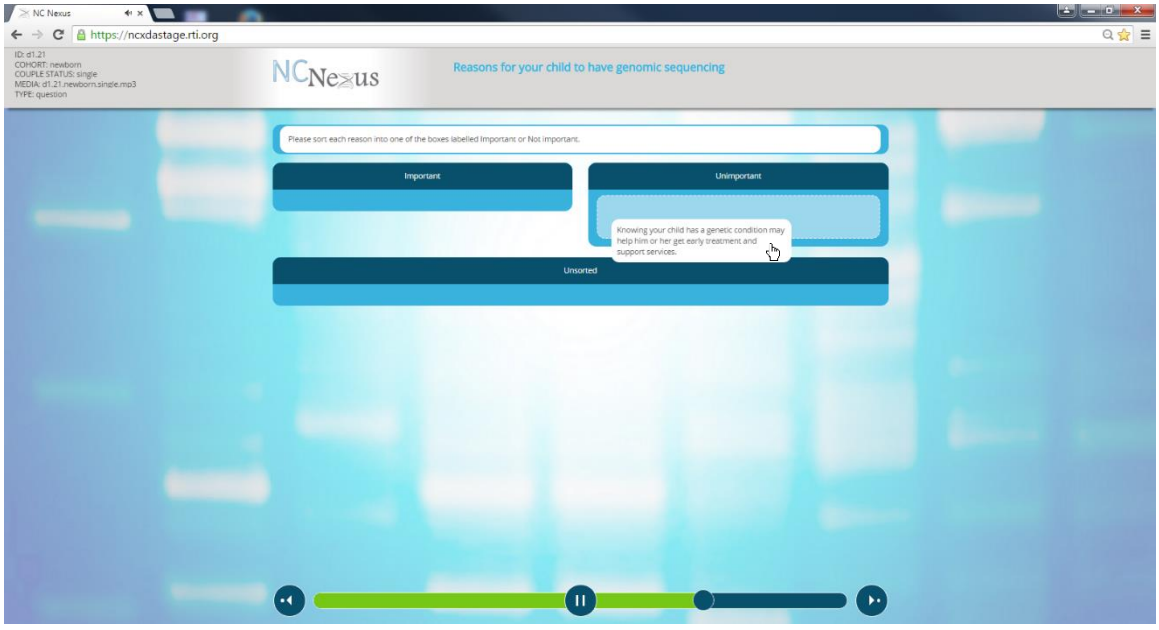
3. ***NC NEXUS Overview and Educational Material –*** DA1 begins with a number of informational screens providing an overview of the goals and procedures of the NC NEXUS study, newborn screening, genomic sequencing, the types of gene variants and conditions that will be studied, and the potential harms and benefits of participating.

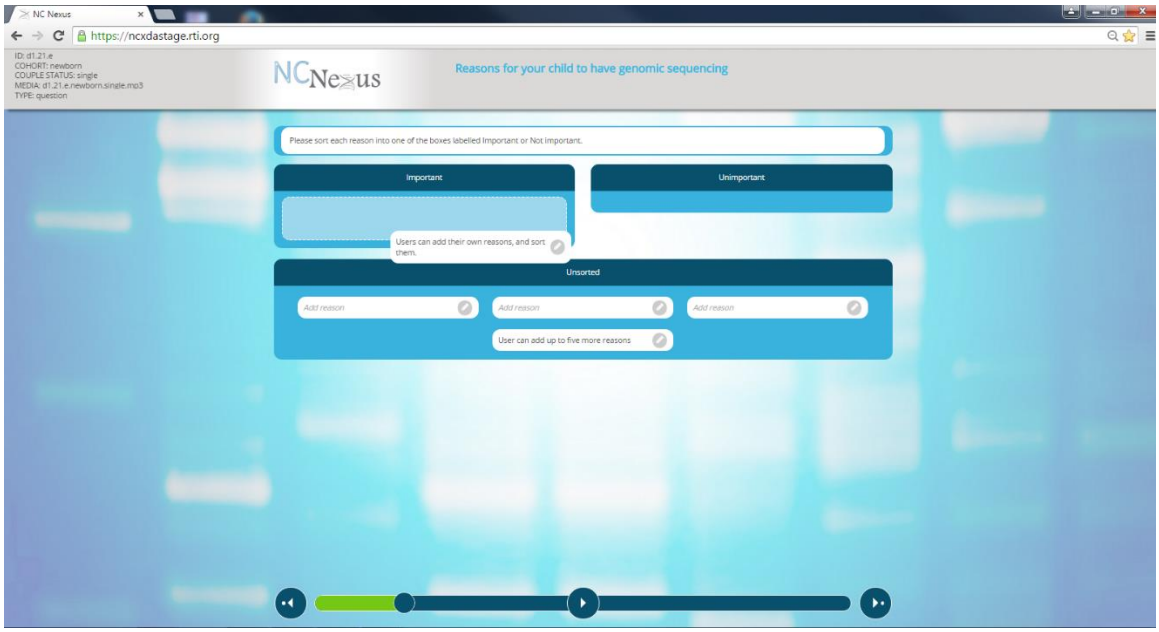


4. ***Interactive Slider Scale –*** Interactive slider scales will be used to assess participants' interest in having genomic sequencing for their child. The same slider format will be used for two questions at different points in the decision aid. About half-way through the guide, parents will be asked, "If you had to decide right now, which way are you leaning about having genomic sequencing for your child as part of NC NEXUS? (*Leaning away, Not sure, Leaning toward*)." Three anchor points along the scale will be labelled, but a more refined numeric value associated with the position on the slider scale will be captured and stored in the site's secure database (e.g., integers ranging 0 – 100).

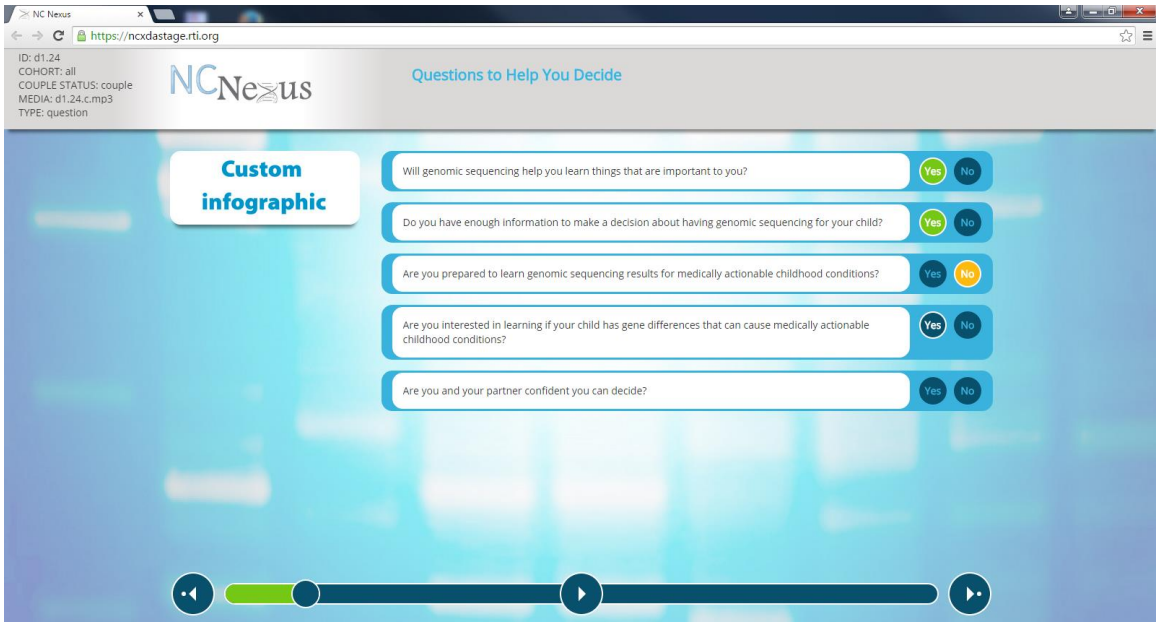


5. ***Values Clarification Sorting Task –*** Users will a set of sorting tasks in DA1. In these tasks, they will be asked to move text boxes containing reasons for and reasons against having genomic sequencing for their child as part of the NC NEXUS study into bins labelled *Important* or *Not important*. Parents who review the decision guide as a couple will have a third option, *We disagree*. We developed a set of predefined reasons through formative research (e.g., parent interviews) that will be presented on the screen and sorted one at a time. In addition to the predefined reasons, users will also be shown five blank text boxes where they can type and sort their own reasons. After both sorting tasks are complete (i.e., *reasons for* and *reasons against*), the site will automatically populate a review screen showing the reasons that the user classified as important.

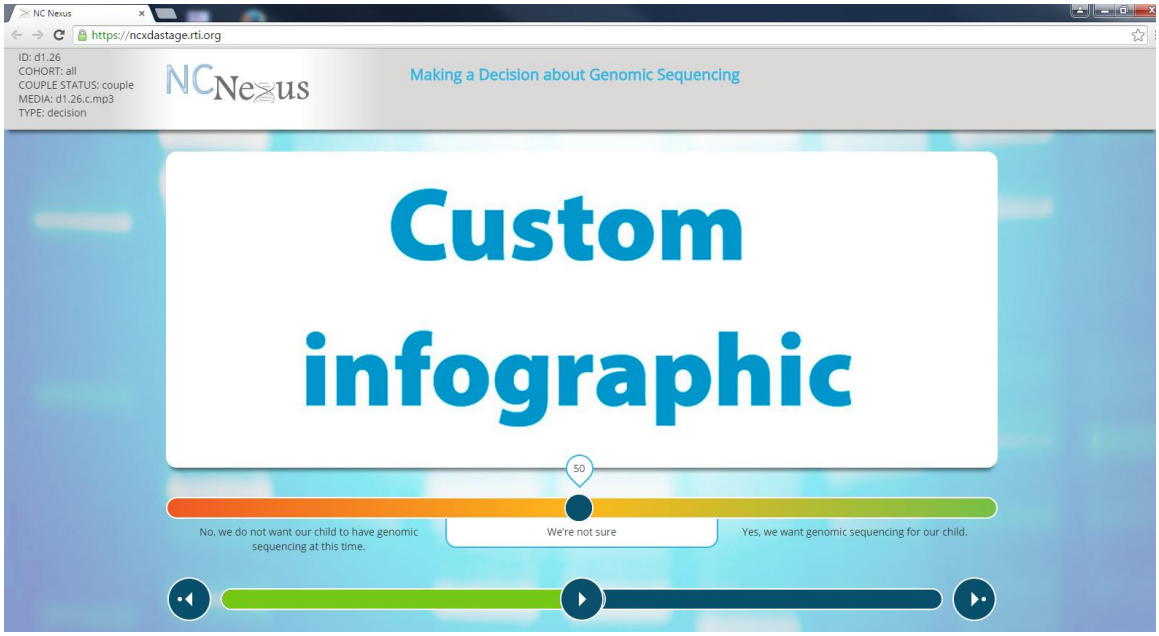




6. ***Questions to help decide –*** After the values clarification sorting task, users will be asked a set of yes/no questions to help them decide if they want their child to have genomic sequencing as part of NC NEXUS. The interactive buttons will change color on roll-over and when clicked. Data from these questions will be captured recorded in the site's secure database.

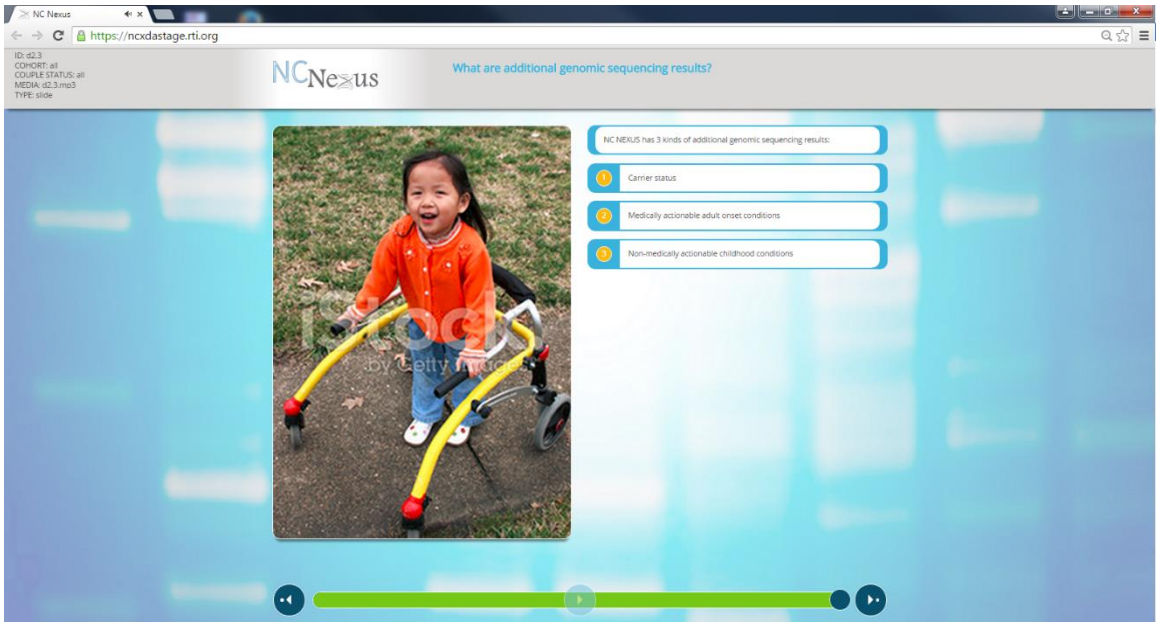


7. ***Decision Screen*** **-** Toward the end of the decision aid, parents will again use a slider scale to answer the question, "Do you want your child to have genomic sequencing for conditions like those found in newborn screening? (*No, Not sure, Yes*)." As with the "which way are you leaning?" screen, three anchor points along the scale will be labelled, but numeric values on a 0 – 100 scale will be captured and stored in the site's secure database. Specific ranges along the 100-point scale will be used to categorize user interest in sequencing and continuing their participation in the study. The data gathered on this screen will be used to determine whether the user(s) should be contacted to schedule a study visit and for evaluation of the decision aid.

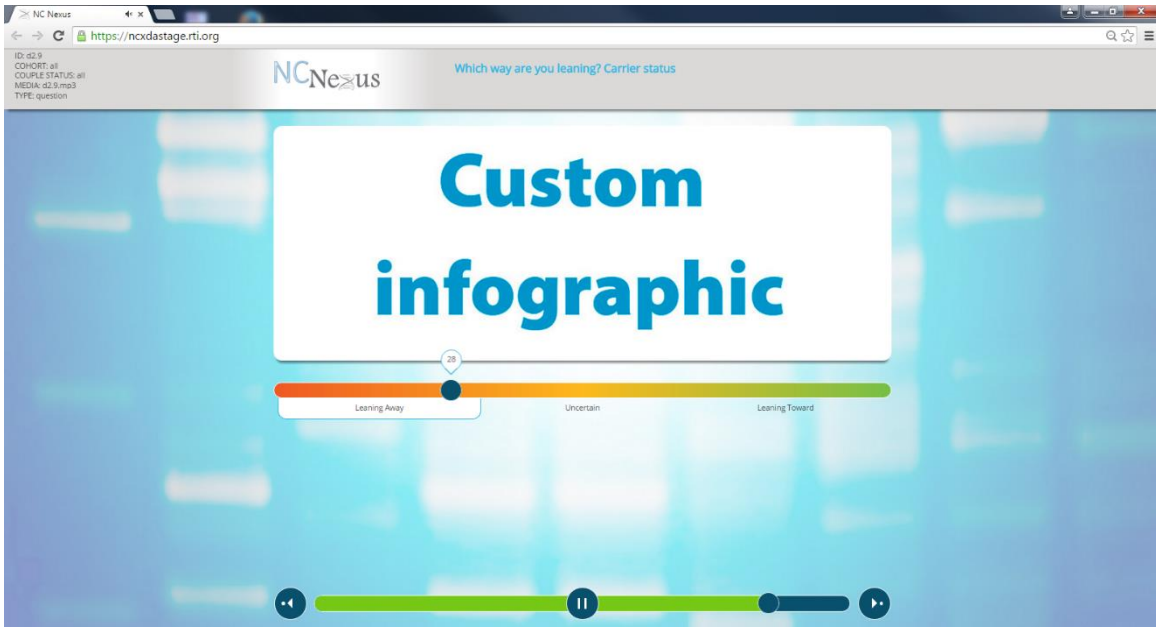


Decision Aid 2

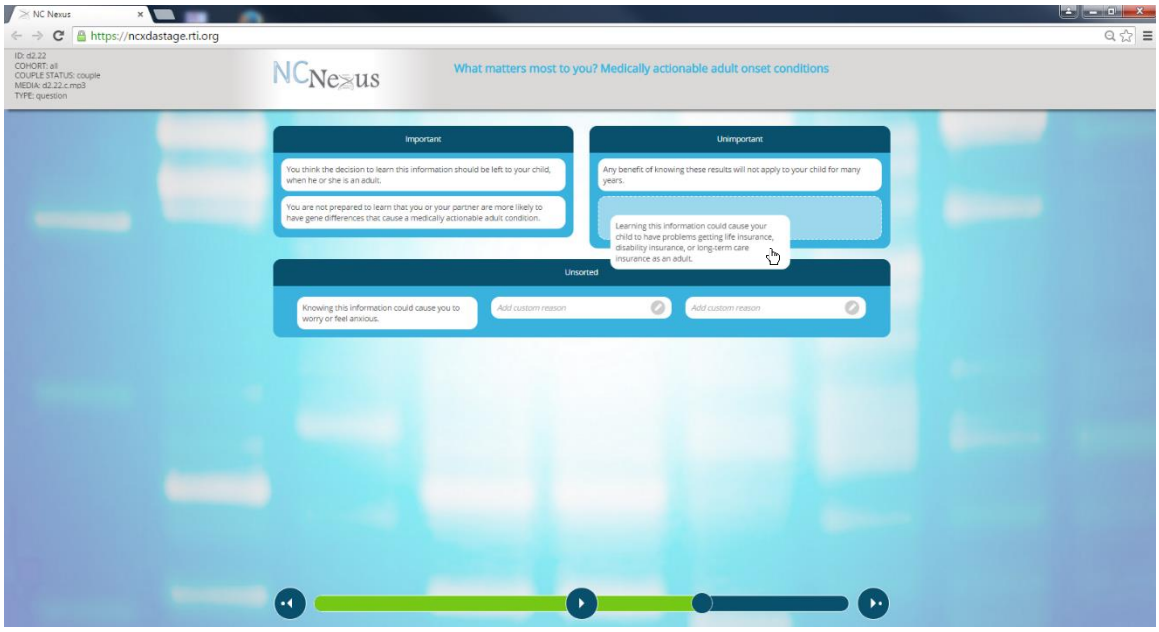
1. ***Additional Sequencing Results Overview*** – Decision Aid 2 (DA2) will only be made available to participants who decide to have genomic sequencing for their child after reviewing DA1 and who have been randomly assigned to a condition where they will be given the option to request up to three kinds of additional sequencing results. DA2 will use the same login interface as DA1. The content of DA2 is separated into three main sections corresponding to the three kinds of additional sequencing results: *carrier status*, *medically actionable adult onset conditions*, and *non-medically actionable childhood conditions*.



2. ***Interactive Sliders –*** Interactive sliders like those in DA1 will be used to assess which way parents are leaning when it comes to learning each kind of additional sequencing result. These screens will appear at the end of each section describing one kind of additional sequencing results.

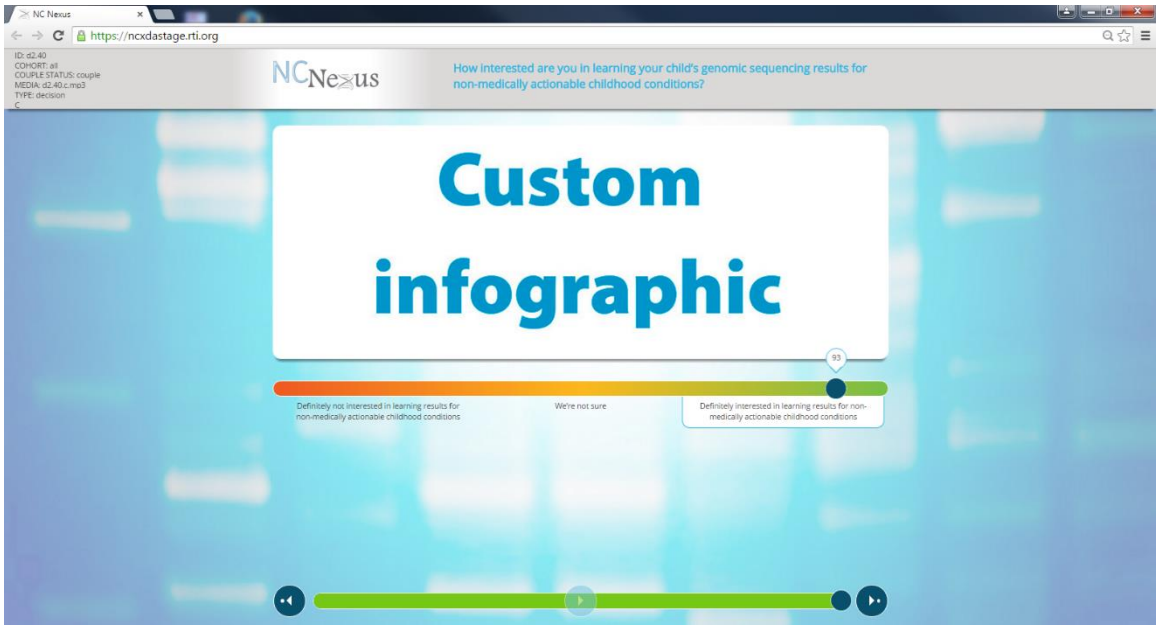


3. ***Values Clarification Sorting Tasks –*** Three separate values clarification sorting tasks will be included in DA2, each corresponding to one kind of additional sequencing result. The basic format of the sorting tasks will be identical to that used in DA1. Like the "Which way are you leaning?" screens, these tasks will be at the end of each additional sequencing results section.



4. ***Questions to help decide –*** After reviewing the information provided about all three kinds of additional sequencing results, users will be shown a set of questions to help them decide. The interactive format of these questions will be identical to that used in DA1.

5. ***Interest Inventory Screens –*** Toward the end of DA2, users will be shown three interactive slider screens, each asking them to express their interest in learning one kind of additional sequencing results. Data from these screens will be used by the NC NEXUS genetic counsellor to prepare for study visits, and by the research team for analysis.



NC NEXUS – Online Decision Aid 1 – Shooting Script
Version: 09/18/2015

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.1	ALL			The North Carolina Newborn Exome Sequencing for Universal Screening Study (NC NEXUS)	Login user name and password fields. Enter button. *NOTE:* There needs to be more of a pause after the user log- in before narration in D1.2 begins *NOTE:* Progress bar for overall DA		(1) Welcome/Login

D1.2	ALL	Welcome to the NC NEXUS decision guide. This decision guide will help you learn more about the NC NEXUS Study, including: • The purpose of the study • How genes can affect your child's health. • Genomic seque	Welcome	Welcome to the NC NEXUS decision guide. (headline) • Purpose of the study • How genes can affect your child's health • Genomic sequencing • Results that might be found • Decide if you want genomic sequencing *NOTE:* Each bullet appears on screen in time with narration. **[? – 'genomic sequencing']** **[? – 'gene']**	Next button Replay button *NOTE: Throughout DA, make sure Replay button brings user back to start of same screen (e.g., Replay on D1.2 restarts D1.2)* Q/A [?] buttons *NOTE: Need to discuss having single Q/A button per screen that links to list of terms needing definitions on that screen*		(3) General content, text list

		ncing, and • The types of results that might be found. The guide will also help you decide if you want to have genomic sequencing for your child.					

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.3	ALL	Before getting started, let's look at the navigation controls you can use to move through the decision guide. Here is the next button to move forward.	A look at the navigation controls, starting with the next button.	How to use this online decision guide. (headline) *Note:* Visual demo pointing out the next button.	Next button Replay button *NOTE: it's not clear which tool the narration is referring to. Image on screen needs to correspond with what is being said in the audio. Eg. Only show the button/tool being referred to at the time it is being talked about in the narration. May need to split this screen out into several sub screens, one per button/tool?*		(2) How to use the website
D1.3.a	ALL	If you need to pause for a moment and come back, click the play/pause button.	This is the play/pause button	How to use this online decision guide. (headline) *Note:* Visual demo pointing out the play/pause button.	Next button Replay button		2) How to use the website

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.3.b	ALL	If you want to listen to information on the screen again, click the replay button. .	This is the replay button	How to use this online decision guide. (headline) *Note:* Visual demo pointing out the replay button.	Next button Replay button		2) How to use the website
D1.3.c	ALL	Some screens have a question-mark button. Clicking this button will show you definitions of key words from that screen.	This is the question button.	How to use this online decision guide. (headline) *Note:* Visual demo pointing out the question button.	Next button Replay button *Note:* We'll need a question button that can appear on some screens, maybe lower right corner? Clicking it will open a window or go to different screen(?) with definitions of key terms.		2) How to use the website

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.3.d	ALL	On some screens you will be asked questions. One way to answer is with a sliding scale. Click and drag the slider, moving it to the point on the scale that best fits your answer. You can choose any point on the scale. Then click the next button to continue.	This is a slider scale	How to use this online decision guide. (headline) *Note:* Infographic pointing out the steps of how to use the interactive slider scale. 1) drag slider, 2) move to point on scale, 3) click next button *NOTE: Make sure that any text in graphic mirrors wording in script. E.g., use "click" not "touch" or "tap"*	Next button Replay button		2) How to use the website
D1.3.e	ALL	Other questions will ask you to sort items. Click and drag each item into the desired box. When you are	This is the sorting task	How to use this online decision guide. (headline) *Note:* Infographic pointing out steps of how to use the sorting task.	Next button Replay button		2) How to use the website

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		done sorting the items, click the next button to continue.		1) drag item, 2) move to sorting box 3) next button. *NOTE: Make sure that any text in graphic mirrors wording in script. E.g., use "click" not "touch" or "tap"*			
D1.3.f	ALL	Some questions will ask you to type in your own thoughts or opinions. Click inside the text box, and then type your answer. When you are done typing, click the checkmark to save what you typed.		How to use this online decision guide. (headline) *Note:* Infographic pointing out steps of how to use the type-in box. 1) inactive text box, 2) click and show cursor 3) type in response, 4) click check mark *NOTE: Make sure that any text in graphic mirrors wording in script. E.g., use "click" not "touch" or "tap"*			2) How to use the website

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.3.g	ALL	Lastly, some questions will ask you to select "yes" or "no." You can answer by clicking the button that matches your selection. Now, if you're ready to begin, please click the next button	These are the yes/no buttons.	How to use this online decision guide. (headline) *Note:* Infographic point out how to use yes/no buttons. *NOTE: Make sure that any text in graphic mirrors wording in script. E.g., use "click" not "touch" or "tap"*	Next button Replay button		2) How to use the website

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.4	ALL	What is NC NEXUS? NC NEXUS is a research study that offers you the option to have genomic sequencing for your child. One goal of NC NEXUS is to find out how well genomic sequencing finds over 30 conditions that all babies in North Carolina are tested for at birth. This test is called *newborn screening*. Another goal is to find out if genomic	NC NEXUS is a research study offering genomic sequencing for your child	*Text on screen:* What is NC NEXUS? (headline) • NC NEXUS is a research study • Find out how well sequencing finds conditions tested for at birth • This test is newborn screening • Find out if sequencings finds other important conditions Word cloud **[? – 'genomic sequencing']** **[? – 'newborn screening]**	Next button Replay button Q/A [?] buttons		(4) General content, text plus image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		sequencing finds hundreds of other important conditions that are not part of newborn screening, but are otherwise similar to them.					
D1.5	ALL	The NC NEXUS study team hopes to learn • How paren		What is the goal of NC NEXUS? (headline)	Next button Replay button Q/A [?] button		(3) General content, text list

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		ts like you decide if they want to have genomic sequencing for their child • The types of information parents want to learn		• To learn how parents decide about genomic sequencing • What parents want to learn from sequencing • How parents react after learning their child's results • If this decision guide helps parents make informed choices **[? – 'genomic sequencing']**			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		from genomic sequencing • How parents react after learning their child's genomic sequencing results, and • Whether this decision		*NOTE: Each bullet appears on screen in time with narration. Unless that is overly complex* *NOTE: Need to ensure that graphics scale to the browser.*			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		guide helps parents make informed choices about genomic sequencing					

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.6	ALL	What is newborn screening? Newborn screening is testing done when a baby is born to find serious conditions before a child becomes sick. The conditions found by newborn screening can cause disability or even death if they are not treated early.	Newborn screening tests for serious conditions.	What is newborn screening? (headline) *Image:* doctor with baby and mom Newborn screening finds serious conditions before a child becomes sick: [*NOTE:* This is a list of signs, symptoms and/or outcomes for many of the conditions tested for with newborn screening. Visuals may be useful here to get at the seriousness of the conditions.] [*NOTE:* Sync this list of signs and symptoms with "The conditions found by newborn screening can cause	Next button Replay button Q/A [?] button		(4) General content, text plus image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				disability…". Enter full list as a block instead of one at a time.] • Intellectual disability • Delayed physical development • Hearing loss • Heart and breathing problems • Seizures • Coma • Early death **[? – 'newborn screening]**			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.7	ALL	Most of the conditions are rare. Only about 13 out of every 10,000 babies born in the United States have a condition that can be found by newborn screening.	Most conditions that are part of newborn screening are rare.	What is newborn screening? (headline) • Conditions found by newborn screening are rare. • 13 out of every 10,000 babies born in the U.S. *Image:* call out of shot of 13 baby icons in row; Full array behind to reveal the 13 babies are part of a grid of 10,000 baby icons. (Here are links to some example risk arrays: conjoint study ex. BRC 1 ex) **[? – 'newborn screening]**	Next button Replay button Q/A [?] button		(4) General content, text plus image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.8.Newborn	IF NEWBORN COHORT	The conditions found by newborn screening have treatments. If a child has one of these conditions, finding out early can help keep him or her from getting sick. It might even save the child's life. If you decide to have genomic sequencing as part of the NC NEXUS study you would still have regular newborn screening when your baby is born.	Newborn screening conditions are treatable	What is newborn screening? (headline) • Conditions found by newborn screening have treatments. • Finding out early can keep the child from getting sick. • It might even save the child's life. *Image*: baby at doctor's office? **[? – 'genomic sequencing']** **[? – 'newborn screening]**	Next button Replay button Q/A [?] button		(4) General content, text plus image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.8.Diagnosed	IF DIAGNOSED COHORT	The conditions found by newborn screening have treatments. If a child has one of these conditions, finding out early can help keep him or her from getting sick. It might even save the child's life.	Newborn screening conditions are treatable	What is newborn screening? (headline) • Conditions found by newborn screening have treatments. • Finding out early can keep the child from getting sick. • It might even save the child's life. *Image*: baby at doctor's office? **[? – 'genomic sequencing']** **[? – 'newborn screening]**	Next button Replay button Q/A [?] button		(4) General content, text plus image
D1.9	ALL	What is genomic sequencing? Each cell in a person's body	DNA contains the instructions your child's body needs to develop and function.	What is genomic sequencing? (headline)	Next button Replay button Q/A [?] buttons		(4) General content, text plus image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		contains a copy of his or her *DNA*. DNA provides the instructions a person's body needs to grow and function. These instructions are divided into genes. Just like how the order of words in a sentence is important for understanding what you read, the order of DNA building blocks is important for telling the body's cells what to do.		• Each cell contains a copy of DNA • DNA provides instructions a body needs to function • These instructions are divided into genes • The order of DNA building blocks tells the body what to do **[? – 'genomic sequencing']** **[? – 'DNA']** **[? – 'gene']** *Image notes:* Illustration of double helix,			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				preferably one that labels the nucleotide bases A, C, T, and G (e.g., double helix is right-most part of diagram on this page. It also uses a bracket to show genes are a part of DNA: http://www.riversideonline.com/health_reference/Tools/DS00549.cfm Other examples: https://www.dnalc.org/resources/genescreen/inheritance.html) *NOTE: timing of text bullets needs to be synced with audio*			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.10	ALL	Differences in a person's DNA can cause people to have different forms of the same gene. Most often these gene differences, or variants, will have no effect on health, but some gene differences can lead to health problems. Genomic sequencing is a way to look for differences in your child's DNA that could cause rare but serious health problems.	Genomic sequencing is a way to look for gene differences that might cause health problems.	What is genomic sequencing? (headline) • People can have different forms of the same gene • Most gene differences have no effect on health • But some lead to health problems • Genomic sequencing is a way to look for gene differences *Image notes:* Show two flattened strings of DNA A,C,T,and Gs arranged one above the other. Most letters in the two sequences are identical, but every so often a letter is different; highlight the differences.	Next button Replay button Q/A [?] buttons		(4) general content, text plus image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				[? – 'gene differences'] **[? – 'genomic sequencing']** **[? – 'DNA']** (E.g. http://www.dana.org/uploadedImages/Images/Content_Images/PR10_CH1_Fig3_Chain_cont.jpg http://performancegenetics.com/wp-content/uploads/2013/10/SNPs.jpg) *NOTE: Check timing of text is synced with audio*			
D1.11	ALL	What Can Genomic Sequencing Tell You About Your Child?		What can genomic sequencing tell you about your child? (headline)	Next button Replay button *NOTE: Check replay button replays current screen.*		(4) General content, text plus image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		In the NC NEXUS study, genomic sequencing will look for gene differences that cause the same conditions that are found through newborn screening, plus more than a hundred other conditions like them.		• Gene differences that cause conditions found through newborn screening • Plus more than 100 conditions like them **[? – 'genomic sequencing']** **[? – 'gene differences']** **[? – 'newborn screening]** *Image*: cute baby.	Q/A [?] button		

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.12.Newborn	IF NEWBORN COHORT	Researchers are still trying to understand how useful genomic sequencing is compared to other tests that tell people about their health. The NC NEXUS study team wants to learn if genomic sequencing can improve current newborn screening. They also want to see if genomic sequencing can be used to find conditions that are not part of current newborn		What can genomic sequencing tell you about your child? (headline) • Trying to understand how useful sequencing is compared to other tests. • NC NEXUS wants to learn if sequencing can improve newborn screening. • Also, if sequencing can find conditions not part of newborn screening. *Image:* someone that looks like a researcher, maybe in a lab coat **[? – 'genomic sequencing']**	Next button Replay button Q/A [?] button		(4) General content, text plus image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		screening, but could be in the future. These are rare conditions that affect children early in life and can be improved with early treatment.		**[? – 'newborn screening]**			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.12.Diagnosed	IF DIAGNOSED COHORT	Researchers are still trying to understand how useful genomic sequencing is compared to other tests that tell people about their health. The NC NEXUS study team wants to learn if genomic sequencing can find gene differences that cause the condition that your child currently has. They also want to see if genomic sequencing can be used to find conditions that are not		What can genomic sequencing tell you about your child? (headline) • Trying to understand how useful sequencing is compared to other tests. • NC NEXUS wants to learn if sequencing can find the condition your child has. • Also, if sequencing can find conditions not part of newborn screening. *Image:* someone that looks like a researcher, maybe in a lab coat **[? – 'genomic sequencing']**	Next button Replay button Q/A [?] button		(4) General content, text plus image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		part of current newborn screening, but could be in the future. These are rare conditions that affect children early in life and can be improved with early treatment.		**[? – 'gene differences']** **[? – 'newborn screening]**			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.13	ALL	What is a medically actionable childhood condition? These are rare but serious genetic conditions that… • Usually begin during childhood and are medically actionable; that is, they • Can be improved with early treatment, and • The benefits of treatment typically	Medically actionable childhood conditions begin during childhood and can be improved with treatment.	What is a medically actionable childhood condition? (headline) *Image:* Doctor with parents and baby. Medically actionable childhood conditions… • Rare and serious • Begin during childhood • Can be improved with early treatment • Benefits of treatment outweigh risks • More than 100 of these conditions	Next button Replay button Q/A [?] button		(3) General content, text plus image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		outweigh the risks. In addition to over 30 conditions that are part of current newborn screening, the NC NEXUS study will look for more than a hundred other conditions like them. The signs and symptoms of medically actionable childhood conditions differ greatly from one to the next.		**[? – 'genetic condition']** **[? – 'medically actionable condition']** *NOTE: 'Rare and serious' bullet still needs to be added.*			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.14	ALL	Pompe disease is one example of a medically actionable childhood condition. Pompe disease affects about 1 out of every 40,000 people in the United States and usually begins in the first few months after birth. Children who have Pompe disease have weak muscles so they are not able to do things like hold their heads up or crawl at the same age as other babies. Other signs of Pompe disease	Pompe disease is an example of a medically actionable childhood condition not currently part of newborn screening.	What is a medically actionable childhood condition? (headline) *Visual notes:* Show risk array for 1 out of 40,000. (example risk arrays: conjoint study ex. BRCA1 ex)*;* time risk array with '1 out of every 40,000' bullet. *Display:* Pompe disease is one example • Affects 1 out of every 40,000 people in the U.S. • Begins the first few months after birth • If untreated, leads to heart	Next button Replay button Q/A [?] button		(4) general content, text plus image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		include an enlarged liver and heart problems. If untreated, Pompe disease often leads to heart failure and death in the first year of life. There are drugs that can prevent some of these problems if given early in a child's life.		failure in the first year of life • Drugs can prevent these problems if given early in a child's life **[? – 'medically actionable condition']** *NOTE: Bulleted list needs to be put higher on screen, side-by-side with image.*			

D1.15	ALL	What can genomic sequencing tell you about medically actionable childhood conditions? The NC NEXUS team will look for gene differences that are known to cause specific conditions. For some medically actionable childhood conditions, these gene differences determine how the condition will affect a child. For other conditions, these gene differences are	NC NEXUS will use genomic sequencing to look for gene differences that lead to specific conditions. For some conditions, these gene differences are the only thing that matters; for other conditions, gene differences are not the *only* cause.	What can genomic sequencing tell you? (headline) *Image:* Another image of two short gene sequences with some differences in A,C,G,Ts, one representing a 'average risk' gene, the other representing an 'increased riskl' gene. Eg. I like how the specific differences are color coded in this example, and the rule line connecting the letters: file://rtints6/hserproj4/0214132%20NEXUS%20Proj%203/Aim%202%20Decision%20Aids/2.1%20DA%20Content/Final%20Decision%20Aid%20Content/working/Visual%20Exampl	Next button Replay button Q/A [?] button		(3) or (4) General text plus image

		not the *only* thing that determines how the condition will affect a child, but they are known to play an important role in a child developing the condition.		e_Gene%20difference_1.png And I like how each letter is assigned a distinct shape in this example: file://rtints6/hserproj4/0214132%20NEXUS%20Proj%203/Aim%202%20Decision%20Aids/2.1%20DA%20Content/Final%20Decision%20Aid%20Content/working/Visual%20Example_Gene%20difference_2.jpg • NC NEXUS will look for specific conditions • For some conditions, gene differences determine how it will affect a child. • For other conditions, gene difference are not the only thing that determines how			

				it will affect a child. • Gene differences play an important role in a child developing the condition **[? – 'gene differences']** **[? – 'genomic sequencing']** **[? – 'medically actionable condition']** *NOTE: Check timing of list on screen* *NOTE: Original D1.5 split into two screens.*			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.15.a	ALL	Finding these gene differences in your child's DNA can tell that he or she is more likely to have one of these conditions during childhood. Still, it is hard to know for sure how severe the condition would be because other factors also play a part in most conditions.		What can genomic sequencing tell you? (headline) *Image:* baby • Tell that a child is more likely to have these conditions. • It is hard to know how severe the condition would be • Other factors play a part in most conditions **[? – 'DNA']** **[? – 'gene differences']** *NOTE: D1.15 and D1.15.were originally on a single screen D1.15, but the content was long and dense, so if possible, we'd like*	Next button Replay button Q/A [?] button		

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				to split it across two screens.			
D1.16	ALL	How common is it for genomic sequencing to find gene differences that cause medically actionable childhood conditions? It is not known for sure how often genomic sequencing will find gene differences that cause these conditions. This is one of the things the NC NEXUS study will try to find out. The best estimate is	The NC NEXUS study team wants to find out how often genomic sequencing will find gene differences that lead to a health problem.	How common is it for genomic sequencing to find gene differences? (headline) • Not known how often sequencing will find these conditions. • The best estimate is in less than 1% of children • Genomic sequencing cannot find all gene differences related to all conditions **[? – 'genomic sequencing']** **[? – 'gene differences']** **[? – 'medically actionable condition']**	Next button Replay button Q/A [?] button		(3) or (4) General content, Text plus image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		that sequencing will find gene differences that cause these conditions in less than 1% of children. Genomic sequencing done by the NC NEXUS study cannot find *all* gene differences related to *all* medically actionable childhood conditions.		*Visual note:* Risk array. Visual to depict that it is unsure exactly how likely it is that a gene difference will be found, but less than 1 out of 100 children tested.			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.17.Newborn	IF NEWBORN COHORT	What will happen if you decide to have genomic sequencing for your child? • If you decide you want your child to have genomic sequencing in NC NEXUS, you will come to UNC Hospitals. The visit will take about one hour. • If you consent to sequencing, we will ask you to	*NOTE:* Reference to the one-hour visit in the first bullet and cheek swab in third bullet were verified by Myra from UNC team, 8/1/2015	What if you decide to have genomic sequencing for your child? (headline) *Image: procedural image, showing a doctor, maybe collecting a cheek swab*. Eg. http://i.ytimg.com/vi/zkPQtNrnt8Q/maxresdefault.jpg • 1 hour visit to UNC Hospitals • Sign a consent form • Your baby's spit will be used for sequencing • Learn results for medically actionable childhood conditions	Next button Replay button Q/A [?] button		(3) General content, text plus image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		sign a consent form. • After your baby is born, you will come back to UNC Hospitals. A small sponge will be lightly rubbed inside your baby's mouth to get saliva, or spit, that will be used for sequencing . • After the sequencing is done,		• Complete online surveys **[? – 'DNA']** **[? – 'medically actionable condition']** **[? – 'genomic sequencing']** **[? – 'newborn screening]**			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		you will learn results for medically actionable childhood conditions found by newborn screening, and many other conditions like them. • All parents in the study will complete several online surveys.					
D1.17.Diagnosed	IF DIAGNOSED	What will happen if you decide to have	*NOTE:* Reference to the one-hour visit in the first bullet and cheek swab in	What if you decide to have genomic	Next button Replay button		(3) General content, text plus image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
	COHORT	genomic sequencing for your child? • If you decide you want your child to have genomic sequencing in NC NEXUS, you will come to UNC Hospitals with your child. The visit will take about one hour. • If you consent to sequencing, we will ask you to sign a	third bullet were verified by Myra from UNC team, 8/1/2015	sequencing for your child? (headline) *Image: procedural image, showing a doctor, maybe collecting a cheek swab*. Eg. http://i.ytimg.com/vi/zkPQtNrnt8Q/maxresdefault.jpg • 1 hour visit to UNC Hospitals • Sign a consent form • Your child's spit will be used for sequencing • Learn results for medically actionable childhood conditions	Q/A [?] button		

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		consent form. • A small sponge will be lightly rubbed inside your child's mouth to get saliva, or spit, that will be used for sequencing . • After the sequencing is done, you will learn results for medically actionable childhood conditions found by		• Complete online surveys **[? – 'DNA']** **[? – 'medically actionable condition']** **[? – 'genomic sequencing']** **[? – 'newborn screening]**			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		newborn screening and many other conditions like them. • All parents in the study will complete several online surveys.					
D1.18	ALL	What if genomic sequencing finds that your child has gene differences that cause these conditions? • The results will be confirmed	*NOTE:* The last bullet point about adding results to health record verified by Myra from UNC team, 8/6/2015	What if genomic sequencing finds these conditions? (headline) • Results will be confirmed with another test • A genetic counselor and a doctor will	Next button Replay button Q/A [?] button		(3) General content, text plus image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		with another test. • A genetic counselor and a doctor will meet with you to discuss the results. • You will be referred for medical or other services your child needs for those conditions. • You will be asked if		discuss the results with you. • You will be referred for medical or other services your child needs • Asked if you want the results added to your child's health record **[? – 'genetic counselor']** *NOTE: Numbered list (instead of bullets)*			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		you want the results added to your child's health record at UNC Hospitals.					
D1.19.Single	IF SINGLE	What else should you know if you choose to have genomic sequencing for your child? • You will not be charged for the study		What else should you know? (headline) *Image:* Clinician or someone who looks like they're in charge of something shaking hands • You will not be charged for study visits or genomic sequencing • You will be given a $20	Next button Replay button		(3) or (4) General content, text plus image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		visits or genomic sequencing. You will be given a $20 Visa card after each survey is completed. You will also get parking vouch		Visa card after each survey is completed • You can stop participation if you don't want to continue *NOTE:* Sync visual with audio; move text next to image			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		ers for study visits. At any point in the study, you can stop participation if you don't want to continue. Your child would still receive regular care from doctors as they usually would.					

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.19.Couple	IF COUPLE	What else should you know if you choose to have genomic sequencing for your child? • You will not be charged for the study visits or genomic sequencing. Each parent will be given		What else should you know? (headline) *Image:* Clinician or someone who looks like they're in charge of something shaking hands • You will not be charged for study visits or genomic sequencing • Each parent will be given a $20 Visa card after each survey is completed • You can stop participation if you	Next button Replay button		(3) or (4) General content, text plus image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		a $20 Visa card for each survey they complete. You will also get parking vouchers for study visits. At any point in the study, you can stop		don't want to continue *NOTE:* Sync visual with audio; move text next to image			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		participation if you don't want to continue. Your child would still receive regular care from doctors as they usually would.					

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.20	ALL	Which way are you leaning? If you had to decide right now, which way are you leaning about having genomic sequencing for your child in NC NEXUS? Click and drag the slider, moving it to the point on the scale that fits your answer. Leaning away from having genomic sequencing Not sure		Which way are you leaning? (headline) *Note:* Interactive scale. *NOTE: Example layout here* file://rtints6/hserproj4/0214132%20NEXUS%20Proj%203/Aim%202%20Decision%20Aids/2.1%20DA%20Content/Final%20Decision%20Aid%20Content/working/DA1_slider%20scale%20example.docx Which way are you leaning about having genomic sequencing for your child? Leaning away from having genomic sequencing------- Not sure	Interactive response scale; ~~Submit button;~~ Next button; Q/A [?] button *NOTE: The page needs to fit to browser so the user doesn't need to scroll down to see the slider scale; may need to remove image at top of page.* *NOTE:* Add more vertical space between the question and the slider scale on screen, so that the scale numerical display doesn't cover the question when the user scrolls.	Capture numerical value associated with position on scale. Treat as scale ranging from 0-100, where anchor points are 0 = left-most position, leaning away 50= center position, Not sure 100=right-most position,	(5) Leaning yes/no screen

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		Leaning toward having genomic sequencing When you are done, click the next button to continue.		-------Leaning toward having genomic sequencing **[? – 'genomic sequencing']** *NOTE: Need to show the three anchor labels on screen at all times. To differentiate the slider from the progress at the bottom of screen, make the slider a pentagon instead of a circle, ex.:* Note: Drop custom infographic, no image on screen.		leaning toward Intermediate values captured as integers. Capture time in milliseconds spent on this screen	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.20.a	ALL	The following screens will show you some reasons for and some reasons against having genomic sequencing for your child. Thinking about which reasons matter most to you can help you make a decision.	Intro to the values clarification task	Reasons for and against genomic sequencing (Headline) *Note:* Visual showing a split screen showing an example of a color coded "reason for" sorting task on the left, and a color coded "reason against" sorting task on the right. **[? – 'genomic sequencing']**	Next button Replay button Q/A [?] button		2) How to use the website

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.21.Newborn.Single	IF NEWBORN COHORT & SINGLE	First, tell us if the following reasons *for* your child to have genomic sequencing in NC NEXUS are important or unimportant to you. Please sort these "reasons for" into the boxes labelled *important* or *not important*. You can sort as many or as few reasons into each box as you want. To sort, click the reason and drag it into a box. . • Knowing your child has a genetic condition		Reasons for genomic sequencing (Headline) Sort "reasons for" into the boxes labelled *Important* or *Not important*. *NOTE:* Label the unsorted box: "Unsorted Reason For" • Knowing your child has a genetic condition may help him or her get early treatment and support services.	Sorting task for users to move boxes with 'reasons for' into two bins labeled 'Important' and 'Not important' ~~Submit button~~ Allow participants to continue to next screen only after the statement is moved into a sorting category. Next button	Capture which bin (i.e., important, unimportant) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		may help him or her get early treatment and support services. When you are done sorting, click the next button to move on to the next reason.		*NOTE: Instead of showing the whole list of reasons on screen in the "unsorted" box, would it instead be possible to show them one at a time in sync with the narration? So, narrator starts reading the reason as it appears on screen, at which point the user can sort that reason, then onto the next reason.* After the pre-defined reasons are read and sorted, the five open-textboxes appear on screen *Note: Color code this reason as a*		not sorted into any box (i.e., values for each statement: important, unimportant, not sorted)	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				'reason for' by giving it a green background and/or border. Basically, we need to do something visually to help the user understand that first we're having them sort reasons for, then reasons against. Use the same green as the 'Yes' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.21.Newborn.Single.a	IF NEWBORN COHORT & SINGLE	• Knowing your child has a genetic condition may help you and your family be prepared if he or she develops the condition.		Reasons for genomic sequencing (Headline) Sort "reasons for" into the boxes labelled *Important* or *Not important*. *NOTE:* Label the unsorted box: "Unsorted Reason For" • Knowing your child has a genetic condition may help you and your family be prepared if he or she develops the condition.	Sorting task for users to move boxes with 'reasons for' into two bins labeled 'Important' and 'Not important' ~~Submit button~~ Allow participants to continue to next screen only after the statement is moved into a sorting category. Next button	Capture which bin (i.e., important, unimportant) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				Note: Color code this reason as a 'reason for' by giving it a green background and/or border. Use the same green as the 'Yes' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes		not sorted into any box (i.e., values for each statement : important, unimportant, not sorted)	
D1.21.Newborn.Single.b	IF NEWBORN COHORT & SINGLE	• Genomic sequencing may help doctors understand genetic conditions better.		Reasons for genomic sequencing (Headline) Sort "reasons for" into the boxes labelled *Important* or *Not important*. *NOTE:* Label the unsorted box: "Unsorted Reason For"	Sorting task for users to move boxes with 'reasons for' into <u>two</u> bins labeled 'Important' and 'Not important' ~~Submit button~~ Allow participants to continue to next screen only after the statement is moved into a sorting category. Next button	Capture which bin (i.e., important, unimportant) user sorts each 'reason for' Capture text user types into interactive text box	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				• Genomic sequencing may help doctors understand genetic conditions better. *Note: Color code this reason as a 'reason for' by giving it a green background and/or border. Use the same green as the 'Yes' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes*		Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it not sorted into any box (i.e., values for each statement: important, unimportant, not sorted)	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.21.Newborn.Single.c	IF NEWBORN COHORT & SINGLE	• Genomic sequencing may help scientists make better tools for finding serious conditions before people get sick.		Reasons for genomic sequencing (Headline) Sort "reasons for" into the boxes labelled *Important* or *Not important*. *NOTE:* Label the unsorted box: "Unsorted Reason For" • Genomic sequencing may help scientists make better tools for finding serious conditions before people get sick.	Sorting task for users to move boxes with 'reasons for' into two bins labeled 'Important' and 'Not important' ~~Submit button~~ Allow participants to continue to next screen only after the statement is moved into a sorting category. Next button	Capture which bin (i.e., important, unimportant) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				Note: Color code this reason as a 'reason for' by giving it a green background and/or border. Use the same green as the 'Yes' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes		not sorted into any box (i.e., values for each statement: important, unimportant, not sorted)	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.21.Newborn.Single.d	IF NEWBORN COHORT & SINGLE	• You would rather not wait to see if any problems occur to find out if your child may have a genetic condition.		Reasons for genomic sequencing (Headline) Sort "reasons for" into the boxes labelled *Important* or *Not important*. *NOTE:* Label the unsorted box: "Unsorted Reason For" • You would rather not wait to see if any problems occur to find out if your child may have a genetic condition. *Note: Color code this reason as a*	Sorting task for users to move boxes with 'reasons for' into two bins labeled 'Important' and 'Not important' ~~Submit button~~ Allow participants to continue to next screen only after the statement is moved into a sorting category. Next button	Capture which bin (i.e., important, unimportant) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				'reason for' by giving it a green background and/or border. Use the same green as the 'Yes' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes		not sorted into any box (i.e., values for each statement: important, unimportant, not sorted)	
D1.21.Newborn.Single.e	IF NEWBORN COHORT & SINGLE	*Are there any other reasons you can think of? Please type them in the text boxes labelled "Add reason"* When you are done sorting, click the next button to continue.		Reasons for genomic sequencing (Headline) Sort "reasons for" into the boxes labelled *Important* or *Not important*. *NOTE:* Label the unsorted box: "Unsorted Reason For" *Are there any other reasons you can think of?*	Sorting task for users to move boxes with 'reasons for' into <u>two</u> bins labeled 'Important' and 'Not important' ~~Submit button~~ 5 interactive textboxes that allows users to write in 5 additional 'reasons for' that is not listed; write-in textboxes are also sortable Next button	Capture which bin (i.e., important, unimportant) user sorts each 'reason for' Capture text user types into interactive text box Capture time in millisecon	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				• *Add reason (x5)* *NOTE: Change label from 'Add custom reason' → 'Add reason'* *Note: Color code this reason as a 'reason for' by giving it a green background and/or border. Use the same green as the 'Yes' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes*		ds spent on this screen Capture which box each statement was sorted into or if it not sorted into any box (i.e., values for each statement : important, unimportant, not sorted)	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.21.Diagnosed. Single	IF DIAGNOSED COHORT & SINGLE	First, tell us if the following reasons *for* your child to have genomic sequencing in NC NEXUS are important or unimportant to you. Please sort these "reasons for" into the boxes labelled *important* or *not important*. You can sort as many or as few reasons into each box as you want. To sort, click the reason and drag it into a box. • Genomic sequencing may help doctors		Reasons for genomic sequencing (Headline) Sort "reasons for" into the boxes labelled *Important* or *Not important*. *NOTE:* Label the unsorted box: "Unsorted Reason For" • Genomic sequencing may help doctors understand your child's condition better. *Note: Color code this reason as a 'reason for' by giving it a green background and/or border. Use the same green as the*	Sorting task for users to move boxes with 'reasons for' into two bins labeled 'Important' and 'Not important' Allow participants to continue to next screen only after the statement is moved into a sorting category. ~~Submit button~~ Next button	Capture which bin (i.e., important, unimportant) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		understand your child's condition better. When you are done sorting, click the next button to move on to the next reason.		*'Yes' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes*		not sorted into any box (i.e., values for each statement: important, unimportant, not sorted)	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.21.Diagnosed.Single.a	IF DIAGNOSED COHORT & SINGLE	• Genomic sequencing for your child may provide information about the risk for others in your family of having a child with the same condition.		Reasons for genomic sequencing (Headline) Sort "reasons for" into the boxes labelled *Important* or *Not important*. *NOTE:* Label the unsorted box: "Unsorted Reason For" • Genomic sequencing for your child may provide information about the risk for others in your family of having a child with the same condition. *Note: Color code this reason as a 'reason for' by*	Sorting task for users to move boxes with 'reasons for' into two bins labeled 'Important' and 'Not important' Allow participants to continue to next screen only after the statement is moved into a sorting category. ~~Submit button~~ Next button	Capture which bin (i.e., important, unimportant) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				giving it a green background and/or border. Use the same green as the 'Yes' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes		not sorted into any box (i.e., values for each statement: important, unimportant, not sorted)	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.21.Diagnosed.Single.b	IF DIAGNOSED COHORT & SINGLE	• Knowing the genetic cause of your child's condition could help your family plan for the future.		Reasons for genomic sequencing (Headline) Sort "reasons for" into the boxes labelled *Important* or *Not important*. *NOTE:* Label the unsorted box: "Unsorted Reason For" • Knowing the genetic cause of your child's condition could help your family plan for the future. *Note: Color code this reason as a 'reason for' by giving it a green background and/or border. Use the*	Sorting task for users to move boxes with 'reasons for' into two bins labeled 'Important' and 'Not important' Allow participants to continue to next screen only after the statement is moved into a sorting category. ~~Submit button~~ Next button	Capture which bin (i.e., important, unimportant) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				same green as the 'Yes' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes		not sorted into any box (i.e., values for each statement: important, unimportant, not sorted)	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.21.Diagnosed.Single.c	IF DIAGNOSED COHORT & SINGLE	• Genomic sequencing may help scientists make better tools for finding serious conditions before people get sick.		Reasons for genomic sequencing (Headline) Sort "reasons for" into the boxes labelled *Important* or *Not important*. *NOTE:* Label the unsorted box: "Unsorted Reason For" • Genomic sequencing may help scientists make better tools for finding serious conditions before people get sick. *Note: Color code this reason as a 'reason for' by giving it a green background and/or border. Use the same green as the*	Sorting task for users to move boxes with 'reasons for' into two bins labeled 'Important' and 'Not important' Allow participants to continue to next screen only after the statement is moved into a sorting category. ~~Submit button~~ Next button	Capture which bin (i.e., important, unimportant) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				'Yes' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes		not sorted into any box (i.e., values for each statement: important, unimportant, not sorted)	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.21.Diagnosed.Single.d	IF DIAGNOSED COHORT & SINGLE	• You want to learn anything you can about your child's condition.		Reasons for genomic sequencing (Headline) Sort "reasons for" into the boxes labelled *Important* or *Not important*. *NOTE:* Label the unsorted box: "Unsorted Reason For" • You want to learn anything you can about your child's condition. *Note: Color code this reason as a 'reason for' by giving it a green background and/or border. Use the same green as the 'Yes' of yes/no buttons. Do not color code the*	Sorting task for users to move boxes with 'reasons for' into two bins labeled 'Important' and 'Not important' Allow participants to continue to next screen only after the statement is moved into a sorting category. ~~Submit button~~ Next button	Capture which bin (i.e., important, unimportant) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				'Important' or 'Not important' boxes		not sorted into any box (i.e., values for each statement: important, unimportant, not sorted)	
D1.21.Diagnosed.Single.e	IF DIAGNOSED COHORT & SINGLE	*Are there any other reasons you can think of? Please type them in the text boxes labelled "Add reason"*		Reasons for genomic sequencing (Headline) Sort "reasons for" into the boxes labelled *Important* or *Not important*.	Sorting task for users to move boxes with 'reasons for' into <u>two</u> bins labeled 'Important' and 'Not important 5 interactive textboxes that allows users to write in 5 additional 'reasons	Capture which bin (i.e., important, unimportant) user sorts each 'reason for'	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		When you are done sorting, click the next button to continue.		*NOTE:* Label the unsorted box: "Unsorted Reason For" *Are there any other reasons you can think of?* • *Add reason (x5)* *NOTE: Change label from 'Add custom reason' → 'Add reason'* *Note: Color code this reason as a 'reason for' by giving it a green background and/or border. Use the same green as the 'Yes' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes*	for' that is not listed; write-in textboxes are also sortable Next button	Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it not sorted into any box (i.e., values for each statement: important, unimporta	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
						nt, not sorted)	
D1.21.Newborn.Couple	IF NEWBORN COHORT & COUPLE	First, tell us if the following reasons *for* your child to have genomic sequencing in NC NEXUS are important or unimportant to you. Please sort these "reasons for" into the boxes labelled *important* or *not important*. If you and your partner disagree about the importance of a reason, you can move it into the box		Reasons for genomic sequencing (Headline) Sort "reasons for" into the boxes labelled *Important, Not important, or We disagree*. *NOTE:* Label the unsorted box: "Unsorted Reason For" • Knowing your child has a genetic condition may help him or her get early treatment and support services.	Sorting task for users to move boxes with 'reasons for' into three bins labeled 'Important,' 'Not important,' and 'We disagree' Allow participants to continue to next screen only after the statement is moved into a sorting category. ~~Submit button~~ Next button	Capture which bin (i.e., important, unimportant, disagree) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		labelled *we disagree*. You can sort as many or as few reasons into each box as you want. To sort, click the reason and drag it into a box. • Knowing your child has a genetic condition may help him or her get early treatment and support services. When you are done sorting, click the next button to move on to		*Note: Color code this reason as a 'reason for' by giving it a green background and/or border. Use the same green as the 'Yes' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes*		Capture which box each statement was sorted into or if it not sorted into any box	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		the next reason.					

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.21.Newborn.Couple.a	IF NEWBORN COHORT & COUPLE	• Knowing your child has a genetic condition may help you and your family be prepared if he or she develops the condition.		Reasons for genomic sequencing (Headline) Sort "reasons for" into the boxes labelled *Important, Not important, or We disagree*. *NOTE:* Label the unsorted box: "Unsorted Reason For" • Knowing your child has a genetic condition may help you and your family be prepared if he or she develops the condition. *Note: Color code this reason as a 'reason for' by giving it a green*	Sorting task for users to move boxes with 'reasons for' into three bins labeled 'Important,' 'Not important,' and 'We disagree' Allow participants to continue to next screen only after the statement is moved into a sorting category. ~~Submit button~~ Next button	Capture which bin (i.e., important, unimportant, disagree) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				background and/or border. Use the same green as the 'Yes' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes		sorted into or if it not sorted into any box	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.21.Newborn.Couple.b	IF NEWBORN COHORT & COUPLE	• Genomic sequencing may help doctors understand genetic conditions better.		Reasons for genomic sequencing (Headline) Sort "reasons for" into the boxes labelled *Important, Not important, or We disagree*. *NOTE:* Label the unsorted box: "Unsorted Reason For" • Genomic sequencing may help doctors understand genetic conditions better. *Note: Color code this reason as a 'reason for' by giving it a green background and/or border. Use the*	Sorting task for users to move boxes with 'reasons for' into <u>three</u> bins labeled 'Important,' 'Not important,' and 'We disagree' Allow participants to continue to next screen only after the statement is moved into a sorting category. Next button	Capture which bin (i.e., important, unimportant, disagree) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				same green as the 'Yes' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes		sorted into or if it not sorted into any box	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.21.Newborn.Couple.c	IF NEWBORN COHORT & COUPLE	• Genomic sequencing may help scientists make better tools for finding serious conditions before people get sick.		Reasons for genomic sequencing (Headline) Sort "reasons for" into the boxes labelled *Important, Not important, or We disagree*. *NOTE:* Label the unsorted box: "Unsorted Reason For" • Genomic sequencing may help scientists make better tools for finding serious conditions before people get sick. *Note: Color code this reason as a 'reason for' by giving it a green background and/or*	Sorting task for users to move boxes with 'reasons for' into three bins labeled 'Important,' 'Not important,' and 'We disagree' Allow participants to continue to next screen only after the statement is moved into a sorting category. ~~Submit button~~ Next button	Capture which bin (i.e., important, unimportant, disagree) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				border. Use the same green as the 'Yes' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes		sorted into or if it not sorted into any box	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.21.Newborn.Couple.d	IF NEWBORN COHORT & COUPLE	• You would rather not wait to see if any problems occur to find out if your child may to have a genetic condition.		Reasons for genomic sequencing (Headline) Sort "reasons for" into the boxes labelled *Important, Not important, or We disagree*. *NOTE:* Label the unsorted box: "Unsorted Reason For" • You would rather not wait to see if any problems occur to find out if your child may to have a genetic condition. *Note: Color code this reason as a 'reason for' by giving it a green background and/or border. Use*	Sorting task for users to move boxes with 'reasons for' into three bins labeled 'Important,' 'Not important,' and 'We disagree' Allow participants to continue to next screen only after the statement is moved into a sorting category. ~~Submit button~~ Next button	Capture which bin (i.e., important, unimportant, disagree) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				the same green as the 'Yes' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes		sorted into or if it not sorted into any box	
D1.21.Newborn.Couple.e	IF NEWBORN COHORT & COUPLE	*Are there any other reasons you can think of? Please type them in the text boxes labelled "Add reason"* When you are done		Reasons for genomic sequencing (Headline) Sort "reasons for" into the boxes labelled *Important, Not important, or We disagree*. *NOTE:* Label the unsorted box:	Sorting task for users to move boxes with 'reasons for' into <u>three</u> bins labeled 'Important,' 'Not important,' and 'We disagree' 5 interactive textboxes that allows users to write in 5 additional 'reasons for' that is not listed; write-in textboxes are also sortable	Capture which bin (i.e., important, unimportant, disagree) user sorts each 'reason for'	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		sorting, click the next button to continue.		"Unsorted Reason For" *Are there any other reasons you can think of?* • *Add reason (x5)* *NOTE: Change label from 'Add custom reason' → 'Add reason'* *Note: Color code this reason as a 'reason for' by giving it a green background and/or border. Use the same green as the 'Yes' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes*	Next button	Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it not sorted into any box (i.e., values for each statement: important, unimportant,	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
						disagree, not sorted)	

D1.21.Diagnosed. Couple	IF DIAGNOSED COHORT & COUPLE	First, tell us if the following reasons *for* your child to have genomic sequencing in NC NEXUS are important or unimportant to you. Please sort these "reasons for" into the boxes labelled *important* or *not important*. If you and your partner disagree about the importance of a reason, you can move it into the box labelled *we disagree*. You can sort as many or as few reasons into each box as you want. To sort, click		Reasons for genomic sequencing (Headline) Sort "reasons for" into the boxes labelled *Important, Not important, or We disagree*. *NOTE:* Label the unsorted box: "Unsorted Reason For" • Genomic sequencing may help doctors understand your child's condition better. *Note: Color code this reason as a 'reason for' by giving it a green background and/or border. Use the same green as the 'Yes' of yes/no*	Sorting task for users to move boxes with 'reasons for' into three bins labeled 'Important,' 'Not important,' and 'We disagree' Allow participants to continue to next screen only after the statement is moved into a sorting category. Next button	Capture which bin (i.e., important, unimportant, disagree) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it	(6) Values clarification, input

		the reason and drag it into a box. • Genomic sequencing may help doctors understand your child's condition better. When you are done sorting, click the next button to move on to the next reason.		*buttons. Do not color code the 'Important' or 'Not important' boxes*		not sorted into any box (i.e., values for each statement: important, unimportant, disagree, not sorted)	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.21.Diagnosed.Couple.a	IF DIAGNOSED COHORT & COUPLE	• Genomic sequencing for your child may provide information about the risk for others in your family of having a child with the same condition.		Reasons for genomic sequencing (Headline) Sort "reasons for" into the boxes labelled *Important, Not important, or We disagree*. *NOTE:* Label the unsorted box: "Unsorted Reason For" • Genomic sequencing for your child may provide information about the risk for others in your family of having a child with the same condition. *Note: Color code this reason as a*	Sorting task for users to move boxes with 'reasons for' into <u>three</u> bins labeled 'Important,' 'Not important,' and 'We disagree' Allow participants to continue to next screen only after the statement is moved into a sorting category. Next button	Capture which bin (i.e., important, unimportant, disagree) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				'reason for' by giving it a green background and/or border. Use the same green as the 'Yes' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes		sorted into or if it not sorted into any box (i.e., values for each statement : important, unimportant, disagree, not sorted)	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.21.Diagnosed.Couple.b	IF DIAGNOSED COHORT & COUPLE	• Knowing the genetic cause of your child's condition could help your family plan for the future.		Reasons for genomic sequencing (Headline) Sort "reasons for" into the boxes labelled *Important, Not important, or We disagree*. *NOTE:* Label the unsorted box: "Unsorted Reason For" • Knowing the genetic cause of your child's condition could help your family plan for the future. *Note: Color code this reason as a 'reason for' by giving it a green background and/or*	Sorting task for users to move boxes with 'reasons for' into three bins labeled 'Important,' 'Not important,' and 'We disagree' Allow participants to continue to next screen only after the statement is moved into a sorting category. Next button	Capture which bin (i.e., important, unimportant, disagree) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				border. Use the same green as the 'Yes' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes		sorted into or if it not sorted into any box (i.e., values for each statement : important, unimportant, disagree, not sorted)	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.21.Diagnosed.Couple.c	IF DIAGNOSED COHORT & COUPLE	• Genomic sequencing may help scientists make better tools for finding serious conditions before people get sick.		Reasons for genomic sequencing (Headline) Sort "reasons for" into the boxes labelled *Important, Not important, or We disagree*. *NOTE:* Label the unsorted box: "Unsorted Reason For" • Genomic sequencing may help scientists make better tools for finding serious conditions before people get sick. *Note: Color code this reason as a 'reason for' by giving it a green background and/or*	Sorting task for users to move boxes with 'reasons for' into <u>three</u> bins labeled 'Important,' 'Not important,' and 'We disagree' Allow participants to continue to next screen only after the statement is moved into a sorting category. Next button	Capture which bin (i.e., important, unimportant, disagree) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				border. Use the same green as the 'Yes' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes		sorted into or if it not sorted into any box (i.e., values for each statement : important, unimportant, disagree, not sorted)	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.21.Diagnosed.Couple.d	IF DIAGNOSED COHORT & COUPLE	• You want to learn anything you can about your child's condition.		Reasons for genomic sequencing (Headline) Sort "reasons for" into the boxes labelled *Important, Not important, or We disagree*. *NOTE:* Label the unsorted box: "Unsorted Reason For" • You want to learn anything you can about your child's condition. *Note: Color code this reason as a 'reason for' by giving it a green background and/or border. Use the same green as the 'Yes' of yes/no buttons. Do not*	Sorting task for users to move boxes with 'reasons for' into <u>three</u> bins labeled 'Important,' 'Not important,' and 'We disagree' Allow participants to continue to next screen only after the statement is moved into a sorting category. Next button	Capture which bin (i.e., important, unimportant, disagree) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				color code the 'Important' or 'Not important' boxes		sorted into or if it not sorted into any box (i.e., values for each statement : important, unimportant, disagree, not sorted)	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.21.Diagnosed.Couple.e	IF DIAGNOSED COHORT & COUPLE	*Are there any other reasons you can think of? Please type them in the text boxes labelled "Add reason"* When you are done sorting, click the next button to continue.		Reasons for genomic sequencing (Headline) Sort "reasons for" into the boxes labelled *Important, Not important, or We disagree*. *NOTE:* Label the unsorted box: "Unsorted Reason For" *Are there any other reasons you can think of?* • *Add reason (x5)* *NOTE: Change label from 'Add custom reason' → 'Add reason'* *Note: Color code this reason as a 'reason for' by*	Sorting task for users to move boxes with 'reasons for' into three bins labeled 'Important,' 'Not important,' and 'We disagree' 5 interactive textboxes that allows users to write in 5 additional 'reasons for' that is not listed; write-in textboxes are also sortable Next button	Capture which bin (i.e., important, unimportant, disagree) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				giving it a green background and/or border. Use the same green as the 'Yes' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes		sorted into or if it not sorted into any box (i.e., values for each statement : important, unimportant, disagree, not sorted)	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.22.Newborn.Single	IF NEWBORN COHORT& SINGLE	Now we would like you to tell us if the following reasons against your child having genomic sequencing are important or unimportant to you. Please sort these "reasons against" into the boxes labelled *important* or *not important*. • Waiting for genomic sequencing results may cause you to worry or feel anxious.		Reasons against genomic sequencing. (Headline) Sort "reasons against" into the boxes labelled *Important* or *Not important*. *NOTE:* Label the unsorted box: "Unsorted Reason Against" • Waiting for genomic sequencing results may cause you to worry or feel anxious.	Sorting task for users to move boxes with 'reasons against' into two bins labeled 'Important' and 'Not important' Allow participants to continue to next screen only after at least one statement is moved into a sorting category. Next button	Capture which bin (i.e., important, unimportant) user sorts each 'reason against' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		When you are done sorting, click the next button to move on to the next reason.		*Note: Color code this reason as a 'reason against' by giving it an orange background and/or border. Use the same orange as the 'No' of yes/no buttons. Basically, we need to do something visually to help the user understand that first we're having them sort reasons for, then reasons against. Do not color code the 'Important' or 'Not important' boxes*		not sorted into any box	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.22.Newborn.Single.a	IF NEWBORN COHORT& SINGLE	• You do not feel prepared to learn that your child may have a genetic condition.		Reasons against genomic sequencing. (Headline) Sort "reasons against" into the boxes labelled *Important* or *Not important*. *NOTE:* Label the unsorted box: "Unsorted Reason Against" • You do not feel prepared to learn that your child may have a genetic condition. *Note: Color code this reason as a*	Sorting task for users to move boxes with 'reasons against' into two bins labeled 'Important' and 'Not important' Allow participants to continue to next screen only after at least one statement is moved into a sorting category. Next button	Capture which bin (i.e., important, unimportant) user sorts each 'reason against' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				'reason against' by giving it an orange background and/or border. Use the same orange as the 'No' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes		not sorted into any box	
D1.22.Newborn.Single.b	IF NEWBORN COHORT& SINGLE	• Knowing that the NC NEXUS study team will have your child's genomic sequencing results makes you uncomfortable.		Reasons against genomic sequencing. (Headline) Sort "reasons against" into the boxes labelled *Important* or *Not important*. *NOTE:* Label the unsorted box: "Unsorted Reason Against" • Knowing that the NC	Sorting task for users to move boxes with 'reasons against' into <u>two</u> bins labeled 'Important' and 'Not important' Allow participants to continue to next screen only after at least one statement is moved into a sorting category. Next button	Capture which bin (i.e., important, unimportant) user sorts each 'reason against' Capture text user types into interactive text box Capture time in millisecon	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				NEXUS study team will have your child's genomic sequencing results makes you uncomfortable. *Note: Color code this reason as a 'reason against' by giving it an orange background and/or border. Use the same orange as the 'No' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes*		ds spent on this screen Capture which box each statement was sorted into or if it not sorted into any box	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.22.Newborn.Single.c	IF NEWBORN COHORT& SINGLE	• You are satisfied with knowing that your child will have standard newborn screening.		Reasons against genomic sequencing. (Headline) Sort "reasons against" into the boxes labelled *Important* or *Not important*. *NOTE:* Label the unsorted box: "Unsorted Reason Against" • You are satisfied with knowing that your child will have standard newborn screening.	Sorting task for users to move boxes with 'reasons against' into <u>two</u> bins labeled 'Important' and 'Not important' Allow participants to continue to next screen only after at least one statement is moved into a sorting category. Next button	Capture which bin (i.e., important, unimportant) user sorts each 'reason against' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				Note: Color code this reason as a 'reason against' by giving it an orange background and/or border. Use the same orange as the 'No' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes		not sorted into any box	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.22.Newborn.Single.d	IF NEWBORN COHORT& SINGLE	• You would rather wait to see if your child has any problems before having genetic testing.		Reasons against genomic sequencing. (Headline) Sort "reasons against" into the boxes labelled *Important* or *Not important*. *NOTE:* Label the unsorted box: "Unsorted Reason Against" • You would rather wait to see if your child has any problems before having genetic testing.	Sorting task for users to move boxes with 'reasons against' into two bins labeled 'Important' and 'Not important' Allow participants to continue to next screen only after at least one statement is moved into a sorting category. Next button	Capture which bin (i.e., important, unimportant) user sorts each 'reason against' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				Note: Color code this reason as a 'reason against' by giving it an orange background and/or border. Use the same orange as the 'No' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes		not sorted into any box	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.22.Newborn.Single.e	IF NEWBORN COHORT& SINGLE	*Are there any other reasons against having genomic sequencing that you can think of? Please type them in the text boxes labelled "Add reason"* When you are done sorting, click the next button to continue.		Reasons against genomic sequencing. (Headline) Sort "reasons against" into the boxes labelled *Important* or *Not important*. *NOTE:* Label the unsorted box: "Unsorted Reason Against" *Are there any other reasons you can think of?* • *Add reason (x5)* *NOTE: Change label from 'Add custom reason' → 'Add reason'* *Note: Color code this reason as a*	Sorting task for users to move boxes with 'reasons against' into <u>two</u> bins labeled 'Important' and 'Not important' Interactive textbox that allows users to write in up to 5 'reason against' not listed; write-in textbox is also sortable Next button	Capture which bin (i.e., important, unimportant) user sorts each 'reason against' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				'reason against' by giving it an orange background and/or border. Use the same orange as the 'No' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes		not sorted into any box	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.22.Diagnosed. Single	IF DIAGNOSED COHORT & SINGLE	Now we would like you to tell us if the following reasons against your child having genomic sequencing are important or unimportant to you. Please sort these "reasons against" into the boxes labelled *important* or *not important*. • Waiting for genomic sequencing results may cause you to worry or feel anxious.		Reasons against genomic sequencing. (Headline) Sort "reasons against" into the boxes labelled *Important* or *Not important*. *NOTE:* Label the unsorted box: "Unsorted Reason Against" • Waiting for genomic sequencing results may cause you to worry or feel anxious. *Note: Color code this reason as a 'reason against' by*	Sorting task for users to move boxes with 'reasons against' into two bins labeled 'Important' and 'Not important' Allow participants to continue to next screen only after at least one statement is moved into a sorting category. Next button	Capture which bin (i.e., important, unimportant) user sorts each 'reason against' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		When you are done sorting, click the next button to move on to the next reason.		*giving it an orange background and/or border. Use the same orange as the 'No' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes*		not sorted into any box	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.22.Diagnosed.Single.a	IF DIAGNOSED COHORT & SINGLE	• You do not feel prepared to learn that your child may have another health problem.		Reasons against genomic sequencing. (Headline) Sort "reasons against" into the boxes labelled *Important* or *Not important*. *NOTE:* Label the unsorted box: "Unsorted Reason Against" • You do not feel prepared to learn that your child may have another health problem. *Note: Color code this reason as a 'reason against' by giving it an orange*	Sorting task for users to move boxes with 'reasons against' into two bins labeled 'Important' and 'Not important' Allow participants to continue to next screen only after at least one statement is moved into a sorting category. Next button	Capture which bin (i.e., important, unimportant) user sorts each 'reason against' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				background and/or border. Use the same orange as the 'No' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes		not sorted into any box	
D1.22.Diagnosed.Single.b	IF DIAGNOSED COHORT & SINGLE	• Knowing that the NC NEXUS study team will have your child's genomic sequencing results makes you uncomfortable.		Reasons against genomic sequencing. (Headline) Sort "reasons against" into the boxes labelled *Important* or *Not important*. *NOTE:* Label the unsorted box: "Unsorted Reason Against"	Sorting task for users to move boxes with 'reasons against' into <u>two</u> bins labeled 'Important' and 'Not important' Allow participants to continue to next screen only after at least one statement is moved into a sorting category. Next button	Capture which bin (i.e., important, unimportant) user sorts each 'reason against' Capture text user types into interactive text box	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				• Knowing that the NC NEXUS study team will have your child's genomic sequencing results makes you uncomfortable. *Note: Color code this reason as a 'reason against' by giving it an orange background and/or border. Use the same orange as the 'No' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes*		Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it not sorted into any box	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.22.Diagnosed.Single.c	IF DIAGNOSED COHORT & SINGLE	• You are satisfied with the medical care your child receives and don't think other information would be helpful.		Reasons against genomic sequencing. (Headline) Sort "reasons against" into the boxes labelled *Important* or *Not important*. *NOTE:* Label the unsorted box: "Unsorted Reason Against" • You are satisfied with the medical care your child receives and don't think other information would be helpful. *Note: Color code this reason as a 'reason against' by giving it an orange*	Sorting task for users to move boxes with 'reasons against' into two bins labeled 'Important' and 'Not important' Allow participants to continue to next screen only after at least one statement is moved into a sorting category. Next button	Capture which bin (i.e., important, unimportant) user sorts each 'reason against' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				background and/or border. Use the same orange as the 'No' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes		not sorted into any box	
D1.22.Diagnosed.Single.d	IF DIAGNOSED COHORT & SINGLE	• You would rather wait to see if your child has any problems before having genetic testing		Reasons against genomic sequencing. (Headline) Sort "reasons against" into the boxes labelled *Important* or *Not important*. *NOTE:* Label the unsorted box: "Unsorted Reason Against"	Sorting task for users to move boxes with 'reasons against' into <u>two</u> bins labeled 'Important' and 'Not important' Allow participants to continue to next screen only after at least one statement is moved into a sorting category. Next button	Capture which bin (i.e., important, unimportant) user sorts each 'reason against' Capture text user types into interactive text box	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				• You would rather wait to see if your child has any problems before having genetic testing *Note: Color code this reason as a 'reason against' by giving it an orange background and/or border. Use the same orange as the 'No' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes*		Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it not sorted into any box	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.22.Diagnosed.Single.e	IF DIAGNOSED COHORT & SINGLE	*Are there any other reasons against having genomic sequencing that you can think of? Please type them in the text boxes labelled "Add reason"* When you are done sorting, click the next button to continue.		Reasons against genomic sequencing. (Headline) Sort "reasons against" into the boxes labelled *Important* or *Not important*. *NOTE:* Label the unsorted box: "Unsorted Reason Against" *Are there any other reasons you can think of?* • *Add reason (x5)* *NOTE: Change label from 'Add custom reason' → 'Add reason'*	Sorting task for users to move boxes with 'reasons against' into <u>two</u> bins labeled 'Important' and 'Not important' Interactive textbox that allows users to write in up to 5 'reason against' not listed; write-in textbox is also sortable Next button	Capture which bin (i.e., important, unimportant) user sorts each 'reason against' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				Note: Color code this reason as a 'reason against' by giving it an orange background and/or border. Use the same orange as the 'No' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes		not sorted into any box	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.22.Newborn.Couple	IF NEWBORN COHORT & COUPLE	Now we would like you to tell us if the following reasons against your child having genomic sequencing are important or unimportant to you. Please sort these "reasons against" into the boxes labelled *important*, *not important,* or *we disagree*. • Waiting for genomic sequencing results may cause you to worry or		Reasons against genomic sequencing. (Headline) Sort "reasons against" into the boxes labelled *Important, Not important, or We disagree*. *NOTE:* Label the unsorted box: "Unsorted Reason Against" • Waiting for genomic sequencing results may cause you to worry or feel anxious. *Note: Color code this reason as a 'reason against' by*	Sorting task for users to move boxes with 'reasons for' into <u>three</u> bins labeled 'Important,' 'Not important,' and 'We disagree' Allow participants to continue to next screen only after at least one statement is moved into a sorting category. Next button	Capture which bin i.e., important, unimportant, disagree) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		feel anxious. When you are done sorting, click the next button to move on to the next reason.		*giving it an orange background and/or border. Use the same orange as the 'No' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes*		sorted into or if it not sorted into any box	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.22.Newborn.Couple.a	IF NEWBORN COHORT & COUPLE	• You do not feel prepared to learn that your child may have a genetic condition.		Reasons against genomic sequencing. (Headline) Sort "reasons against" into the boxes labelled *Important, Not important, or We disagree*. *NOTE:* Label the unsorted box: "Unsorted Reason Against" • You do not feel prepared to learn that your child may have a genetic condition. *Note: Color code this reason as a 'reason against' by giving it an orange*	Sorting task for users to move boxes with 'reasons for' into three bins labeled 'Important,' 'Not important,' and 'We disagree' Allow participants to continue to next screen only after at least one statement is moved into a sorting category. Next button	Capture which bin i.e., important, unimportant, disagree) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				background and/or border. Use the same orange as the 'No' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes		sorted into or if it not sorted into any box	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.22.Newborn.Couple.b	IF NEWBORN COHORT & COUPLE	• Knowing that the NC NEXUS study team will have your child's genomic sequencing results makes you uncomfortable.		Reasons against genomic sequencing. (Headline) Sort "reasons against" into the boxes labelled *Important, Not important, or We disagree*. *NOTE:* Label the unsorted box: "Unsorted Reason Against" • Knowing that the NC NEXUS study team will have your child's genomic sequencing results makes you uncomfortable.	Sorting task for users to move boxes with 'reasons for' into three bins labeled 'Important,' 'Not important,' and 'We disagree' Allow participants to continue to next screen only after at least one statement is moved into a sorting category. Next button	Capture which bin i.e., important, unimportant, disagree) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				Note: Color code this reason as a 'reason against' by giving it an orange background and/or border. Use the same orange as the 'No' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes		sorted into or if it not sorted into any box	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.22.Newborn.Couple.c	IF NEWBORN COHORT & COUPLE	• You are satisfied with knowing that your child will have standard newborn screening.		Reasons against genomic sequencing. (Headline) Sort "reasons against" into the boxes labelled *Important, Not important, or We disagree*. *NOTE:* Label the unsorted box: "Unsorted Reason Against" • You are satisfied with knowing that your child will have standard newborn screening. *Note: Color code this reason as a*	Sorting task for users to move boxes with 'reasons for' into <u>three</u> bins labeled 'Important,' 'Not important,' and 'We disagree' Allow participants to continue to next screen only after at least one statement is moved into a sorting category. Next button	Capture which bin i.e., important, unimportant, disagree) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				'reason against' by giving it an orange background and/or border. Use the same orange as the 'No' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes		sorted into or if it not sorted into any box	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.22.Newborn.Couple.d	IF NEWBORN COHORT & COUPLE	• You would rather wait to see if your child has any problems before having genetic testing.		Reasons against genomic sequencing. (Headline) Sort "reasons against" into the boxes labelled *Important, Not important, or We disagree*. *NOTE:* Label the unsorted box: "Unsorted Reason Against" • You would rather wait to see if your child has any problems before having genetic testing. *Note: Color code this reason as a 'reason against' by*	Sorting task for users to move boxes with 'reasons for' into three bins labeled 'Important,' 'Not important,' and 'We disagree' Allow participants to continue to next screen only after at least one statement is moved into a sorting category. Next button	Capture which bin i.e., important, unimportant, disagree) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				giving it an orange background and/or border. Use the same orange as the 'No' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes		sorted into or if it not sorted into any box	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.22.Newborn.Couple.e	IF NEWBORN COHORT & COUPLE	*Are there any other reasons against having genomic sequencing that you can think of? Please type them in the text boxes labelled "Add reason"* When you are done sorting, click the next button to continue.		Reasons against genomic sequencing. (Headline) Sort "reasons against" into the boxes labelled *Important, Not important, or We disagree*. *NOTE:* Label the unsorted box: "Unsorted Reason Against" *Are there any other reasons you can think of?* • *Add reason (x5)* *NOTE: Change label from 'Add custom reason' → 'Add reason'*	Sorting task for users to move boxes with 'reasons for' into three bins labeled 'Important,' 'Not important,' and 'We disagree' 5 interactive textboxes that allows users to write in 5 additional 'reasons for' that is not listed; write-in textboxes are also sortable Next button	Capture which bin i.e., important, unimportant, disagree) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				NOTE: Please underline the word "not" in the headline to help emphasize the distinction between this and the reasons for task. *Note: Color code this reason as a 'reason against' by giving it an orange background and/or border. Use the same orange as the 'No' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes*		sorted into or if it not sorted into any box	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.22.Diagnosed. Couple	IF DIAGNOSED COHORT & COUPLE	Now we would like you to tell us if the following reasons against your child having genomic sequencing are important or unimportant to you. Please sort these "reasons against" into the boxes labelled *important*, *not important,* or *we disagree*. • Waiting for genomic sequencing results may cause you to worry or		Reasons against genomic sequencing. (Headline) Sort "reasons against" into the boxes labelled *Important, Not important, or We disagree*. *NOTE:* Label the unsorted box: "Unsorted Reason Against" • Waiting for genomic sequencing results may cause you to worry or feel anxious. *Note: Color code this reason as a 'reason against' by*	Sorting task for users to move boxes with 'reasons for' into <u>three</u> bins labeled 'Important,' 'Not important,' and 'We disagree' Allow participants to continue to next screen only after at least one statement is moved into a sorting category. ~~Submit button~~ Next button	Capture which bin (i.e., important, unimportant, disagree) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		feel anxious. When you are done sorting, click the next button to move on to the next reason.		*giving it an orange background and/or border. Use the same orange as the 'No' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes*		sorted into or if it not sorted into any box	
D1.22.Diagnosed. Couple.a	IF DIAGNOSED COHORT & COUPLE	• You do not feel prepared to learn that your child may have another health problem.		Reasons against genomic sequencing. (Headline) Sort "reasons against" into the boxes labelled *Important, Not important, or We disagree*.	Sorting task for users to move boxes with 'reasons for' into three bins labeled 'Important,' 'Not important,' and 'We disagree' Allow participants to continue to next screen only after at least one statement is moved into a sorting category.	Capture which bin (i.e., important, unimportant, disagree) user sorts each 'reason for'	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				NOTE: Label the unsorted box: "Unsorted Reason Against" • You do not feel prepared to learn that your child may have another health problem. *Note: Color code this reason as a 'reason against' by giving it an orange background and/or border. Use the same orange as the 'No' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes*	~~Submit button~~ Next button	Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it not sorted into any box	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.22.Diagnosed.Couple.b	IF DIAGNOSED COHORT & COUPLE	• Knowing that the NC NEXUS study team will have your child's genomic sequencing results makes you uncomfortable.		Reasons against genomic sequencing. (Headline) Sort "reasons against" into the boxes labelled *Important, Not important, or We disagree*. *NOTE:* Label the unsorted box: "Unsorted Reason Against" • Knowing that the NC NEXUS study team will have your child's genomic sequencing results makes you uncomfortable.	Sorting task for users to move boxes with 'reasons for' into <u>three</u> bins labeled 'Important,' 'Not important,' and 'We disagree' Allow participants to continue to next screen only after at least one statement is moved into a sorting category. ~~Submit button~~ Next button	Capture which bin (i.e., important, unimportant, disagree) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				Note: Color code this reason as a 'reason against' by giving it an orange background and/or border. Use the same orange as the 'No' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes		sorted into or if it not sorted into any box	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.22.Diagnosed.Couple.c	IF DIAGNOSED COHORT & COUPLE	• You are satisfied with the medical care your child receives and don't think other information would be helpful.		Reasons against genomic sequencing. (Headline) Sort "reasons against" into the boxes labelled *Important, Not important, or We disagree*. *NOTE:* Label the unsorted box: "Unsorted Reason Against" • You are satisfied with the medical care your child receives and don't think other information would be helpful. *Note: Color code this reason as a*	Sorting task for users to move boxes with 'reasons for' into three bins labeled 'Important,' 'Not important,' and 'We disagree' Allow participants to continue to next screen only after at least one statement is moved into a sorting category. ~~Submit button~~ Next button	Capture which bin (i.e., important, unimportant, disagree) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				'reason against' by giving it an orange background and/or border. Use the same orange as the 'No' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes		sorted into or if it not sorted into any box	
D1.22.Diagnosed. Couple.d	IF DIAGNOSED COHORT & COUPLE	• You would rather wait to see if your child has any problems before having genetic testing		Reasons against genomic sequencing. (Headline) Sort "reasons against" into the boxes labelled *Important, Not important, or We disagree*.	Sorting task for users to move boxes with 'reasons for' into three bins labeled 'Important,' 'Not important,' and 'We disagree' Allow participants to continue to next screen only after at least one statement is moved into a sorting category.	Capture which bin (i.e., important, unimportant, disagree) user sorts each 'reason for'	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				NOTE: Label the unsorted box: "Unsorted Reason Against" • You would rather wait to see if your child has any problems before having genetic testing *Note: Color code this reason as a 'reason against' by giving it an orange background and/or border. Use the same orange as the 'No' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes*	~~Submit button~~ Next button	Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it not sorted into any box	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.22.Diagnosed.Couple.e	IF DIAGNOSED COHORT & COUPLE	*Are there any other reasons against having genomic sequencing that you can think of? Please type them in the text boxes labelled "Add reason"* When you are done sorting, click the next button to continue.		Reasons against genomic sequencing. (Headline) Sort "reasons against" into the boxes labelled *Important, Not important, or We disagree*. *NOTE:* Label the unsorted box: "Unsorted Reason Against" *Are there any other reasons you can think of?* • *Add reason (x5)* *NOTE: Change label from 'Add custom reason' → 'Add reason'*	Sorting task for users to move boxes with 'reasons for' into <u>three</u> bins labeled 'Important,' 'Not important,' and 'We disagree' 5 interactive textboxes that allows users to write in 5 additional 'reasons for' that is not listed; write-in textboxes are also sortable Next button	Capture which bin (i.e., important, unimportant, disagree) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				Note: Color code this reason as a 'reason against' by giving it an orange background and/or border. Use the same orange as the 'No' of yes/no buttons. Do not color code the 'Important' or 'Not important' boxes		sorted into or if it not sorted into any box	
D1.23.Newborn.Single	IF NEWBORN COHORT & SINGLE	Here are the reasons for and against genomic sequencing for your child that are important to you. This is a summary of what you just sorted. When you are done reviewing		Here are the reasons that are important to you. (Headline) <u>Two</u> boxes on screen. One is labelled "Reasons for having genomic sequencing in NC NEXUS." In this box, list the	Visually present whether user sorted 'reasons for' as important on screen D1.21.Newborn.Single and 'reasons against' as important on screen D1.22.Newborn.Single Any statement that was not sorted into a category is not displayed on review screen.		(7) Values clarification, review

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		these reasons, click the next button to continue.		reasons that the user sorted into the 'important' box from screen D1.21.Newborn.Single *NOTE: Color code this box green* "Reasons against having genomic sequencing in NC NEXUS" In this box, list the reasons that the user sorted into the 'important' box from screen D1.22.Newborn.Single *NOTE: Color code this box orange*	Replay button Next button		

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.23.Diagnosed.Single	IF DIAGNOSED COHORT & SINGLE	Here are the reasons for and against genomic sequencing for your child that are important to you. This is a summary of what you just sorted. When you are done reviewing these reasons, click the next button to continue.		Here are the reasons that are important to you. (Headline) Two boxes on screen. One is labelled "Reasons for having genomic sequencing in NC NEXUS." In this box, list the reasons that the user sorted into the 'important' box from screen D1.21.Diagnosed.Single *NOTE: Color code this box green* "Reasons against having genomic sequencing in NC NEXUS" In this box, list the reasons that the user sorted into the 'important' box from screen	Visually present whether user sorted 'reasons for' as important on screen D1.21.Diagnosed.Single and 'reasons against' as important on screen D1.22.Diagnosed.Single Any statement that was not sorted into a category is not displayed on review screen. Replay button Next button		(7) Values clarification, review

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				D1.22.Diagnosed.Single *NOTE: Color code this box orange*			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.23.Newborn.Couple	IF NEWBORN COHORT & COUPLE	Here are the reasons for and against genomic sequencing for your child that are important to you. This is a summary of what you just sorted. When you are done reviewing these reasons, click the next button to continue.		Here are the reasons that are important to you. (Headline) Three boxes on screen. One is labelled "Reasons for having genomic sequencing in NC NEXUS." In this box, list the reasons that the user sorted into the 'important' box from screen D1.21.Newborn.Couple *NOTE: Color code this box green* "Reasons against having genomic sequencing in NC NEXUS" In this box, list the reasons that the user sorted into the 'important' box from screen	Visually present whether user sorted 'reasons for' as important on screen D1.21.Newborn.Couple, the 'reasons against' as important on screen D1.22.Newborn.Couple, or any reasons sorted into 'we disagree' on D1.21.Newborn.Couple or D1.22.Newborn.Couple Any statement that was not sorted into a category is not displayed on review screen. Replay button Next button		(7) Values clarification, review

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				D1.22.Newborn.Couple *NOTE: Color code this box orange* "Reasons that you and your partner disagree about" In this box, list the reasons that the user sorted into the 'We disagree' box from screen D1.21.Newborn.Couple or D1.22.Newborn.Couple			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.23.Diagnosed.Couple	IF DIAGNOSED COHORT & COUPLE	Here are the reasons for and against genomic sequencing for your child that are important to you. This is a summary of what you just sorted. When you are done reviewing these reasons, click the next button to continue.		Here are the reasons that are important to you. (Headline) Three boxes on screen. One is labelled "Reasons for having genomic sequencing in NC NEXUS." In this box, list the reasons that the user sorted into the 'important' box from screen D1.21.Diagnosed.Couple *NOTE: Color code this box green* "Reasons against having genomic sequencing in NC NEXUS" In this box, list the reasons that the user sorted into the 'important' box from screen	Visually present whether user sorted 'reasons for' as important on screen D1.21.Diagnosed.Couple, the 'reasons against' as important on screen D1.22.Diagnosed.Couple, or any reasons sorted into 'we disagree' on D1.21.Diagnosed.Couple or D1.22.Diagnosed.Couple Replay button Next button		(7) Values clarification, review

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				D1.22.Diagnosed.Couple *NOTE: Color code this box orange* "Reasons that you and your partner disagree about" In this box, list the reasons that the user sorted into the 'We disagree' box from screen D1.21.Diagnosed.Couple or D1.22.Diagnosed.Couple			
D1.24.Single	IF SINGLE	Here are some questions that can help you decide if you want your		Questions to help you decide (headline) Yes No	Check boxes/buttons for users to select yes or no for each question ~~Submit button~~	Capture y/n answers to each question;	(9) Questions to help decide, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		child to have genomic sequencing in NC NEXUS. Please answer "yes" or "no" to the following questions. You can pick your answers by clicking the button that matches your selection. • Will having genomic sequencing for your child help you learn		❒ ❒ Will genomic sequencing help you learn things that are important to you? ❒ ❒ Do you have enough	Next button; *NOTE:* Would it be possible to grey-out the list of questions when the page loads, and then have color appear as each is being read? Alternatively, have the questions appear one at a time, in sync with the narration.	Capture time in milliseconds spent on this screen	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		things that are important to you? • Do you have enough information to make a decision about having genomic sequencing for your child?		information to make a decision about having genomic sequencing for your child? ❒ ❒ Are you prepar			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		• Are you prepared to learn genomic sequencing results for medically actionable childhood conditions? • Are you interested in learning if your child has gene		ed to learn genomic sequencing results for medically actionable childhood conditions? ❒ ❒ Are you inte			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		differences that can cause medically actionable childhood conditions? • Are you confident you can make the decision that is right for you and your		rested in learning if your child has gene differences that can cause medically actionable childho			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		family ? When you are done, click the next button to continue.		od conditions ? ❒ ❒ Are you confident you can decide ? *NOTE: Please add the words 'Yes' and 'No' on screen, perhaps at top of columns with yes/no buttons* *NOTE: Color code 'yes' buttons green and 'no' buttons dark orange (same orange from slider scales)*			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.24.Couple	IF COUPLE	Here are some questions that can help you decide if you want your child to have genomic sequencing in NC NEXUS Please answer "yes" or "no" to the following questions. You can pick your answers by clicking the button that matches your selection: • Will having genomic sequencing for		Questions to help you decide (headline) Yes ❒ No ❒ Will genomic sequencing help you learn things that are important to you?	Check boxes/buttons for users to select yes or no for each question ~~Submit button~~ Next button; *NOTE:* Would it be possible to grey-out the list of questions when the page loads, and then have color appear as each is being read? Alternatively, have the questions appear one at a time, in sync with the narration.	Capture y/n answers to each question; Capture time in milliseconds spent on this screen	(9) Questions to help decide, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		your child help you learn things that are important to you? • Do you have enough information to make a decision about having genomic seque		❒ ❒ Do you have enough information to make a decision about having genomic sequencing for your			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		ncing for your child? • Are you prepared to learn genomic sequencing results for medically actionable childhood conditions? • Are you interested in learning if		child? ❒ ❒ Are you prepared to learn genomic sequencing results for medically actionable childhood con			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		your child has gene differences that can cause medically actionable childhood conditions? • Are you and your partner confident you can make the decisi		ditions? ❒ ❒ Are you interested in learning if your child has gene differences that can cause medic			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		on that is right for you and your family? When you are done, click the next button to continue.		ally actionable childhood conditions? ❒ ❒ Are you and your partner confident you can decide? *NOTE: Please add the words 'Yes' and*			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				'No' on screen, perhaps at top of columns with yes/no buttons *NOTE: Color code 'yes' buttons green and 'no' buttons dark orange (same orange from slider scales)*			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.25.Single	IF SINGLE	If you answered *Yes* to more of these questions, maybe you are ready for your child to have genomic sequencing. If you answered *No* to more, maybe this is not the right decision for your family at this time. Or you might still need more time or information to decide. You should make the decision that is best for you and your family. There		Questions to help you decide (headline) • Will genomic sequencing help you learn things that are important to you? • Do you have enough information to make a decision about having genomic sequencing for your child? • Are you prepared to learn genomic sequencing	Visually show whether user selected yes/no for each question from screen 'D1.24.Single' Replay button Next button		(10) Questions to help decide, review

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		are no right or wrong choices.		results for medically actionable childhood conditions? • Are you interested in learning if your child has gene differences that can cause medically actionable childhood conditions? • Are you confident you can decide?			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.25.Couple	IF COUPLE	If you answered *Yes* to more of these questions, maybe you are ready for your child to have genomic sequencing. If you answered *No* to more, maybe this is not the right decision for your family at this time. Or you might still need more time or information to decide. You should make the decision that is best for you and your family. There		Questions to help you decide (headline) • Will genomic sequencing help you learn things that are important to you? • Do you have enough information to make a decision about having genomic sequencing for your child? • Are you prepared to learn genomic sequencing	Visually show whether user selected yes/no for each question from screen 'D1.24.Couple' Replay button Next button		(10) Questions to help decide, review

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		are no right or wrong choices.		results for medically actionable childhood conditions? • Are you interested in learning if your child has gene differences that can cause medically actionable childhood conditions? • Are you and your partner confident you can decide?			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.26.Single	IF SINGLE	You have a decision to make at this time. Do you want your child to have genomic sequencing for conditions like those found in newborn screening? Click and drag the slider, moving it to the point on the scale that best fits your answer. • No, I do not want my child to have genomic sequencing at this time		Making a decision about genomic sequencing (headline) *Note: Interactive scale* Do you want your child to have genomic sequencing for conditions like those found in newborn screening? • No, I do not want my child to have genomic sequencing. • I'm not sure • Yes, I want my child to have genomic sequencing. *NOTE: Example layout here* file://rtints6/hserpr	Interactive response scale (slider); ~~Submit button~~ Next button; Q/A [?] button	Capture selection-yes/no/not sure Capture numerical value associated with position on scale. Treat as scale ranging from 0-100. In addition to capturing integer values in dataset, values will also be used for conditional piping on screens"D1.27…" 3-	(11) Decision choices

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		for conditions like those found in newborn screening. I do not want to schedule a study visit. • I'm not sure if I want my child to have genomic sequencing or not, but I want to schedule a study visit with a genetic counselor at UNC		oj4/0214132%20NEXUS%20Proj%203/Aim%202%20Decision%20Aids/2.1%20DA%20Content/Final%20Decision%20Aid%20Content/working/DA1_slider%20scale%20example.docx *NOTE: To differentiate the slider from the progress at the bottom of screen, make the slider a pentagon instead of a circle, ex.:* **[? – 'genetic counselor']**		point categorical values, where anchor points are 0-33 = left third, No 34-66= center third, Not sure 67-100= right third, Yes Capture time in milliseconds spent on this screen	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		Hospitals to discuss the decision. • Yes, I want my child to have genomic sequencing for conditions like those found in newborn screening. I want to schedule a study visit with a genetic counselor at UNC Hospitals. If you select "Yes" or "I'm					

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		not sure," a member of the NC NEXUS study team will contact you to schedule a study visit at UNC Hospitals. Remember, even if you decide to schedule a study visit, you can change your mind and stop participation in this study at any point in time. When you are done making your decision, click the next					

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		button to continue.					

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.26.Couple	IF COUPLE	You have a decision to make at this time. Do you want your child to have genomic sequencing for conditions like those found in newborn screening? Click and drag the slider, moving it to the point on the scale that best fits your answer. • No, we do not want our child to have genomic sequencing		Making a decision about genomic sequencing (headline) *NOTE: Interactive scale* Do you want your child to have genomic sequencing for conditions like those found in newborn screening? • No, we do not want our child to have genomic sequencing. • We're not sure • Yes, we want our child to have genomic sequencing. *NOTE: Example layout here* file://rtints6/hserproj4/0214132%20NE	Interactive response scale (slider); ~~Submit button~~ Next button; Q/A [?] button	Capture selection-yes/no/not sure Capture numerical value associated with position on scale. Treat as scale ranging from 0-100. In addition to capturing integer values in dataset, values will also be used for conditional piping on screens"D1.27…" 3-	(11) Decision choices

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		at this time for conditions like those found in newborn screening. We do not want to schedule a study visit. • We're not sure if we want our child to have genomic sequencing or not, but we want to schedule a study visit with a genetic counselor		XUS%20Proj%203/Aim%202%20Decision%20Aids/2.1%20DA%20Content/Final%20Decision%20Aid%20Content/working/DA1_slider%20scale%20example.docx *NOTE: Need to show the three anchor labels on screen at all times. Change gradient in the slider line three separate color blocks, corresponding to the three response options. To differentiate the slider from the progress at the bottom of screen, make the slider a pentagon instead of a circle, ex.:*		point categorical values, where anchor points are 0-33 = left third, No 34-66= center third, Not sure 67-100= right third, Yes Capture time in milliseconds spent on this screen *Note:* Make sure the conditiona	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		at UNC Hospitals to discuss the decision. • Yes, we want our child to have genomic sequencing for conditions like those found in newborn screening. We want to schedule a study visit with a genetic counselor at UNC Hospitals.		**[? – 'genetic counselor']**		l screens for D1.27 function correctly based on selection made on this screen.	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		If you select "Yes" or "We're not sure," a member of the NC NEXUS study team will contact you to schedule a study visit at UNC Hospitals. Remember, even if you decide to schedule a study visit, you can change your mind and stop participation in this study at any point in time. When you are done making your decision, click the next					

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		button to continue.					
D1.27.Single.No	IF D1.26.Single=No &	What happens next?		What happens next? (headline)	Replay button Next/Exit button		(12) Closing

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
	SINGLE	You have decided not to have genomic sequencing for your child. Within the next week or so, you will be asked to complete an online survey about this decision because understanding why you made this decision is important. You will be sent a $20 VISA card for completing the survey. After completing this survey, you will end your		• You will be asked to complete an online survey • You will be sent a $20 Visa card for completing the survey • Your participation in the NC NEXUS study will end Thank you!			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		participation in the NC NEXUS study.					
D1.27.Couple.No	IF D1.26.Couple= No & COUPLE	What happens next? You and your partner have decided not to have genomic sequencing for your child. Within the next week or so, you and your partner will both be asked to complete an online survey about this decision because understanding why you made		What happens next? (headline) • You will be asked to complete an online survey • You will each be sent a $20 Visa card after you complete the survey • Your participation in the NC NEXUS study will end Thank you!	Replay button Next/Exit button		(12) Closing

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		this decision is important. You will each be sent a $20 VISA card for completing the survey. After completing the survey, you will end your participation in the NC NEXUS study.					

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.27.Single.MaybeYes	IF D1.26.Single=Not sure OR IF D1.26.Single=Yes & SINGLE	What happens next? • A member of the NC NEXUS study team will contact you to schedule a study visit at UNC Hospitals. • At the study visit, you will meet with a genetic counselor to discuss why you may or may not want to have genomic sequencing for your		What happens next? (headline) • Schedule a visit at UNC Hospitals • Meet with a genetic counselor to discuss genomic sequencing for your child • Visit will last about 1 hour • Decide if you want to consent to genomic sequencing for your child **[? – 'genetic counselor]**	Replay button Exit/Close button Q/A [?] button		(12) Closing

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		child. This visit will last about 1 hour. • You will then be asked if you want to consent to having genomic sequencing for your child. If you come to the study visit, it does not mean you have to consent to genomic sequencing.					

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.27.Single.MaybeYes.a	IF D1.26.Single=Not sure OR IF D1.26.Single=Yes & SINGLE	• If you choose to have genomic sequencing for your child, you will sign a consent form and continue your participation in the NC NEXUS study. You will learn your child's genomic		What happens next? (headline) If you choose to have genomic sequencing for your child: • Sign a consent form • Continue participation in NC NEXUS • Learn sequencing results for conditions found with newborn screening • Complete three online surveys	Replay button Next button		(12) Closing

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		sequencing results for medically actionable childhood conditions, like those found with newborn screening. You will be asked to complete three online surveys over the					

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		next several months. You will be sent a $20 Visa card for each survey you complete.					
D1.27.Single.MaybeYes.b	IF D1.26.Single=Not sure OR IF D1.26.Single=Yes & SINGLE	You will also be sorted into one of two groups by a random drawing. One group will be asked to decide if they want to request additional analysis of their child's		What happens next? (headline) If you choose to have genomic sequencing for your child: • You will be sorted into two groups • One group will decide if they want to	Replay button Next button		(12) Closing

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		genetic information. They will use another decision guide to help decide if learning this additional information is right for them and their families. The other group will not be asked to make this decision.		• request additional genetic information • Other group will not be asked to make this decision			
D1.27.Single.MaybeYes.c	IF D1.26.Single=Not sure OR IF D1.26.Single=Yes & SINGLE	• If you choose not to have genomic sequencing for your child at that time, you will		What happens next? (headline) If you choose <u>not</u> to have genomic sequencing for your child: • Complete one online survey • Participation in NC NEXUS will end	Replay button Next button		(12) Closing

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		complete an online survey asking about this decision and then your participation in the NC NEXUS study will end. You will be sent a $20 VISA card after completing the survey.					

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.27.Couple.MaybeYes	IF D1.26.Couple=Not sure OR IF D1.26.Couple=Yes & COUPLE	What happens next? • A member of the NC NEXUS study team will contact you to schedule a study visit at UNC Hospitals. • At the study visit, you and your partner will meet with a genetic		What happens next? (headline) • Schedule a visit at UNC Hospitals • Meet with a genetic counselor to discuss genomic sequencing for your child • Visit will last about 1 hour • Decide if you want to consent to genomic	Replay button Next/Exit button Q/A [?] button		(12) Closing

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		counselor to discuss why you may or may not want to have genomic sequencing for your child. This visit will last about 1 hour. • You will then be asked if you want to consent to having genomic sequencing for your child. If you come to the study visit, it does not		sequencing for your child **[? – 'genetic counselor]**			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		mean you have to consent to genomic sequencing.					
D1.27.Couple.MaybeYes.a	IF D1.26.Couple=Not sure OR IF D1.26.Couple=Yes & COUPLE	• If you choose to have genomic sequencing for your child, both you and your partner		What happens next? (headline) If you choose to have genomic sequencing for your child: • Sign a consent form • Continue participatio			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		will sign a consent form and continue your participation in the NC NEXUS study. You will learn your child's genomic sequencing results for medically actionable childho		n in NC NEXUS • Learn sequencing results for conditions found with newborn screening • Complete three online surveys			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		od conditions, like those found with newborn screening. You and your partner will each be asked to complete three online surveys over the next several months. You will each be					

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		sent a $20 Visa card for each survey you complete.					
D1.27.Couple.MaybeYes.b	IF D1.26.Couple=Not sure OR IF D1.26.Couple=Yes & COUPLE	• You will also be sorted into one of two groups by a random drawing. One group will be asked to decide if they want to request addition		What happens next? (headline) If you choose to have genomic sequencing for your child: • You will be sorted into two groups • One group will decide if they want to request additional genetic information • Other group will			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		al analysis of their child's genetic information. They will use another decision guide to help decide if learning this additional information is right for them and their families. The		not be asked to make this decision			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		other group will not be asked to make this decision.					
D1.27.Couple.MaybeYes.c	IF D1.26.Couple=Not sure OR IF D1.26.Couple=Yes & COUPLE	• If you and your partner choose not to have genomic sequencing for your child at that time, you will complet		What happens next? (headline) If you choose not to have genomic sequencing for your child: • Complete one online survey • Participation in NC NEXUS will end			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		e an online survey asking about this decision and then your particip ation in the NC NEXUS study will end. You will each be sent a $20 VISA card after complet ing the survey.					

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.28	IF EXPERIMENT ARM	The work to develop this decision guide was funded by a grant from the Eunice Kennedy Shriver National Institute of Child Health and Human Development and the National Human Genome Research Institute at the National		The work to develop this decision guide was funded by a grant from the Eunice Kennedy Shriver National Institute of Child Health and Human Development and the National Human Genome Research Institute at the National Institutes of Health. <UNC and RTI logos> 			(3) General content, Text

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		Institutes of Health.		RTI INTERNATIONAL			

Term	Script	Visual Notes	Screen(s)
Gene	*Genes* are pieces of DNA that carry information needed for specific traits, like height or eye color. Every person has two copies of each gene, one from each parent. [1,2]	*Genes*— Pieces of DNA that carry information needed for specific traits. Every person has two copies of each gene, one from each parent.	D1.2; D1.9
Genomic Sequencing	*Genomic sequencing* is a way to map out a person's DNA. It can be used to find changes in genes that cause genetic conditions.[1]	*Genomic Sequencing*— A way to map out a person's DNA. It can be used to find changes in genes that cause genetic conditions.	D1.2; D1.4; D1.5; D1.8; D1.9; D1.10; D1.11; D1.12.Newborn; D1.12.Diagnosed; D1.15; D1.16; D1.17.Newborn; D1.17.Diagnosed; D1.20; D1.20.a
Newborn Screening	*Newborn screening* is a test done when a baby is born. The test looks for rare but serious genetic conditions before a child becomes sick.	*Newborn Screening*— A test done when a baby is born. The test looks for rare but serious genetic conditions before a child becomes sick.	D1.4; D1.6; D1.7; D1.8.Newborn; D1.8.Diagnosed; D1.11; D1.12.Newborn; D1.12.Diagnosed; D1.17.Newborn; D1.17.Diagnosed
DNA	*DNA* is the material inside cells that holds the genetic instructions a living thing needs to function. Parents pass their DNA to their children.[1]	*DNA*— The material inside cells that holds the genetic instructions a living thing needs to function. Parents pass their DNA to their children.	D1.9; D1.10; D1.15.a; D1.17.Newborn; D1.17.Diagnosed
Gene Differences	*Gene differences*, or variants, are changes in the DNA building blocks of a gene. These differences are what makes every person unique.[3]	*Gene Differences*— Changes in the DNA building blocks of a gene. These differences are what makes every person unique	D1.10; D1.11; D1.12.Diagnosed; D1.15; D1.15.a; D1.16
Genetic Condition	*Genetic conditions* are rare but serious conditions caused by differences in one or more genes.[4]	*Genetic Conditions*— Rare but serious conditions caused by differences in one or more genes.	D1.13; D1.21.Newborn.Single;
Medically Actionable Condition	*Medically actionable conditions* are conditions that can be improved with treatment, and the benefits of treatment typically outweigh the risks.	*Medically Actionable Conditions*— Conditions that can be improved with treatment, and the benefits of treatment typically outweigh the risks.	D1.13; D1.14; D1.15; D1.16; D1.17.Newborn; D1.17.Diagnosed;
Non-medically Actionable Condition	*Non-medically actionable conditions* are conditions that may have some symptoms that can be	*Non-medically Actionable Conditions*— Conditions that may have some symptoms that can be	

Term	Script	Visual Notes	Screen(s)
	treated, but there are no effective medical treatments to prevent or improve the condition itself.	treated, but there are no effective medical treatments to prevent or improve the condition itself.	
Genetic counselor	A *genetic counselor* is a health expert with special training in medical genetics and counseling.	*Genetic Counselor*— A health expert with special training in medical genetics and counseling.	D1.18; D1.26.Single; D1.26.Couple; D1.27.Single.MaybeYes; D1.27.Couple.MaybeYes;
Genetic testing	*Genetic testing* is testing done on blood or other tissue to find changes in genes that are linked to genetic conditions.[5,6]	*Genetic Testing*— Testing done on blood or other tissue to find changes in genes linked to genetic conditions.	
Carrier	A *carrier* is a person who has gene differences linked to a genetic condition from only **one** parent. A carrier usually shows no signs of the condition. However, carriers can pass gene differences to their children. [1,7]	*Carrier*— A person who has gene differences linked to a genetic condition from only **one** parent. A carrier usually shows no signs of the condition. Carriers can pass gene differences to their children.	
Carrier testing	*Carrier testing* is a genetic test to find *carriers* of a gene difference linked to a genetic condition. [7]	*Carrier testing*— A genetic test to find *carriers* of a gene difference linked to a genetic condition.	D2.8

References

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University of North Carolina at Chapel Hill
Consent to Participate in a Research Study: Phase II of NC NEXUS
Parental Permission for Child Participants: Diagnosed Cohort
Biomedical Form

Consent Form Version Date: 5/15/2015
Title of Study: North **C**arolina **N**ewborn **Ex**ome **S**equencing as **U**niversal **S**creening **(NC NEXUS)**
Principal Investigators: Cynthia Powell, M.D. and Jonathan S. Berg, MD, PhD
UNC-Chapel Hill Department: Genetics
UNC-Chapel Hill Phone number: 919-966-7043
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Co-Investigators: Donald Bailey, Karen Weck, Kirk Wilhelmsen
Funding Source: National Human Genome Research Institute and National Institutes of Child Health and Development (National Institutes of Health)
Study Contact:
Study Contact telephone number:
Study Contact email: IRB Study #

What are some general things you should know about research?

The goal of research is to learn information that may help other people in the future. You and your child may ***not*** receive any direct benefit from joining this study and there may be risks.

You may refuse for your child to take part in this study. If your child is a patient with an illness, he or she does not have to be in a study to get treatment. Joining the study is voluntary.

It is important for you to understand the information in this consent form so that you can make an informed choice. You will be given a copy. You have the right to ask, and have answered, any questions you may have about this research. Please contact the researchers listed at the top of this form.

What is the purpose of the NC NEXUS study?

The purpose of this study is to learn whether a new kind of testing, called "genomic sequencing" can help identify children who have or are likely to develop some kinds of genetic conditions.

Newborn screening is done to look for conditions that can be successfully treated when they are found early. Screening can identify some, but not all, genetic conditions.

Genomic sequencing looks for **genetic** differences, called "variants," that cause conditions like the ones identified by newborn screening and many more. The technology that allows many genes to be studied at once is called "Next-generation sequencing." It is a new way to test for these conditions.

We are interested in knowing how parents decide whether or not to have sequencing of their child and, if so, how they understand and respond to the different kinds of information they can learn from testing.

How long will your and your child's part in this study last?
We ask that you and your child to join for a total of up to **4** years. Periodically, we plan to review the information from your child's sequencing. If we find new information that would affect your child's medical care, or your willingness to continue participation in the study, we will contact you.

How many people will take part in this study?
We expect **~ 400** children will have genomic sequencing in this study.

You are currently participating in Phase I of NC NEXUS.
You have been sent this consent form because you have been scheduled for the first study visit.

What will happen at the first study visit?
You will be asked a few questions about your understanding of genetics. A genetic counselor will review this consent form with you and discuss sequencing and the types of results you could learn.

What is genomic sequencing?
Genomic sequencing looks for differences, called "variants", in many genes at once to identify those that cause genetic conditions.

What types of information could you learn from the genomic sequencing done in NC NEXUS?

- Although all of your child's genes will be sequenced, only a selected group of genes will be analyzed and interpreted.

- Genomic sequencing might find genetic variants that provide information about the condition that made your child eligible for NC NEXUS.

  We will look for variants in those genes that have been reported as being connected with your child's diagnosis. Variants in these genes will be classified in 1 of 3 categories:

  1. Positive result: a gene variant is identified that explains your child's diagnosis.

  2. Uncertain result: a gene variant is identified that **might** explain your child's diagnosis but we are **uncertain** if it explains it or not. This is called a "variant of uncertain significance" or "VUS."

  3. Negative result: no variant has been identified that explains your child's health condition in the group of genes that was studied.

- If your child's sequencing identifies a variant of uncertain clinical significance (VUS), we might be able to clarify the meaning by testing family members. Genomic sequencing will not be done on the family samples but rather they will **only** be tested to study the meaning of the VUS found in your child's sample. This testing is part of the research study and you will not be charged for it.

- Genomic sequencing might also find variants in a group of genes that provide information about **other** genetic conditions like those identified by newborn screening.

    - In this part of the NC NEXUS study, we will analyze and interpret variants in those genes that provide information about conditions like those identified by newborn screening. These conditions have symptoms that begin in infancy or childhood and have treatments. We call this genetic screening, "**NGS-NBS**."

    - **Everyone** who consents to sequencing of their child will learn about the results of the **NGS-NBS screen**. Only "positive" results that strongly indicate the presence of a genetic disorder will be reported. Most participants in the study will screen "negative" for these conditions.

- We call this analysis, the "**Next-generation Sequencing Newborn Screen" (NGS-NBS Screen).**

- **In the NGS-NBS Screen, we will analyze and interpret gene variants to provide information about genetic conditions that are very similar to those found by newborn screening.**

    - Newborn screening identifies children who have, or are likely to develop conditions that

        - can be successfully treated when they are found early.

        - have symptoms that begin in infancy or childhood.

    - **All** parents who consent to sequencing of their child will learn results from the **NGS-NBS Screen**.

    - Only those results that **strongly** indicate the presence of a genetic condition as described above will be reported. These results are considered to be "**positive."**

    - Most children will have "**negative**" results for these conditions.

- It is possible that your child's results will indicate that other family members are at risk. When genetic testing for that condition is clinically available, it would *not* be paid for by the study.

What will happen if you and your child join Phase II of NC NEXUS?

(1) After the first study visit, you will **complete a questionnaire** on line that asks questions about your decision to consent for your child to be sequenced. You *may* choose *not* to answer a question for any reason. We will send you a $20 Visa card for completing it.

(2) We will **obtain the sample at this visit** or **schedule a visit to obtain the sample.** An experienced nurse will use up to 5 sponges to swab the inside of your baby's cheeks and along the gums.

(3) After your child's results from the **NGS-NBS Screen** have been analyzed, we will **schedule the second study visit** to discuss them with you. You will be **randomized** to one of two study groups and told which group you are in (described below).

What will happen at the second study visit?
We will explain the results of your child's sequencing and provide genetic counseling to help you understand the meaning and implications of their results.

Most children will have a **negative** result.

If your child has a **positive** result:

> We will confirm it in the CLIA-approved, Medical Genetics Laboratory (MGL) at UNC Hospitals. Once confirmed, we will give you a clinical report that can be placed into your child's UNC Hospitals electronic medical record (EMR).
>
> We will ask you to decide whether or not you want the clinically confirmed positive results to become a permanent part of your child's EMR.
>
> If you choose to do so, we will enter the report so that other health care providers taking care of your child can be aware of this result.
>
> If you choose **not** to do that, we will **not** enter the report into your child's EMR.
>
> We will ask you to sign a form to indicate your decision.

(4) Randomization Procedure
Before the second study visit, parents will be randomized into two groups. Both parents will be in the same group. We will tell you which group you are in when we schedule the visit with you and give you more information at that time.

- The "decision" group will use an electronic decision guide to help them make decisions about whether or not to learn information from three additional categories.
- The "control" group will not make decisions about learning additional information.

(5) After learning these results, you will **complete two more questionnaires** over the next 3 months.
You will be asked questions about your decision to consent for your child to be sequenced. You *may* choose *not* to answer a question for any reason. We will send you a $20 Visa card for completing it.

(6) Some parents will be asked if they will agree to be interviewed by phone and to have the study visits observed. You can decline these optional activities but still remain in the NC NEXUS study. Declining to participate in these optional activities will *not* affect your child's medical care at UNC.

There are a few other things you should know about this study:

- We will ask you to sign a HIPAA authorization so we can access your child's medical records and other health-related information from visits to UNC Hospitals. This information will include his or her health history, family history, and relevant laboratory test results.

- Since our knowledge about genomics is growing quickly, we plan to periodically review the information from your child's sequencing. If we learn new information that would affect your child's medical care, we will contact you for a follow-up visit that will be part of the study.

- NC NEXUS researchers may observe the study visits to help us improve our explanations.

What will happen to your child's sample?
We will code your child's sample with a unique participant identifier (ID) so that his or her personal health information will *not* be available to the study personnel that process and analyze the sample.

One half of the sample will be sent to the UNC Biospecimen Processing Facility to extract and store the DNA. Some of this sample will be sent to Dr. Jonathan S. Berg's laboratory for sequencing.

The other half of the sample will be sent to the UNC Hospitals' Clinical Laboratory to extract and store the DNA. It will be used to confirm the variants found by sequencing and for quality control testing.

We will use the samples for an undetermined period of time but may choose to destroy them when the study is complete.

Who owns the specimens?
Any samples or sequence data obtained for this study become the exclusive property of the UNC-Chapel Hill. The researchers may retain, preserve or dispose of these specimens and may use these specimens for research that may result in commercial applications.

There are *no* plans to compensate you or your baby for any future commercial use of these coded specimens.

What are the possible benefits to you?

Research is designed to benefit society by gaining new knowledge. There is little chance you or your child will benefit from being in this research study. Your and your child's participation will contribute to our understanding of how to use this new genomic test in the future and help us learn how people might respond to learning different kinds of information from this testing.

What are the possible risks or discomforts involved with participation in this study?

(1) Physical risk: The physical risks in this study are minimal. An experienced nurse will collect the cheek swab. It should only take a few minutes to obtain but might cause your child some discomfort.

(2) Psychological Risks: Genetic testing can provide information about the risk for health conditions in a family. This knowledge may affect your or your child's emotional well-being. Some people may experience stress, anxiety and/or depression. We will explain your child's results to you and provide genetic counseling to help you understand their meaning and implications for family members.

To study how parents respond to genomic sequencing and learning the results, we will ask you questions about your experiences in the study. You can choose *not* to answer any question at any time.

(3) DNA Storage: The foreseeable risks of storing your child's genetic material are low.

(4) Risk to Confidentiality and Privacy: Som**e** parents of children who get positive results may want to keep that information private. This study has many protections to protect the privacy of your and your child's participation in the study and to protect information arising from the study.

> **Use of Participant ID Numbers:** We will code your child's samples and all study materials with a unique participant ID number. The link between the ID number and your child's personal identifying information will be kept in a secured database with restricted access. Electronic information, including that from your child's medical records, will also be stored on secure drives in password-protected databases with restricted access.
>
> **Paper Documents:** Paper documents, including this signed consent form, will be stored in a locked filing cabinet in a locked office at UNC.
>
> **Report of Positive Results**: We will ask for your consent before putting any clinically confirmed positive results into your child's UNC electronic medical record.

Publications about the Research:

When we report findings from this research, we will not identify you nor your child.

We will make every effort to keep research records private but there may be times when federal or state law requires their disclosure, including of personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, the information could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

(5) Risk for Genetic Discrimination

A Federal law called the "Genetic Information Nondiscrimination Act" (GINA) generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against someone based on their genetic information.

GINA does ***not*** protect people against discrimination based on an already-diagnosed genetic condition or disease. The Americans with Disabilities Act (ADA) applies to them.

The Affordable Care Act (ACA) prohibits health insurance companies from discriminating against patients with genetic diseases by refusing coverage because of 'pre-existing conditions'.

GINA and the ACA do ***not*** protect people against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance

(6) Other risks to study participation: There may be uncommon or other risks that we don't know about. You should report any concerns to the researchers listed on the first page of this form.

Who is sponsoring and paying for this research?

NC NEXUS is being paid for by a grant from the National Human Genome Research Institute and the National Institutes of Child Health and Development at the National Institutes of Health (NIH). The researchers are paid to carry out the study but they do ***not*** have a direct financial interest with the sponsor or in the final study results.

Data Sharing with Qualified Researchers

By signing this consent form, you are allowing us to share the DNA or the sequence data obtained from your child's samples with researchers at UNC or other institutions to study the clinical use of sequencing. Your child's personal identifying information will ***not*** be included and will ***not*** be sent.

The NIH is the government agency that funds most of medical research in the US. By collecting the genetic information obtained from many research centers, the NIH and

other data banks will store it so other qualified researchers can use it to do more studies. Researchers can be from the government, academic, or a commercial site and studies may be done at many places at the same time.

Risks to Privacy and Confidentiality by Data Sharing
We think that the risks to your privacy and confidentiality by sharing your child's genetic information with other databanks is low; however, we cannot predict how genetic information will be used in the future. These databases have safeguards to protect information while it is stored and used for research. If your child has a genetic condition, this information will be sent with only a code number and personal identifying information will *not* be included and will *not* be sent.

You will not receive any results produced from your child's participation in the national databases unless it is considered medically relevant. If you no longer want your child's data in these databases, you can choose to withdraw your consent at anytime with no penalty. However, data that has already been sent to researchers cannot be retrieved from them.

Will researchers seek approval from you to do future studies involving the specimens?
A committee called the Institutional Review Board (IRB) protects the rights and welfare of research participants in current and future research.

For your child's data to be used in a future research study, the IRB may require that you be re-contacted and asked for your consent. You have the right, at that future time, to refuse to allow your child to participate. This refusal will *not* affect your or your child's medical care or result in loss of benefits to which you are or your child is entitled. In other cases, the IRB may determine that future research on your child's specimen is acceptable without re-contacting you. For example, your child's uniquely coded specimen and sequence data may be useful for other genetic research studies *not* directly related to genomic sequencing in children.

You may opt-out of future genetic research studies unrelated to this consent form by initialing:

_____I do *not* want my child's sample or data to be used in future genetic studies unrelated to those described in this consent form

Can you withdraw from participation in this study?
You can withdraw from this study at any time, without penalty by contacting the researchers listed on the front page of this form. We will then destroy any remaining samples. If you withdraw after you have consented for your child's results to be entered into the UNC electronic medical record, this report cannot be removed and will remain a permanent part of the medical record. Analyses that are complete or in progress when you withdraw will continue to be used in the study.

What will happen if you are or your child is injured by this research?
All research involves a chance that something bad might happen to participants. This may include the risk of personal injury. In spite of all safety measures, your child might develop a reaction or injury from having the sample collected. UNC-Chapel Hill has *not* set aside funds to pay for any such reactions or injuries, or for the related medical care. However, by signing this form, you do *not* give up any of your or your child's legal rights.

Will there be any cost to you for participating in NC NEXUS?
You will *not* be charged for the visits or the sequencing done as part of the study.

Will you receive anything for your participation?
We will not pay you nor your child for allowing the samples to be taken or for coming to the visits. You will receive parking vouchers and a $20 VISA card for completing each questionnaire for a total of $80.

What if you have questions about your rights as a research participant?
The IRB reviews all research on human volunteers in order to protect your rights and welfare. If you have questions or concerns about your rights as a research participant you may contact, the IRC at 919-966-3113 or to IRB_subjects@unc.edu. You do not have to use your name.

Participant Agreement:
I have read the information provided above and have asked all the questions I have at this time. I voluntarily agree to my and my child's participation in **the N**orth **C**arolina **N**ewborn **Ex**ome **S**equencing as **U**niversal **S**creening **(NC NEXUS); Principal Investigators:** Cynthia Powell, MD and Jonathan S. Berg, MD, PhD

_____ _____
Signature of Research Participant's Parent or Guardian Date

_____
Printed Name of Research Participant's Parent or Guardian and Relationship

_____ _____
Signature of Research Participant's Parent or Guardian Date

_____
Printed Name of Research Participant's Parent or Guardian and Relationship

_____ _____
Signature of Research Team Member Obtaining Consent Date

_____
Printed Name of Research Team Member

University of North Carolina at Chapel Hill
Consent to Participate in a Research Study: NC NEXUS: Phase II
Parental Permission for Child Participants: Well-Child Cohort
Biomedical Form

Consent Form Version Date: 5/15/2015
Title of Study: North **C**arolina **N**ewborn **Ex**ome **S**equencing as **U**niversal **S**creening **(NC NEXUS)**
Principal Investigators: Cynthia Powell, M.D. and Jonathan S. Berg, MD, PhD
UNC-Chapel Hill Department: Genetics
UNC-Chapel Hill Phone number: 919-966-7043
Email Address: powellcm@med.unc.edu; jsberg@med.unc.edu
Co-Investigators: Donald Bailey, Karen Weck, Kirk Wilhelmsen
Funding Source: National Human Genome Research Institute and National Institutes of Child Health and Development (National Institutes of Health)
Study Contact:
Study Contact telephone number:
Study Contact email:

What are some general things you should know about research?

The goal of research is to learn information that may help other people in the future. You and your child may ***not*** receive any direct benefit from joining this study and there may be risks.

You may refuse for your child to take part in this study. If your child is a patient with an illness, he or she does not have to be in a study to get treatment. Joining the study is voluntary.

It is important for you to understand the information in this consent form so that you can make an informed choice. You will be given a copy. You have the right to ask, and have answered, any questions you may have about this research. Please contact the researchers listed at the top of this form.

What is the purpose of the NC NEXUS study?

The purpose of this study is to learn whether genomic sequencing can help identify children who have, or are likely to develop, some kinds of genetic conditions.

After a baby is born, newborn screening is done to look for conditions that can be successfully treated when they are found early. Screening can identify some, but not all, genetic conditions.

Genomic sequencing looks for **genetic** differences, called "variants," that cause conditions like the ones identified by newborn screening and many more. The technology that allows many genes to be studied at once is called "Next-generation sequencing" and is a new way to test for these conditions.

We are interested in knowing how parents decide whether or not to have sequencing of their child and, if so, how they understand and respond to the different kinds of information they can learn from testing.

How long will your and your child's part in this study last?
We ask that you and your child to join for a total of up to **4** years. Periodically, we plan to review the information from your child's sequencing. If we find new information that would affect your child's medical care, or your willingness to continue participation in the study, we will contact you.

How many people will take part in this study?
We expect **~ 400** children will have genomic sequencing in this study.

You are currently participating in Phase I of NC NEXUS.
You have been sent this consent form because you have been scheduled for the first study visit.

What will happen at the first study visit?
You will be asked a few questions about your understanding of genetics. A genetic counselor will review this consent form with you and discuss sequencing and the types of results you could learn.

What is genomic sequencing?
Genomic sequencing looks for differences, called "variants", in many genes at once to identify those that cause genetic conditions.

What types of information could you learn from the genomic sequencing done in NC NEXUS?

- Although all of your child's genes will be sequenced, only a selected group of genes will be analyzed and interpreted.
- We call this analysis, the "**Next-generation Sequencing Newborn Screen" (NGS-NBS Screen).**
- **In the NGS-NBS Screen, we will analyze and interpret gene variants to provide information about genetic conditions that are very similar to those found by newborn screening.**
    - Newborn screening identifies children who have, or are likely to develop, health conditions that
        - can be successfully treated when they are found early.
        - have symptoms that begin in infancy or childhood.

- **All** parents who consent to sequencing of their child will learn results from the **NGS-NBS Screen**.

- Only those results that **strongly** indicate the presence of a genetic condition as described above will be reported. These results are considered to be "**positive."**

- Most children will have "**negative**" results for these conditions.

- It is possible that your child's results will indicate that other family members are at risk. When genetic testing for that condition is clinically available, it would *not* be paid for by the study.

Parents who consent to genomic sequencing for their child will join Phase II of NC NEXUS.

Parents who do not consent to sequencing will complete a questionnaire that asks about this decision. We will send them a $20 VISA card and this will end their participation in the study.

What will happen if you and your child participate in Phase II of NC NEXUS?

(1) After the first study visit, you will **complete a questionnaire** on line that asks questions about your decision to consent for your child to be sequenced. You *may* choose *not* to answer a question for any reason. We will send you a $20 Visa card for completing it.

(2) After your baby is born, we will **schedule a visit to obtain the sample.** An experienced nurse will use up to 5 sponges to swab the inside of your baby's cheeks and along the gums.

(3) After your child's results from the **NGS-NBS Screen** have been analyzed, we will **schedule the second study visit** to discuss them with you. You will be **randomized** to one of two study groups and told which group you are in (described below).

What will happen at the second study visit?
We will explain the results of your child's sequencing and provide genetic counseling to help you understand the meaning and implications of their results.

Most children will have a **negative** result.

If your child has a **positive** result:

We will confirm it in the CLIA-approved, Medical Genetics Laboratory (MGL) at UNC Hospitals. Once confirmed, we will give you a clinical report that can be placed into your child's UNC Hospitals electronic medical record (EMR).

We will ask you to decide whether or not you want the clinically confirmed positive results to become a permanent part of your child's EMR.

If you choose to do so, we will enter the report so that other health care providers taking care of your child can be aware of this result.

If you choose **not** to do that, we will **not** enter the report into your child's EMR.

We will ask you to sign a form to indicate your decision.

(4) Randomization Procedure
Before the second study visit, parents will be randomized into two groups. Both parents will be in the same group. We will tell you which group you are in when we schedule the visit with you and give you more information at that time.

- The "experimental" group will use an electronic decision guide to help them make decisions about whether or not to learn information from three additional categories.

- The "control" group will not make decisions about learning additional information.

(5) After learning the results, you will **complete two more questionnaires** in the next 3 months.
You will be asked questions about your decision to consent for your child to be sequenced. You *may* choose *not* to answer a question for any reason. We will send you a $20 Visa card for completing it.

(6) Some parents will be asked if they will agree to be interviewed by phone and to have the study visits observed. You can decline these optional activities but still remain in the NC NEXUS study. Declining to participate in these optional activities will *not* affect your child's medical care at UNC.

There are a few other things you should know about this study:

- We will ask you to sign a HIPAA authorization so we can access your child's medical records and other health-related information from visits to UNC Hospitals. This information will include his or her health history, family history, and relevant laboratory test results.

- Since our knowledge about genomics is growing quickly, we plan to periodically review the information from your child's sequencing. If we learn new information that would affect your child's medical care, we will contact you for a

follow-up visit that will be part of the study.

- NC NEXUS researchers may observe the study visits to help us improve our explanations.

What will happen to your child's sample?
We will code your child's sample with a unique participant identifier (ID) so that his or her personal health information will *not* be available to the study personnel that process and analyze the sample.

One half of the sample will be sent to the UNC Biospecimen Processing Facility to extract and store the DNA. Some of this sample will be sent to Dr. Jonathan S. Berg's laboratory for sequencing.

The other half of the sample will be sent to the UNC Hospitals' Clinical Laboratory to extract and store the DNA. It will be used to confirm the variants found by sequencing and for quality control testing.

We will use the samples for an undetermined period of time but may choose to destroy them when the study is complete.

Who owns the specimens?
Samples or sequence data obtained for this study become the exclusive property of the UNC-Chapel Hill. The researchers may retain, preserve or dispose of these specimens and may use these specimens for research that may result in commercial applications. There are *no* plans to compensate you or your baby for any future commercial use of these coded specimens.

What are the possible benefits to you?
Research is designed to benefit society by gaining new knowledge. There is little chance you or your child will benefit from being in this research study. Your and your child's participation will contribute to our understanding of how to use this new genomic test in the future and help us learn how people might respond to learning different kinds of information from this testing.

What are the possible risks or discomforts involved with participation in this study?

(1) Physical risk: The physical risks in this study are minimal. An experienced nurse will collect the cheek swab. It should only take a few minutes to obtain but might cause your child some discomfort.

(2) Psychological Risks: Learning that your baby has a **positive** result may affect your emotional well being and some people may experience stress, anxiety and/or depression. We will explain your child's results to you and provide genetic counseling to help you understand their meaning and implications for family members.

Learning that your baby has a **negative** result is not expected to affect your emotional well being.

To study how parents respond to genomic sequencing and learning the results, we will ask you questions about your experiences in the study. You can choose *not* to answer any question at any time.

(3) DNA Storage: The foreseeable risks of storing your child's genetic material are low.

(4) Risk to Confidentiality and Privacy: Some parents of children who get positive results may want to keep that information private. This study has many protections to protect the privacy of your and your child's participation in the study and to protect information arising from the study.

Use of Participant ID Numbers: We will code your child's samples and all study materials with a unique participant ID number. The link between the ID number and your child's personal identifying information will be kept in a secured database with restricted access. Electronic information, including that from your child's medical records, will also be stored on secure drives in password-protected databases with restricted access.

Paper Documents: Paper documents, including this signed consent form, will be stored in a locked filing cabinet in a locked office at UNC.

Report of Positive Results: We will ask for your consent before putting any clinically confirmed positive results into your child's UNC electronic medical record.

Publications about the Research:
When we report findings from this research, we will not identify you nor your child.

We will make every effort to keep research records private but there may be times when federal or state law requires their disclosure, including of personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, the information could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

(5) Risk for Genetic Discrimination
A Federal law called the "Genetic Information Nondiscrimination Act" (GINA) generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against someone based on their genetic information.

GINA does *not* protect people against discrimination based on an already-diagnosed genetic condition or disease. The Americans with Disabilities Act (ADA) applies to them.

The Affordable Care Act (ACA) prohibits health insurance companies from discriminating against patients with genetic diseases by refusing coverage because of 'pre-existing conditions'.

GINA and the ACA do *not* protect people against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance

(6) Other risks to study participation: There may be uncommon or other risks that we don't know about. You should report any concerns to the researchers listed on the first page of this form.

Who is sponsoring and paying for this research?

NC NEXUS is being paid for by a grant from the National Human Genome Research Institute and the National Institutes of Child Health and Development at the National Institutes of Health (NIH). The researchers are paid to carry out the study but they do *not* have a direct financial interest with the sponsor or in the final study results.

Data Sharing with Qualified Researchers

By signing this consent form, you are allowing us to share the DNA or the sequence data obtained from your child's samples with researchers at UNC or other institutions to study the clinical use of sequencing. Your child's personal identifying information will *not* be included and will *not* be sent.

The NIH is the government agency that funds most of medical research in the US. By collecting the genetic information obtained from many research centers, the NIH and other data banks will store it so other qualified researchers can use it to do more studies. Researchers can be from the government, academic, or a commercial site and studies may be done at many places at the same time.

Risks to Privacy and Confidentiality by Data Sharing

We think that the risks to your privacy and confidentiality by sharing your child's genetic information with other databanks is low; however, we cannot predict how genetic information will be used in the future. These databases have safeguards to protect information while it is stored and used for research. If your child has a genetic condition, this information will be sent with only a code number and personal identifying information will *not* be included and will *not* be sent.

You will not receive any results produced from your child's participation in the national databases unless it is considered medically relevant. If you no longer want your child's data in these databases, you can choose to withdraw your consent at anytime with no penalty. However, data that has already been sent to researchers cannot be retrieved from them.

Will researchers seek approval from you to do future studies involving the specimens?

A committee called the Institutional Review Board (IRB) protects the rights and welfare of research participants in current and future research.

For your child's data to be used in a future research study, the IRB may require that you be re-contacted and asked for your consent. You have the right, at that future time, to refuse to allow your child to participate. This refusal will *not* affect your or your child's medical care or result in loss of benefits to which you are or your child is entitled. In other cases, the IRB may determine that future research on your child's specimen is acceptable without re-contacting you. For example, your child's uniquely coded specimen and sequence data may be useful for other genetic research studies *not* directly related to genomic sequencing in children.

You may opt-out of future genetic research studies unrelated to this consent form by initialing:

_____I do *not* want my child's sample or data to be used in future genetic studies unrelated to those described in this consent form

Can you withdraw from participation in this study?

You can withdraw from this study at any time, without penalty by contacting the researchers listed on the front page of this form. We will then destroy any remaining samples. If you withdraw after you have consented for your child's results to be entered into the UNC electronic medical record, this report cannot be removed and will remain a permanent part of the medical record. Analyses that are complete or in progress when you withdraw will continue to be used in the study.

What will happen if you are or your child is injured by this research?

All research involves a chance that something bad might happen to participants. This may include the risk of personal injury. In spite of all safety measures, your child might develop a reaction or injury from having the sample collected. UNC-Chapel Hill has *not* set aside funds to pay for any such reactions or injuries, or for the related medical care. However, by signing this form, you do *not* give up any of your or your child's legal rights.

Will there be any cost to you for participating in NC NEXUS?

You will *not* be charged for the visits or the sequencing done as part of the study.

Will you receive anything for your participation?

We will not pay you nor your child for allowing the samples to be taken or for coming to the visits. You will receive parking vouchers and a $20 VISA card for completing each questionnaire for a total of $80.

What if you have questions about your rights as a research participant?
The IRB reviews all research on human volunteers in order to protect your rights and welfare. If you have questions or concerns about your rights as a research participant you may contact, the IRC at 919-966-3113 or to IRB_subjects@unc.edu. You do not have to use your name.

Participant Agreement:
I have read the information provided above and have asked all the questions I have at this time. I voluntarily agree to my and my child's participation in **the N**orth **C**arolina **N**ewborn **Ex**ome **S**equencing as **U**niversal **S**creening **(NC NEXUS); Principal Investigators:** Cynthia Powell, MD and Jonathan S. Berg, MD, PhD

_____ _____
Signature of Research Participant's Parent or Guardian Date

_____
Printed Name of Research Participant's Parent or Guardian and Relationship

_____ _____
Signature of Research Participant's Parent or Guardian Date

_____
Printed Name of Research Participant's Parent or Guardian and Relationship

_____ _____
Signature of Research Team Member Obtaining Consent Date

_____
Printed Name of Research Team Member Obtaining Consent

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.1	IF EXPERIMENT ARM			The North Carolina Newborn Exome Sequencing for Universal Screening Study (NC NEXUS)	Login user name and password fields. Enter button. *NOTE:* Progress bar for overall DA		(1) Welcome/Login
D2.2	IF EXPERIMENT ARM	Welcome back to the NC NEXUS decision guide. This part of the decision guide will tell you… • About three kinds of additional genomic sequencing results in the NC NEXUS study. The guide will also help you decide if you want the NC NEXUS study team to look at your child's genomic sequencing results and tell you about findings in any of the additional categories. The success of this research study does not depend on which decisions you make. We are most interested in	Welcome	Welcome back! (headline) The decision guide will… • Tell you about three kinds of additional results • Decide if you want findings in any of these categories **[? – 'genomic sequencing']**	Replay button Next button Q/A [?] button *NOTE:* Need more of a pause between D2.1 and D2.2; may just be short pause before narration begins.		(3) General content, text plus image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		finding out what information people choose to learn, and how they decide.					

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.3	IF EXPERIMENT ARM	What are additional genomic sequencing results? When you decided to have genomic sequencing for your child, you agreed to learn results for conditions currently found with newborn screening and other conditions like them. Now, you can also decide if you want to request three kinds of additional genomic sequencing results: 1. *Carrier status.* Almost all of us are carriers of genetic conditions. Carriers have gene differences that do not usually affect their own health, but that increase the risk for health problems in their children and others in future generations. 2. *Medically actionable adult onset conditions*. These are rare but	NC NEXUS has 3 kinds of additional genomic sequencing results. *NOTE:* The sentence "Adults who know they are at risk can take definite steps to protect their health" was verbiage recommended by the steering committee on 6/1/2015. It replaced the phrase used up to	What are additional genomic sequencing results? (headline) • You agreed to learn results for conditions found with newborn screening • Now, you can request three kinds of additional results: (NOTE: Show 3 'bins', labelled as follows) 1. Carrier status 2. Medically actionable adult onset conditions 3. Non-medically actionable childhood conditions **[? – 'newborn screening']** **[? – 'genetic condition]** **[? – 'carrier']** **[? – 'medically actionable condition']** **[? – 'non-medically actionable condition']**	Replay button Next button Q/A [?] button		(3) (4) general content, text plus image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		serious genetic conditions that typically don't begin until adulthood. Adults who know they are at risk can take definite steps to protect their health. And… 3. *Non-medically actionable childhood conditions*. These are rare but serious genetic conditions that typically begin in childhood or the teen years, but there are no medical treatments that will improve them.	that point, "…for which there are treatments that can help."				

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.3.a	IF EXPERIMENT ARM	In this part of the decision guide, you will learn about carrier status		Carrier Status	Replay button Next button		(X) Display Section Title Screen *Note*: Need new screen format, Header should be blank, and text on screen will be centered and large display type.
D2.4	IF EXPERIMENT ARM	~~Carrier Status~~ What does it mean to be a carrier? Genes are passed on in families from one generation to the next. Everyone has two copies of most genes. One copy is from their mother and the other is from their father. Some gene differences cause rare genetic conditions in people who	Carriers have two copies of a gene. One copy contains a gene difference that causes a condition.	What does it mean to be a carrier? (headline) *Note:* Show image of inheritance chart, with some kind of emphasis on the carriers. Ben – I linked to an example here • Genes are passed on in families • Everyone has two copies of most genes • Some gene differences cause	Replay button Next button Q/A [?] button		(4) general content, image plus text

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		have the difference in both copies of the gene. People who have the gene difference in only one copy of the gene are called *carriers*.		conditions in people who have it in both copies. • People who have the gene difference in only one copy are *carriers* **[? – 'genes']** **[? – 'gene differences']** **[? – 'genetic condition']** **[? – 'carrier']**			
D2.5	IF EXPERIMENT ARM	What can carrier status results tell you about your child? The kinds of genetic conditions your child might be a carrier for differ greatly from one to the next. Some may be preventable or treatable, while others may not be. If your child is a carrier, he or she will <u>not</u> usually have signs of the condition. But your child might pass on the gene differences to his or her children. It is only when both parents are carriers	Carriers do not usually have the condition, but may pass on a gene difference that causes it in their children.	What can carrier status results tell you about your child? (headline) *Visual note:* Word cloud • The conditions differ from one to the next • Some may be treatable, others may not be • A carrier will <u>not</u> usually have signs of the condition • Only when both parents are carriers is a child at risk **[? – 'genetic condition']** **[? – 'carrier']**	Replay button Next button Q/A [?] button		(4) general content, image plus text

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		that their child is at risk of having the condition. We do not know if learning this information about your child will be more helpful or more harmful.					
D2.6	IF EXPERIMENT ARM	How common is it for genomic sequencing to find carrier status results? On average, everyone is a carrier for about 3 to 5 gene differences that could cause conditions in future generations. Genomic sequencing done by the NC NEXUS study cannot find all gene differences and will not find all carriers for all conditions.	Everyone is a carrier for around 3 to 5 gene differences that cause conditions	How common is it to find carrier status results? (headline) • Everyone is a carrier for about 3 to 5 conditions. • Genomic sequencing cannot find all carriers for all conditions *Image:* Visual to depict that on average, everyone is a carrier for 3 to 5 gene differences that cause conditions. **[? – genomic sequencing]** **[? – 'carrier']**	Replay button Next button Q/A [?] button		(4) general content, text plus image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.7	IF EXPERIMENT ARM	What would knowing your child is a carrier mean for you? Learning your child is a carrier usually means that you or your child's other parent is also a carrier of the gene difference. Most parents do not know they are carriers until they have a child who develops a certain condition. If both parents are carriers for the same genetic condition, then they could have a child with that condition. Genomic sequencing done by the NC NEXUS study cannot tell for sure if you are a carrier. If you want to learn this information about yourself, there are many labs that offer this testing.	If your child is a carrier for a specific gene difference, then you or your partner are too.	What would knowing your child is a carrier mean for you? (headline) • You or your child's other parent is also a carrier • If both parents are carriers, they could have a child with that condition • NC NEXUS cannot tell for sure if you are a carrier. • There are labs that offer this testing. *Image:* Visual of inheritance chart. Multi step visual, like frames in comic book): Close shot on carrier. Pan up the family tree to dad and mom (in this case, both are carriers). Then pan down to affected sibling. **[? – genomic sequencing]** **[? – 'genetic condition']** **[? – 'carrier']** **[? – 'carrier testing]**	Replay button Next button Q/A [?] button		(4) general content, text plus image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.8	IF EXPERIMENT ARM	What happens if your child is a carrier? If you request these results and genomic sequencing finds that your child is a carrier: • These results will be confirmed with another test. • A genetic counselor and a doctor will meet with you to discuss the results. • You will be given information about how you can have testing to learn if you are a carrier. • You will be asked if you want the results added to your child's health record at UNC Hospitals.		What happens if your child is a carrier? (headline) ~~If genomic sequencing finds that your child is a carrier~~ • Results will be confirmed with another test • A genetic counselor and a doctor will discuss the results with you. • You will be given information about testing to learn if you are a carrier • Asked if you want the results added to your child's health record **[? – genomic sequencing]** **[? – genetic counselor]** **[? – 'carrier']** **[? – 'carrier testing]**	Replay button Next button Q/A [?] button		(3) General content, text plus image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.9	IF EXPERIMENT ARM	If you had to decide right now, which way are you leaning about learning your child's carrier status results? Click and drag the slider, moving it to the point on the scale that fits your answer. Leaning away from learning these results Not sure Leaning toward learning these results When you are done, click the next button to continue.		Which way are you leaning? (headline) *Note:* Interactive scale *NOTE: Example layout here* file://rtints6/hserproj4/0214132%20NEXUS%20Proj%203/Aim%202%20Decision%20Aids/2.1%20DA%20Content/Final%20Decision%20Aid%20Content/working/DA1_slider%20scale%20example.docx Which way are you leaning about learning your child's carrier status results? Leaning away from learning these results-------Not sure-------Leaning toward learning these results *NOTE: Need to show the three anchor labels on screen at all times. To differentiate the slider from the progress at the bottom of screen, make the slider a pentagon instead of a circle, ex.:* **[? – 'carrier']**	Interactive response scale; ~~Submit button;~~ Next button; *NOTE: The page needs to fit to browser so the user doesn't need to scroll down to see the slider scale; may need to remove image at top of page.* *Note: Joe - Default to middle point of the scale at beginning of screen.* Q/A [?] button 	Capture numerical value associated with position on scale. Treat as continuous scale ranging from 0-100, where anchor points are 0 = left-most position, leaning away 50= center position, Not sure 100=right-most position, leaning toward	(5) Leaning yes/no screen

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				Note: Drop custom infographic, no image on screen. *NOTE:* Add display window to show number associated with position of slider		Intermediate values captured as integers. Capture time in milliseconds spent on this screen	

D2.10. Single	IF EXPERIMENT ARM & SINGLE	What matters most to you when deciding if you should learn your child's carrier status results? There are lots of things to think about when deciding whether you want to learn your child's carrier status results. Are these reasons important or unimportant to you? • You want information about your family's risk for genetic conditions, even if it won't affect your child's health. • You could help scientists better understand the effects on children who grow up knowing their carrier status. • You are curious to know if your child is a carrier. • You do not see any harm in learning this information. • You could help scientists better		What matters most to you? (headline) Reasons to learn your child's carrier status results • You want information about your family's risk for genetic conditions, even if it won't affect your child's health. • You could help scientists better understand the effects on children who grow up knowing their carrier status. • You are curious to know if your child is a carrier. • You do not see any harm in learning this information. • You could help scientists better understand how parents respond to learning a child's carrier status. • *Are there any other reasons you can think of? Please type them here.*	Sorting task for users to move boxes with 'reasons for' into <u>two</u> bins labeled 'Important' and 'Unimportant' 2 interactive textboxes that allows users to write in 2 additional 'reasons for' that is not listed; write-in textbox is also sortable Allow participants to continue to next screen only after at least one statement is moved into a sorting category. ~~Submit button~~ Next button Q/A [?] button	Capture which bin (i.e., important, unimportant) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it not sorted into any box (i.e., values for each statement: important, unimporta	(6) Values clarification, input

		understand how parents respond to learning a child's carrier status. • *Are there any other reasons you can think of? Please type them here.* Move at least one statement to continue.		Move at least one statement to continue. **[? – 'genetic condition']** **[? – 'carrier']**		nt, not sorted)	

D2.10. Couple	IF EXPERIMENT ARM & COUPLE	What matters most to you when deciding if you should learn your child's carrier status results? There are lots of things to think about when deciding whether you want to learn your child's carrier status results. Are these reasons important or unimportant to you? If you and your partner disagree about the importance of a reason, you can move it into the box labelled "We disagree." • You want information about your family's risk for genetic conditions, even if it won't affect your child's health. • You could help scientists better understand the effects on children who grow up knowing their carrier status. • You are curious to know if your child is a carrier.		What matters most to you? (headline) Reasons to learn your child's carrier status results • You want information about your family's risk for genetic conditions, even if it won't affect your child's health. • You could help scientists better understand the effects on children who grow up knowing their carrier status. • You are curious to know if your child is a carrier. • You do not see any harm in learning this information. • You could help scientists better understand how parents respond to learning a child's carrier status. • *Are there any other reasons you can think of? Please type them here.*	Sorting task for users to move boxes with 'reasons for' into three bins labeled 'Important,' 'Unimportant,' and 'We disagree' 2 interactive textboxes that allows users to write in 2 additional 'reasons for' that is not listed; write-in textbox is also sortable Allow participants to continue to next screen only after at least one statement is moved into a sorting category. ~~Submit button~~ Next button Q/A [?] button	Capture which bin (i.e., important, unimportant) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it not sorted into any box (i.e., values for each statement: important, unimporta	(6) Values clarification, input

		• You do not see any harm in learning this information. • You could help scientists better understand how parents respond to learning a child's carrier status. • *Are there any other reasons you can think of? Please type them here.* Move at least one statement to continue.		Move at least one statement to continue. **[? – 'genetic condition']** **[? – 'carrier']**		nt, not sorted)	

D2. 11.Single	IF EXPERIMENT ARM & SINGLE	Are the following reasons to *not* learn your child's carrier status results important or unimportant to you? Please sort each of the following reasons into the boxes labelled *important or not important* as they appear on screen. • You are worried your child could face discrimination because of his or her carrier status. • You would rather wait until your child can make his or her own decision about learning this information. • Knowing your child is a carrier could cause you to worry or feel anxious. • You are worried that if your child is a carrier, it may lead you to treat him or her differently. • The idea of learning your child's carrier status makes you uncomfortable.		What matters most to you? (headline) Reasons not to learn your child's carrier status results • You are worried your child could face discrimination because of his or her carrier status. • You would rather wait until your child can make his or her own decision about learning this information. • Knowing your child is a carrier could cause you to worry or feel anxious. • You are worried that if your child is a carrier, it may lead you to treat him or her differently. • The idea of learning your child's carrier status makes you uncomfortable. • *Add reason (x5)* **[? – 'carrier']**	Sorting task for users to move boxes with 'reasons against' into two bins labeled 'Important' and 'Not important' 5 interactive textboxes that allows users to write in 5 additional 'reasons against' that is not listed; write-in textbox is also sortable ~~Allow participants to continue to next screen only after at least one statement is moved into a sorting category.~~ ~~Submit button~~ Next button Q/A [?] button	Capture which bin (i.e., important, unimportant) user sorts each 'reason against' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it not sorted into any box (i.e., values for each statement: important,	(6) Values clarification, input

		• *Are there any other reasons not to learn your child's carrier status results that you can think of? Please type them in the text boxes labelled "Add reason"* When you are done sorting, click the next button to move forward. You must move at least one reason to continue.				unimportant, not sorted)	

D2. 11.Couple	IF EXPERIMENT ARM & COUPLE	Are the following reasons to *not* learn your child's carrier status results important or unimportant to you? Or do you and your partner disagree? Please sort each of the following reasons into the boxes labelled *important to us, not important to us, or we disagree* as they appear on screen. • You are worried your child could face discrimination because of his or her carrier status. • You would rather wait until your child can make his or her own decision about learning this information. • Knowing your child is a carrier could cause you to worry or feel anxious. • You are worried that if your child is a carrier, it may lead you to treat him or her differently.		What matters most to you? (headline) Reasons not to learn your child's carrier status results • You are worried your child could face discrimination because of his or her carrier status. • You would rather wait until your child can make his or her own decision about learning this information. • Knowing your child is a carrier could cause you to worry or feel anxious. • You are worried that if your child is a carrier, it may lead you to treat him or her differently. • The idea of learning your child's carrier status makes you uncomfortable. • *Add reason (x5)* ~~Move at least one statement to continue.~~ **[? – 'carrier']**	Sorting task for users to move boxes with 'reasons for' into three bins labeled 'Important to us,' 'Not important to us' and 'We disagree' 5 interactive textboxes that allows users to write in 5 additional 'reasons against' that is not listed; write-in textbox is also sortable ~~Allow participants to continue to next screen only after at least one statement is moved into a sorting category.~~ ~~Submit button~~ Next button Q/A [?] button	Capture which bin (i.e., important, unimportant, disagree) user sorts each 'reason for' Capture text user types into interactive text box Capture time in millisecond s spent on this screen Capture which box each statement was sorted into or if it not sorted into any box (i.e., values for each statement:	(6) Values clarification, input

		• The idea of learning your child's carrier status makes you uncomfortable. • *Are there any other reasons not to learn your child's carrier status results that you can think of? Please type them in the text boxes labelled "Add reason"* When you are done sorting, click the next button to move forward. You must move at least one reason to continue.				important, unimportant, disagree, not sorted)	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.12. Single	IF EXPERIMENT ARM & SINGLE	Here are the reasons for and against learning your child's carrier status results that matter most to you. When you are done reviewing these reasons, click the next button to move forward.		Here are the reasons that matter most to you. ((Headline) Two boxes on screen. One is labelled "Reasons to learn your child's carrier status results." In this box, list the reasons that the user sorted into the 'important' box from screen D2.10.Single "Reasons not to learn your child's carrier status results" In this box, list the reasons that the user sorted into the 'important' box from screen D2.11.Single	Visually present whether user sorted 'reasons for' as important on screen D2.10.Single and 'reasons against' as important on screen D2.11.Single Any statement that was not sorted into a category is not displayed on review screen. Replay button Next button		(7) Values clarification, review

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.12. Couple	IF EXPERIMENT ARM & COUPLE	Here are the reasons for and against learning your child's carrier status results that matter most to you. When you are done reviewing these reasons, click the next button to move forward.		Here are the reasons that matter most to you. (Headline) Two boxes on screen. One is labelled "Reasons to learn your child's carrier status results." In this box, list the reasons that the user sorted into the 'important' box from screen D2.10.Couple "Reasons not to learn your child's carrier status results" In this box, list the reasons that the user sorted into the 'important' box from screen D2.11.Couple	Visually present whether user sorted 'reasons for' as important on screen D2.10.Couple and 'reasons against' as important on screen D2.11.Couple Any statement that was not sorted into a category is not displayed on review screen. Replay button Next button		(7) Values clarification, review

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.12.a	IF EXPERIMENT ARM	In this part of the decision guide, you will learn about medically actionable adult onset conditions.		Medically Actionable Adult Onset Conditions	Replay button Next button		(X) Display Section Title Screen *Note*: Need new screen format, Header should be blank, and text on screen will be centered and large display type.
D2.13	IF EXPERIMENT ARM	What is a medically actionable adult onset condition? These are rare but serious genetic conditions that… • Usually do not begin until adulthood. • Can be improved with treatment, and • The benefits of treatment typically outweigh the risks.	Medically actionable adult onset conditions begin in adulthood and can be improved with treatment.	What is a medically actionable adult onset condition? (headline) *Image note:* Show pictures that indicate medical treatment for adult conditions. Medically actionable adult onset conditions… • Rare and serious • Begin in adulthood • Can be improved with treatment	Replay button Next button Q/A [?] button		(3) General content, text plus image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		The NC NEXUS study will look for more than a hundred of these conditions The signs and symptoms of medically actionable adult onset conditions differ greatly from one to the next.		• Benefits of treatment outweigh risks • More than 100 of these conditions **[? – 'genetic condition']** **[? – 'medically actionable condition']**			
D2.14	IF EXPERIMENT ARM	One example of these conditions is Lynch syndrome. People with Lynch syndrome are more likely to get colon cancer, as well as several other types of cancer. Cancers caused by Lynch syndrome usually begin between the ages of 40 and 60. These cancers can often be prevented by early screening or surgery. About 5 to 15 out of every 10,000 people in the United States have Lynch syndrome.	Lynch syndrome is an example of a medically actionable adult condition.	What is a medically actionable adult onset condition? (headline) *Visual notes:* Show risk array for 5 to 15 out of 10,000. Timed to display with last bullet (Here are links to some example risk arrays: conjoint study ex. BRCA1 ex) *Display:* One example is Lynch syndrome • More likely to get colon cancer • Begin between the ages of 40 and 60	Replay button Next button		(4) general content, text plus image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				• Can often be prevented by early screening or surgery • 5 to 15 out of every 10,000 people in the U.S. have Lynch syndrome			
D2.15	IF EXPERIMENT ARM	What can genomic sequencing tell you about medically actionable adult onset conditions? If you request these results, the NC NEXUS team will look for gene differences that cause this type of genetic condition. Finding these gene differences in your child's DNA can tell if he or she is	NC NEXUS will use genomic sequencing to look for gene differences that lead to specific conditions. These gene differences	What can genomic sequencing tell you? (headline) *Visual note:* We need a visual that somehow depicts the conditions. • NC NEXUS will look for this type of condition • Tell if your child is more likely to get one of these conditions as an adult • Will not know if or when the condition will set in	Replay button Next button Q/A [?] button		(3) or (4) General content, text plus image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		more likely to get one of these conditions as an adult. Still, we will not know for sure if or when signs of the condition will set in and how severe it will be because other factors also play a part in most conditions.	are not the *only* cause.	• Other factors play a part in most conditions **[? – DNA]** **[? – 'genetic condition']** **[? – 'medically actionable condition']**			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.16	IF EXPERIMENT ARM	How common is it for genomic sequencing to find gene differences that lead to a medically actionable adult onset condition? It is not known for sure how often genomic sequencing will find gene differences that cause these conditions. This is one of the things the NC NEXUS study will try to find out. The best estimate is that sequencing will find one of these gene differences in about 2% or 3% of children. Genomic sequencing done by the NC NEXUS study cannot find *all* gene differences related to *all* medically actionable adult onset conditions.	The NC NEXUS study team wants to find out how often genomic sequencing will find gene differences that lead to a health problem.	How common is it for genomic sequencing to find gene differences? (headline) • Not known how often sequencing will find these conditions. • The best estimate is in about 2% or 3% of children • Genomic sequencing cannot find all gene differences related to all conditions *Image notes:* Risk array. Visual to depict that it is unsure exactly how likely it is that a gene difference will be found, but about 2 or 3 out of 100 children. Maybe a risk array. **[? – genomic sequencing]** **[? – gene differences]** **[? – 'medically actionable condition']**	Replay button Next button Q/A [?] button		(3) or (4) General content, text plus image
D2.17	IF EXPERIMENT ARM	What would finding gene differences that lead to a medically actionable adult		What would finding gene differences mean? (headline)	Replay button Next button		(3) or (4) General content, text plus image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		onset condition mean for your child? Learning that your child has these gene differences will not affect your child's health right now. But it could help your child's doctors in the future recommend ways to prevent or delay a condition that would likely begin in adulthood. Some people think it is wrong for parents to learn whether their children have gene differences that cause conditions that cannot be treated until adulthood because it takes away the choice from the children to decide to learn these things themselves. One possible risk is that your child could face discrimination based on this type of finding. We do not know if learning this information about your child will be more helpful or more harmful.		*Visual note*: Picture of older teenager young adult in doctor's office. • Will not affect your child's health right now • Could help your child's doctors in the future • Takes away the choice from children to learn these things • Your child could face discrimination based on this type of finding • We do not know if learning this information will be more helpful or more harmful **[? – gene differences]** **[? – 'medically actionable condition']**	Q/A [?] button		

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.18	IF EXPERIMENT ARM	What would finding gene differences that lead to a medically actionable adult onset condition mean for you? The gene differences that cause many of these conditions are passed on in such a way that finding them in a child could mean that one of the parents has the same gene differences. If your child has a gene difference that causes a medically actionable adult condition, you might think about having testing for yourself. In this way, your child's genomic sequencing results could lead you to receive early treatment or prevention services.	If your child has a gene difference that causes a medically actionable adult condition, you could mean that one of the parents will have the condition.	What would finding gene differences mean for you? (headline) • Could mean one of the parents has the same gene differences • You might think about testing for yourself • Your child's results could lead you to early treatment or prevention **[? – gene differences]** **[? – genetic testing]** **[? – 'medically actionable condition']**	Replay button Next button Q/A [?] button		(3) or (4) general content, text plus image

D2.19	IF EXPERIMENT ARM	~~What happens if your child has a gene difference that causes a medically actionable adult onset condition?~~ If you request these results and genomic sequencing finds that your child has gene differences that cause a medically actionable adult onset condition: • These results will be confirmed with another test • A genetic counselor and a doctor will meet with you to discuss the results and how your child should be followed up as an adult. • You will be given information about how you can have testing for yourself. • You will be asked if you want the results added to your child's health record at UNC Hospitals.		What if genomic sequencing finds these conditions? (headline) ~~If genomic sequencing finds gene differences that cause a medically actionable adult onset condition~~ • Results will be confirmed with another test • A genetic counselor and a doctor will discuss the results with you. • You will be given information about testing for yourself • Asked if you want the results added to your child's health record **[? – genomic sequencing]** **[? – gene differences]** **[? – genetic counselor]** **[? – genetic testing]** **[? – 'medically actionable condition']** *NOTE: Keep as numbered list (instead of bullets)*	Replay button Next button Q/A [?] button		(3) general content, text plus image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.20	IF EXPERIMENT ARM	If you had to decide right now, which way are you leaning about learning your child's results for medically actionable adult onset conditions? Click and drag the slider, moving it to the point on the scale that fits your answer. Leaning away from learning these results Not sure Leaning toward learning these results When you are done, click the next button to continue.		Which way are you leaning? (headline) *Note:* Interactive scale. *NOTE: Example layout here* file://rtints6/hserproj4/0214132%20NEXUS%20Proj%203/Aim%202%20Decision%20Aids/2.1%20DA%20Content/Final%20Decision%20Aid%20Content/working/DA1_slider%20scale%20example.docx Which way are you leaning about learning your child's results for medically actionable adult onset conditions? Leaning away from learning these results------- Not sure-------Leaning toward learning these results *NOTE: To differentiate the slider from the progress at the bottom of screen, make the slider a pentagon instead of a circle, ex.:* **[? – 'carrier']**	Interactive response scale; ~~Submit button;~~ Next button; Q/A [?] button *NOTE: The page needs to fit to browser so the user doesn't need to scroll down to see the slider scale; may need to remove image at top of page.* *Note: Joe - Default to middle point of the scale at beginning of screen.*	Capture numerical value associated with position on scale. Treat as continuous scale ranging from 0-100, where anchor points are 0 = left-most position, leaning away 50= center position, Not sure 100=right-most position, leaning toward	(5) Leaning yes/now screen

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				[? – 'medically actionable condition'] Note: Drop custom infographic, no image on screen.		Intermediate values captured as integers. Capture time in milliseconds spent on this screen	
D2.21. Single	IF EXPERIMENT ARM & SINGLE	What matters most to you when deciding if you should learn your child's results for medically actionable adult onset conditions? There are lots of things to think about when deciding whether you want to learn your child's sequencing results for medically actionable adult onset conditions. Are these reasons important or unimportant to you? • Your child's future doctors might be helped by knowing this information when your child is an adult		What matters most to you? (Headline) Reasons to learn your child's genomic sequencing results for medically actionable adult onset conditions • Your child's future doctors might be helped by knowing this information when your child is an adult • The results may help you prepare your child for the future. • You want to know if you are at greater risk for one of these conditions. • You might benefit from early treatment if you	Sorting task for users to move boxes with 'reasons for' into <u>two</u> bins labeled 'Important' and 'Unimportant' 2 interactive textboxes that allows users to write in 2 additional 'reasons for' that is not listed; write-in textbox is also sortable Allow participants to continue to next screen only after at least one statement is	Capture which bin (i.e., important, unimportant) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		• The results may help you prepare your child for the future. • You want to know if you are at greater risk for one of these conditions. • You might benefit from early treatment if you learn you have one of these conditions. • You could help scientists better understand the effects on children who grow up knowing their results before they have signs of the condition. • *Are there any other reasons you can think of? Please type them here.*		learn you have one of these conditions. • You could help scientists better understand the effects on children who grow up knowing their results before they have signs of the condition. • *Are there any other reasons you can think of? Please type them here.* **[? – genomic sequencing]** **[? – 'medically actionable condition']**	moved into a sorting category. ~~Submit button~~ Next button Q/A [?] button	each statement was sorted into or if it not sorted into any box (i.e., values for each statement: important, unimportant, not sorted)	

D2.21. Couple	IF EXPERIMENT ARM & COUPLE	What matters most to you when deciding if you should learn your child's results for medically actionable adult onset conditions? There are lots of things to think about when deciding whether you want to learn your child's sequencing results for medically actionable adult onset conditions. Are these reasons important or unimportant to you? If you and your partner disagree about the importance of a reason, you can move it into the box labelled "We disagree." • Your child's future doctors might be helped by knowing this information when your child is an adult • The results may help you prepare your child for the future. • You want to know if you or your partner are at greater risk for one of these conditions.		What matters most to you? (Headline) Reasons to learn your child's genomic sequencing results for medically actionable adult onset conditions • Your child's future doctors might be helped by knowing this information when your child is an adult • The results may help you prepare your child for the future. • You want to know if you or your partner are at greater risk for one of these conditions. • You or your partner might benefit from early treatment if you learn that you have one of these conditions. • You could help scientists better understand the effects on children who grow up knowing their results before they have signs of the condition.	Sorting task for users to move boxes with 'reasons for' into three bins labeled 'Important,' 'Unimportant,' and 'We disagree' 2 interactive textboxes that allows users to write in 2 additional 'reasons for' that is not listed; write-in textbox is also sortable Allow participants to continue to next screen only after at least one statement is moved into a sorting category. ~~Submit button~~ Next button Q/A [?] button	Capture which bin (i.e., important, unimportant, disagree) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it not sorted into any box (i.e., values for each statement:	(6) Values clarification, input

		• You or your partner might benefit from early treatment if you learn that you have one of these conditions. • You could help scientists better understand the effects on children who grow up knowing their results before they have signs of the condition. • *Are there any other reasons you can think of? Please type them here.* Move at least one statement to continue.		• *Are there any other reasons you can think of? Please type them here.* Move at least one statement to continue. **[? – genomic sequencing]** **[? – 'medically actionable condition']**		important, unimportant, disagree, not sorted)	

D2.22. Single	IF EXPERIMENT ARM & SINGLE	Are the following reasons <u>not</u> to learn your child's genomic sequencing results for medically actionable adult onset conditions important or unimportant to you? Please sort each of the following reasons into the boxes labelled *important* or *not important* as they appear on screen. Are these reasons important or unimportant to you? • You think the decision to learn this information should be left to your child, when he or she is an adult. • Any benefit of knowing these results will not apply to your child for many years. • Knowing this information could cause you to worry or feel anxious. • Learning this information could cause your child to have		What matters most to you? (Headline) Reasons <u>not</u> to learn your child's genomic sequencing results for medically actionable adult onset conditions • You think the decision to learn this information should be left to your child, when he or she is an adult. • Any benefit of knowing these results will not apply to your child for many years. • Knowing this information could cause you to worry or feel anxious. • Learning this information could cause your child to have problems getting life insurance, disability insurance, or long-term care insurance as an adult. • You are not prepared to learn that you are more likely to have gene differences that cause a	Sorting task for users to move boxes with 'reasons against' into <u>two</u> bins labeled 'Important' and 'Not important' Interactive textbox that allows users to write in up to 5 'reason against' that is not listed; write-in textbox is also sortable ~~Allow participants to continue to next screen only after at least one statement is moved into a sorting category.~~ ~~Submit button~~ Next button Q/A [?] button *NOTE: The 'Add reason' box is*	Capture which bin (i.e., important, unimportant) user sorts each 'reason against' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it not sorted into any box (i.e., values for each statement: important,	(6) Values clarification, input

		problems getting life insurance, disability insurance, or long-term care insurance as an adult. • You are not prepared to learn that you are more likely to have gene differences that cause a medically actionable adult condition. • *Are there any other reasons* not to learn results for medically actionable adult onset conditions that *you can think of? Please type them in the text boxes labelled "Add reason"* When you are done sorting, click the next button to move forward. You must move at least one reason to continue.		medically actionable adult condition. • *Add reason (x5)* *NOTE: Change label from 'Add custom reason' → 'Add reason'* Move at least one reason to continue. **[? – genomic sequencing]** **[? – gene differences]** **[? – 'medically actionable condition']** *NOTE: Unsorted reasons cannot be positioned underneath the important, unimportant, etc. boxes…the way the columns line up, it looks like the reasons are already sorted. Maybe put the unsorted box as column on left side of screen or above the other boxes?*	*complicated b/c it requires users need to click the pencil before they can enter text. Can this be changed to function like a more standard textbox, where they just need to click on the text field itself to enter text?*	unimportant, not sorted)	

D2.22. Couple	IF EXPERIMENT ARM & COUPLE	Are the following reasons <u>not</u> to learn your child's genomic sequencing results for medically actionable adult onset conditions important or unimportant to you? Or do you and your partner disagree? Please sort each of the following reasons into the boxes labelled *iImportant to us, Not important to us, or We disagree* as they appear on screen. • You think the decision to learn this information should be left to your child, when he or she is an adult. • Any benefit of knowing these results will not apply to your child for many years. • Knowing this information could cause you to worry or feel anxious. • Learning this information could cause your child to have problems getting life		What matters most to you? (Headline) Reasons <u>not</u> to learn your child's genomic sequencing results for medically actionable adult onset conditions • You think the decision to learn this information should be left to your child, when he or she is an adult. • Any benefit of knowing these results will not apply to your child for many years. • Knowing this information could cause you to worry or feel anxious. • Learning this information could cause your child to have problems getting life insurance, disability insurance, or long-term care insurance as an adult. • You are not prepared to learn that you or your partner are more likely to have gene differences that cause a medically actionable adult condition.	Sorting task for users to move boxes with 'reasons for' into <u>three</u> bins labeled 'Important to us,' 'Not important to us' and 'We disagree' 5 interactive textboxes that allows users to write in 5 additional 'reasons against' that is not listed; write-in textbox is also sortable ~~Allow participants to continue to next screen only after at least one statement is moved into a sorting category.~~ ~~Submit button~~ Next button Q/A [?] button	Capture which bin i.e., important, unimportant, disagree) user sorts each 'reason for' Capture text user types into interactive text box Capture time in millisecond s spent on this screen Capture which box each statement was sorted into or if it not sorted into any box (i.e., values for each statement:	(6) Values clarification, input

		insurance, disability insurance, or long-term care insurance as an adult. • You are not prepared to learn that you or your partner are more likely to have gene differences that cause a medically actionable adult condition. • *Are there any other reasons* not to learn results for medically actionable adult onset conditions that *you can think of? Please type them in the text boxes labelled "Add reason"* When you are done sorting, click the next button to move forward. You must move at least one reason to continue.		• *Add reason (x5)* Move at least one reason to continue. **[? – genomic sequencing]** **[? – gene differences]** **[? – 'medically actionable condition']**		important, unimportant, disagree, not sorted)	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.23. Single	IF EXPERIMENT ARM & SINGLE	Here are the reasons for and against learning genomic sequencing results for medically actionable adult onset condition that matter most to you. When you are done reviewing these reasons, click the next button to move forward.		Here are the reasons that matter most to you. (Headline) Two boxes on screen. One is labelled "Reasons to learn your child's results for medically actionable adult onset conditions." In this box, list the reasons that the user sorted into the 'important' box from screen D2.21.Single "Reasons not to learn your child's results for medically actionable adult onset condition." In this box, list the reasons that the user sorted into the 'important' box from screen D2.22.Single	Visually present whether user sorted 'reasons for' as important on screen D2.21.Single and 'reasons against' as important on screen D2.22.Single Any statement that was not sorted into a category is not displayed on review screen. Replay button Next button		(7) Values clarification, review

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.23. Couple	IF EXPERIMENT ARM & COUPLE	Here are the reasons for and against learning genomic sequencing results for medically actionable adult onset condition that matter most to you. When you are done reviewing these reasons, click the next button to move forward.		Here are the reasons that matter most to you. (Headline) Two boxes on screen. One is labelled "Reasons to learn your child's results for medically actionable adult onset conditions." In this box, list the reasons that the user sorted into the 'important' box from screen D2.21.Couple "Reasons not to learn your child's results for medically actionable adult onset condition." In this box, list the reasons that the user sorted into the 'important' box from screen D2.22.Couple	Visually present whether user sorted 'reasons for' as important on screen D2.21.Couple and 'reasons against' as important on screen D2.22.Couple Any statement that was not sorted into a category is not displayed on review screen. Replay button Next button		(7) Values clarification, review

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.23.a	IF EXPERIMENT ARM	In this part of the decision guide, you will learn about non-medically actionable childhood conditions.		Non-Medically Actionable Childhood Conditions	Replay button Next button		(X) Display Section Title Screen *Note*: Need new screen format, Header should be blank, and text on screen will be centered and large display type.
D2.24	IF EXPERIMENT ARM	~~Non-Medically Actionable Childhood Conditions~~ What is a non-medically actionable childhood condition? These conditions are rare but serious genetic conditions that… • Usually begin early in a child's life. • May have some symptoms that can be treated, but	Non-medically actionable childhood conditions begin early in a child's life and there are no medical treatments that can cure the condition.	What is a non-medically actionable childhood condition? (headline) *Visual note:* Show pictures that indicate medical treatment. Child in doctors Non-medically actionable childhood conditions… • Rare and serious • Begin early in a child's life • Some symptoms can be treated • No treatments to improve the condition itself	Replay button Next button Q/A [?] button		(3) General content, text plus image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		• There are no effective medical treatments to prevent or improve the condition itself. **The NC NEXUS study will look** for about 3,000 of **these conditions** The signs and symptoms of non-medically actionable childhood conditions differ greatly from one to the next.		• About 3,000 of these conditions *NOTE:* There is a typo in web app in 'Rare and serious' line **[? – 'genetic condition']** **[? – 'non-medically actionable condition']**			
D2.25	IF EXPERIMENT ARM	Mowat-Wilson syndrome is one example of a non-medically actionable childhood condition. Children with this genetic condition have distinctive facial features, intellectual disabilities, and many have seizures. They are also not able to sit, stand, and walk at the same age as other children. Many children who have Mowat-Wilson syndrome can understand what others say, but only learn to speak a few words themselves. There are no treatments to prevent or improve the child's	Mowat-Wilson syndrome is an example of a non-medically actionable childhood condition.	What is a non-medically actionable childhood condition? (headline) *Visual note.* Risk array 1 to 2 out of 100,000 babies. Timed to display with last bullet Mowat-Wilson syndrome is one example • Distinctive facial features and intellectual disabilities • Not able to sit, stand, and walk at the same age as other children • No treatments prevent or improve the child's intellectual disability	Replay button Next button Q/A [?] button		(4) General content, text plus image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		intellectual disability. About 1 to 2 out of every 100,000 babies born in the United States have Mowat-Wilson syndrome.		• 1 to 2 out of every 100,000 babies born in the U.S. have Mowat-Wilson syndrome **[? – 'genetic condition']**			
D2.26	IF EXPERIMENT ARM	What can genomic sequencing tell you about non-medically actionable childhood conditions? If you request these results, the NC NEXUS team will look for gene differences that cause specific conditions. Finding these gene differences in your child's DNA can tell that he or she is much more likely to have one of these conditions during childhood. Still, it is hard to know for sure how severe the condition would be because other factors		What can genomic sequencing tell you? (headline) *Image:* We need a visual that somehow depicts these conditions. *Display:* • NC NEXUS will look for specific conditions • Tell that a child is more likely to have one of these conditions • It is hard to know how severe the condition would be • Other factors play a part in most conditions **[? – DNA]**	Replay button Next button Q/A [?] button		(3) or (4) General, text plus image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		also play a part in most conditions.		**[? – gene differences]** **[? – 'non-medically actionable condition']**			
D2.27	IF EXPERIMENT ARM	How common is it for genomic sequencing to find gene differences that lead to a non-medically actionable childhood condition? It is not known for sure how often genomic sequencing will find gene differences that cause these conditions. This is one of the things the NC NEXUS study will try to find out. The best estimate is that sequencing will find one of these gene differences in less than 1% of children. Genomic sequencing done by the NC NEXUS study cannot find *all* gene differences related to *all* non-medically actionable childhood conditions.	The NC NEXUS study team wants to find out how often genomic sequencing will find gene differences that lead to a health problem.	How common is it for genomic sequencing to find gene differences? (headline) • Not known how often sequencing will find these conditions. • The best estimate is in less than 1% of children • Genomic sequencing cannot find all gene differences related to all conditions *Visual note:* Risk array. Visual to depict that it is unsure exactly how likely it is that a gene difference will be found, but less than 1 out of 100. Timed to come up with second bullet. **[? – genomic sequencing]** **[? – gene differences]** **[? – 'non-medically actionable condition']**	Replay button Next button Q/A [?] button		(3) or (4) General content, Text plus image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.28	IF EXPERIMENT ARM	What would finding gene differences that lead to a non-medically actionable childhood condition mean for your child? Learning that your child has these gene differences will not allow your child's doctor to take specific steps to prevent it. That's because, right now, there are no definite ways to use the information to help protect your child's health. Parents may have different views on whether or not learning this information about their child is harmful and distressing or valuable and helpful.		What would finding gene differences mean? (headline) Note. Visuals to depict that different parents will have differing views • Will not allow your child's doctor take steps to prevent it. • No ways to use the information to help protect your child's health • Different views on whether this information is harmful or helpful **[? – gene differences]** **[? – 'non-medically actionable condition']**	Replay button Next button Q/A [?] button		(3) or (4) General content
D2.29	IF EXPERIMENT ARM	Some parents would prefer not to learn about these gene differences in their child. They may be concerned that the information will make them worry about their child's future health. Other parents might be concerned that not knowing for sure	Knowing might cause some parents to worry excessively. Knowing might cause some	What would finding gene differences mean? (headline) *Image note:* Concerned/stressed-looking parents • Some parents prefer not to learn about these gene differences	Replay button Next button Q/A [?] button		(3) or (4) General content, image and text

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		when and how the condition will develop would cause them to think their child is sick even when he or she is healthy.	parents might treat a healthy child like s/he is sick.	• Worry about their child's future health. • Think their child is sick when healthy **[? – gene differences]**			

D2.30	IF EXPERIMENT ARM	Some parents might think it is useful to learn this information. Even though these conditions are not preventable right now, new treatments may become available in the future. Knowing your child has gene differences that cause a non-medically actionable childhood condition may help you and your child's doctor prepare for the condition if symptoms appear, refer your child to support services, and get new treatments sooner if they become available.	Knowing might help parents prepare for the condition and act fast if new treatments are developed.	What would finding gene differences mean? (headline)
				
				• Some parents might think it is useful
				  • New treatments may become available in the future
				  • May help you prepare for the condition
				  • Refer child to support services
				  • Get new treatments sooner
				
				[? – 'non-medically actionable condition']
					Replay button
					
					Next button
					
					Q/A [?] button
							(3) or (4) General content, image and text

D2.30.a	IF EXPERIMENT ARM	Some diseases are very difficult for doctors to diagnose, even after symptoms appear. Learning that your child has gene differences for these		What would finding gene differences mean? (headline) • Some diseases are difficult to diagnose	Replay button Next button		(3) or (4) General content, image and text

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		conditions may lower the number of tests your child's doctor would need to explain the symptoms.		• Lower number of tests to explain symptoms			
D2.31	IF EXPERIMENT ARM	~~What happens if your child has a gene difference that causes a non-medically actionable childhood condition?~~ If you request these results and genomic sequencing finds that your child has gene differences that cause these conditions: • These results will be confirmed with another test. • A genetic counselor and a doctor will meet with you to discuss the results. • You will be given information about other testing, if it is needed. • You will be asked if you want the results added to your child's health record at UNC Hospitals.		What if genomic sequencing finds these conditions? (headline) • Results will be confirmed with another test • A genetic counselor and a doctor will discuss the results with you. • You will be given information about other testing, if needed • Asked if you want the results added to your child's health record **[? – genomic sequencing]** **[? – gene differences]** **[? – genetic counselor]** **[? – 'non-medically actionable condition']**	Replay button Next button Q/A [?] button		(3) general content, Text plus image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.32	IF EXPERIMENT ARM	If you had to decide right now, which way are you leaning about learning your child's results for non-medically actionable childhood conditions? Click and drag the slider, moving it to the point on the scale that fits your answer. Leaning away from learning these results Not sure Leaning toward learning these results When you are done, click the next button to continue.		Which way are you leaning? (headline) *Note:* Interactive scale *NOTE: Example layout here* file://rtints6/hserproj4/0214132%20NEXUS%20Proj%203/Aim%202%20Decision%20Aids/2.1%20DA%20Content/Final%20Decision%20Aid%20Content/working/DA1_slider%20scale%20example.docx Which way are you leaning about learning your child's results for non-medically actionable childhood conditions? Leaning away from learning these results------- Not sure---------Leaning toward learning these results *NOTE: To differentiate the slider from the progress at the*	Interactive response scale; ~~Submit button~~ Next button; Q/A [?] button *Note: Joe - Default to middle point of the scale at beginning of screen.*	Capture numerical value associated with position on scale. Treat as continuous scale ranging from 0-100, where anchor points are 0 = left-most position, leaning away 50= center position, Not sure	(5) Leaning yes/now screen

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				bottom of screen, make the slider a pentagon instead of a circle, ex.: **[? – 'non-medically actionable conditions']** Note: Drop custom infographic, no image on screen.		100=right-most position, leaning toward Intermediate values captured as integers. Capture time in milliseconds spent on this screen	

D2.33. Single	IF EXPERIMENT ARM & SINGLE	What matters most to you when deciding if you should learn your child's genomic sequencing results for non-medically actionable childhood conditions? There are lots of things to think about when deciding if you want to learn your child's sequencing results for non-medically actionable childhood conditions. Are the following reasons important or unimportant to you? • The results may help your child's doctor diagnose your child's symptoms. • Children with these gene differences will be referred to support services. • Knowing this information could help you get new treatments for your child if they become available. • You could help scientists better understand how		What matters most to you? (Headline) Reasons to learn your child's genomic sequencing results for non-medically actionable childhood conditions • The results may help your child's doctor diagnose your child's symptoms. • Children with these gene differences will be referred to support services. • Knowing this information could help you get new treatments for your child if they become available. • You could help scientists better understand how these conditions affect children before signs appear. • The results may help you learn your chances of having other children with the same condition. • *Are there any other reasons you can think of? Please type them here.*	Sorting task for users to move boxes with 'reasons for' into <u>two</u> bins labeled 'Important' and 'Unimportant' 2 interactive textboxes that allows users to write in 2 additional 'reasons for' that is not listed; write-in textbox is also sortable Allow participants to continue to next screen only after at least one statement is moved into a sorting category. ~~Submit button~~ Next button Q/A [?] button	Capture which bin (i.e., important, unimportant) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it not sorted into any box (i.e., values for each statement: important, unimporta	(6) Values clarification, input

		these conditions affect children before signs appear. • The results may help you learn your chances of having other children with the same condition. • *Are there any other reasons you can think of? Please type them here.* Move at least one statement to continue.		Move at least one statement to continue. **[? – genomic sequencing]** **[? – gene differences]** **[? – 'non-medically actionable condition']**		nt, not sorted)	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.33. Couple	IF EXPERIMENT ARM & COUPLE	What matters most to you when deciding if you should learn your child's genomic sequencing results for non-medically actionable childhood conditions? There are lots of things to think about when deciding if you want to learn your child's sequencing results for non-medically actionable childhood conditions. Are the following reasons important or unimportant to you? If you and your partner disagree about the importance of a reason, you can move it into the box labelled "We disagree." • The results may help your child's doctor diagnose your child's symptoms. • Children with these gene differences will be referred to support services.		What matters most to you? (Headline) Reasons to learn your child's genomic sequencing results for non-medically actionable childhood conditions • The results may help your child's doctor diagnose your child's symptoms. • Children with these gene differences will be referred to support services. • Knowing this information could help you get new treatments for your child if they become available. • You could help scientists better understand how these conditions affect children before signs appear. • The results may help you learn your chances of having other children with the same condition. • *Are there any other reasons you can think of? Please type them here.*	Sorting task for users to move boxes with 'reasons for' into three bins labeled 'Important,' 'Unimportant,' and 'We disagree' 2 interactive textboxes that allows users to write in 2 additional 'reasons for' that is not listed; write-in textbox is also sortable Allow participants to continue to next screen only after at least one statement is moved into a sorting category. ~~Submit button~~ Next button Q/A [?] button	Capture which bin (i.e., important, unimportant, disagree) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it not sorted into any box (i.e., values for	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		• Knowing this information could help you get new treatments for your child if they become available. • You could help scientists better understand how these conditions affect children before signs appear. • The results may help you learn your chances of having other children with the same condition. • *Are there any other reasons you can think of? Please type them here.* Move at least one statement to continue.		Move at least one statement to continue. **[? – genomic sequencing]** **[? – gene differences]** **[? – 'non-medically actionable condition']**		each statement: important, unimportant, disagree, not sorted)	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.34. Single	IF EXPERIMENT ARM & SINGLE	Are the following reasons important or unimportant to you? • Knowing this information will not help you and your child's doctor prevent the condition. • Learning this information might make you think your child is sick even when he or she is healthy. • Knowing this information could make you feel less connected to your child. • Learning this information could cause your child to have problems getting disability insurance or long-term care insurance. • You would rather not know this information because it is not sure if or when symptoms would begin. • *Are there any other reasons you can think of? Please type them here.*		What matters most to you? (Headline) Reasons not to learn your child's genomic sequencing results for non-medically actionable childhood conditions • Knowing this information will not help you and your child's doctor prevent the condition. • Learning this information might make you think your child is sick even when he or she is healthy. • Knowing this information could make you feel less connected to your child. • Learning this information could cause your child to have problems getting disability insurance or long-term care insurance. • You would rather not know this information because it is not sure if or when symptoms would begin.	Sorting task for users to move boxes with 'reasons against' into <u>two</u> bins labeled 'Important' and 'Unimportant' Interactive textbox that allows users to write in a 'reason against' that is not listed; write-in textbox is also sortable Allow participants to continue to next screen only after at least one statement is moved into a sorting category. ~~Submit button~~ Next button Q/A [?] button	Capture which bin (i.e., important, unimportant) user sorts each 'reason against' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it not sorted into any box (i.e., values for each	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		Move at least one statement to continue.		• *Are there any other reasons you can think of? Please type them here.* Move at least one statement to continue. **[? – genomic sequencing]** **[? – 'non-medically actionable condition']**		statement: important, unimportant, not sorted)	
D2.34. Couple	IF EXPERIMENT ARM & COUPLE	Are the following reasons important or unimportant to you? Or do you and your partner disagree? • Knowing this information will not help you and your		What matters most to you? (Headline) Reasons not to learn your child's genomic sequencing results for non-medically	Sorting task for users to move boxes with 'reasons for' into three bins labeled 'Important,'	Capture which bin i.e., important, unimportant, disagree)	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		child's doctor prevent the condition. • Learning this information might make you think your child is sick even when he or she is healthy. • Knowing this information could make you feel less connected to your child. • Learning this information could cause your child to have problems getting disability insurance or long-term care insurance. • You would rather not know this information because it is not sure if or when symptoms would begin. • *Are there any other reasons you can think of? Please type them here.* Move at least one statement to continue.		actionable childhood conditions • Knowing this information will not help you and your child's doctor prevent the condition. • Learning this information might make you think your child is sick even when he or she is healthy. • Knowing this information could make you feel less connected to your child. • Learning this information could cause your child to have problems getting disability insurance or long-term care insurance. • You would rather not know this information because it is not sure if or when symptoms would begin. • *Are there any other reasons you can think of? Please type them here.* **[? – genomic sequencing]** **[? – 'non-medically actionable condition']**	'Unimportant,' and 'We disagree' 2 interactive textboxes that allows users to write in 2 additional 'reasons against' that is not listed; write-in textbox is also sortable Allow participants to continue to next screen only after at least one statement is moved into a sorting category. ~~Submit button~~ Next button Q/A [?] button	user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it not sorted into any box (i.e., values for each statement: important, unimportant, disagree, not sorted)	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				Move at least one statement to continue.			
D2.35. Single	IF EXPERIMENT ARM & SINGLE	Here are the reasons for and against learning genomic sequencing results for non-medically actionable childhood condition that matter most to you.		What matters most to you? (Headline) Two boxes on screen. One is labelled "Reasons to learn your child's results for non-medically actionable childhood condition." In this box, list the reasons that the user sorted into the 'important' box from screen D2.33.Single "Reasons not to learn your child's results for medically actionable adult condition" In this box, list the reasons that the user sorted into the 'important' box from screen D2.34.Single	Visually present whether user sorted 'reasons for' as important on screen D2.33.Single and 'reasons against' as important on screen D2.34.Single Any statement that was not sorted into a category is not displayed on review screen. Replay button Next button		(7) Values clarification, review

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.35. Couple	IF EXPERIMENT ARM & COUPLE	Here are the reasons for and against learning genomic sequencing results for non-medically actionable childhood condition that matter most to you.		What matters most to you? (Headline) Two boxes on screen. One is labelled "Reasons to learn your child's results for non-medically actionable childhood condition." In this box, list the reasons that the user sorted into the 'important' box from screen D2.33.Couple "Reasons not to learn your child's results for medically actionable adult condition" In this box, list the reasons that the user sorted into the 'important' box from screen D2.34.Couple	Visually present whether user sorted 'reasons for' as important on screen D2.33.Couple and 'reasons against' as important on screen D2.34.Couple Any statement that was not sorted into a category is not displayed on review screen. Replay button Next button		(7) Values clarification, review

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.35.a	IF EXPERIMENT ARM	Now you have some decisions to make about the additional genomic sequencing results you just learned about. You may choose to request results for all three kinds of conditions, only one or two of them, or none of them. All of these options are up to you and, if you want, you can change your mind even after you have made your decision.		Decisions about additional genomic sequencing results			X) Display Section Title Screen *Note*: Need new screen format, Header should be blank, and text on screen will be centered and large display type.

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.36. Single	IF EXPERIMENT ARM AND SINGLE	Here are some questions that will help you decide if you want to learn one or more kinds of additional genomic sequencing results: Please answer "yes" or "no" to the following questions. You can pick your answers by clicking the button that matches your selection. • Will learning additional genomic sequencing results help you learn things that are important to you? • Do you have enough information to make a decision about learning one or more of the three kinds of additional sequencing results? • Are you prepared to learn one or more kinds of additional results from your child's genomic sequencing? • Are you interested in learning one or more kinds of additional		Questions to help you decide (headline) Yes No ❒ ❒ Will learning additional genomic sequencing results help you learn things that are important to you? ❒ ❒ Do you have enough information to make a decision about learning one or more of the three kinds of additional sequencing results? ❒ ❒ Are you prepared to learn one or more kinds of additional results from your child's	Check boxes/buttons for users to select yes or no for each question ~~Submit button~~ Next button; Q/A [?] button *NOTE:* Would it be possible to grey-out the list of questions when the page loads, and then have color appear as each is being read? Alternatively, have the questions appear one at a time, in sync with the narration.	Capture y/n answers to each question; Capture time in milliseconds spent on this screen	(8) Questions to help decide, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		genomic sequencing results? • Are you confident you can make the decision that is right for you and your family? When you are done, click the next button to move forward.		genomic sequencing? ❒ ❒ Are you interested in learning one or more kinds of additional genomic sequencing results? ❒ ❒ Are you confident you can decide? **[? – genomic sequencing]** *NOTE: Please add the words 'Yes' and 'No' on screen, perhaps at top of columns with yes/no buttons*			
D2.36. Couple	IF EXPERIMENT ARM	Here are some questions that will help you decide if you want to learn one or more kinds of additional		Questions to help you decide (headline)	Check boxes/buttons for users to select yes	Capture y/n answers to	(8) Questions to help decide, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
	AND COUPLE	genomic sequencing results: Please answer "yes" or "no" to the following questions. You can pick your answers by clicking the button that matches your selection: • Will learning additional genomic sequencing results help you learn things that are important to you? • Do you have enough information to make a decision about learning one or more of the three kinds of additional sequencing results? • Are you prepared to learn one or more kinds of additional results from your child's genomic sequencing? • Are you interested in learning one or more kinds of additional genomic sequencing results? • Are you and your partner confident you can make		Yes No ❒ ❒ Will learning additional genomic sequencing results help you learn things that are important to you? ❒ ❒ Do you have enough information to make a decision about learning one or more of the three kinds of additional sequencing results? ❒ ❒ Are you prepared to learn one or more kinds of additional results from your child's genomic sequencing? ❒ ❒ Are you interested in learning one or	or no for each question ~~Submit button~~ Next button; Q/A [?] button *NOTE:* Would it be possible to grey-out the list of questions when the page loads, and then have color appear as each is being read? Alternatively, have the questions appear one at a time, in sync with the narration.	each question; Capture time in millisecond s spent on this screen	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		the decision that is right for you and your family? When you are done, click the next button to move forward.		more kinds of additional genomic sequencing results? ❒ ❒ Are you and your partner confident you can decide? **[? – genomic sequencing]** *NOTE: Please add the words 'Yes' and 'No' on screen, perhaps at top of columns with yes/no buttons*			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.37. Single	IF EXPERIMENT ARM AND SINGLE	If you answered ***Yes*** to more of these questions, maybe you are ready to learn one or more kinds of additional genomic sequencing results. If you answered ***No*** to more, maybe this is not the right decision for your family at this time. You should make the decision that is best for you and your family. There are no right or wrong choices.		Questions to help you decide (headline) • Will learning additional genomic sequencing results help you learn things that are important to you? • Do you have enough information to make a decision about learning one or more of the three kinds of additional sequencing results? • Are you prepared to learn one or more kinds of additional results from your child's genomic sequencing? • Are you interested in learning one or more kinds of additional genomic sequencing results? • Are you confident you can decide?	Visually show whether user selected yes/no for each question from screen 'D2.36.Single' Replay button Next button		(9) Questions to help decide, review

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.37. Couple	IF EXPERIMENT ARM AND COUPLE	If you answered ***Yes*** to more of these questions, maybe you are ready to learn one or more kinds of additional genomic sequencing results. If you answered ***No*** to more, maybe this is not the right decision for your family at this time. You should make the decision that is best for you and your family. There are no right or wrong choices.		Questions to help you decide (headline) • Will learning additional genomic sequencing results help you learn things that are important to you? • Do you have enough information to make a decision about learning one or more of the three kinds of additional sequencing results? • Are you prepared to learn one or more kinds of additional results from your child's genomic sequencing? • Are you interested in learning one or more kinds of additional genomic sequencing results? • Are you and your partner confident you can decide?	Visually show whether user selected yes/no for each question from screen 'D2.36.Couple' Replay button Next button		(9) Questions to help decide, review

D2.38. Single	IF EXPERIMENT ARM & Single	How interested are you in learning your child's sequencing results for carrier status? Click and drag the slider, moving it to the point on the scale that fits your answer. • I'm definitely not interested in these results • I'm not sure • I'm definitely interested in in these results When you are done, click the next button to continue.		Making a decision about carrier status (Headline) *Note:* Interactive scale How interested are you in learning your child's sequencing results for carrier status? • Definitely not interested in these results • I'm not sure • Definitely interested in these results **[? – genomic sequencing]** **[? – 'carrier']** *NOTE: Example layout here* file://rtints6/hserproj4/0214132%20NEXUS%20Proj%203/Aim%202%20Decision%20Aids/2.1%20DA%20Content/Final%20Decision%20Aid%20Content/working/DA1_slider%20scale%20example.docx *NOTE: Change gradient in the slider line three separate color blocks, corresponding to the three response options. To*	Interactive response scale (slider); ~~Submit button~~ Next button; Q/A [?] button *Note: Joe - Default to middle point of the scale at beginning of screen.*	Capture numerical value associated with position on scale. Treat as scale ranging from 0-100, where anchor points are 0 = left-most position, Definitely not interested 50= center position, Not sure 100=right-most position, Definitely interested Intermediate values captured as integers.	(12) Interest inventory

				differentiate the slider from the progress at the bottom of screen, make the slider a pentagon instead of a circle, ex.: *NOTE:* Add display window to show number associated with position of slider		Capture time in milliseconds spent on this screen	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.39. Single	IF EXPERIMENT ARM & Single	How interested are you in learning your child's sequencing results for *medically actionable adult onset conditions*? • I'm definitely not interested in these results • I'm not sure • I'm definitely interested in in these results When you are done, click the next button to continue.		Making a decision about medically actionable adult onset conditions (Headline) *Note:* Interactive scale How interested are you in learning your child's sequencing results for medically actionable adult onset conditions? • Definitely not interested in these results • I'm not sure • Definitely interested in these results **[? – genomic sequencing]** **[? – 'medically actionable condition']** *NOTE: Example layout here* file://rtints6/hserproj4/0214132%20NEXUS%20Proj%203/Aim%202%20Decision%20Aids/2.1%20DA%20Content/Final%20Decision%20Aid%20Content/working/DA1_slider%20scale%20example.docx	Interactive response scale (slider); ~~Submit button~~ Next button; Q/A [?] button *Note: Joe - Default to middle point of the scale at beginning of screen.*	Capture numerical value associated with position on scale. Treat as scale ranging from 0-100, where anchor points are 0 = left-most position, Definitely not interested 50= center position, Not sure 100=right-most position, Definitely interested Intermediate values	(12) Interest inventory

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				NOTE: Add display window to show number associated with position of slider		captured as integers. Capture time in millisecond s spent on this screen	
D2.40. Single	IF EXPERIMENT ARM & Single	How interested are you in learning your child's sequencing results for non-*medically actionable childhood conditions*? • I'm definitely not interested in these results • I'm not sure • I'm definitely interested in in these results When you are done, click the next button to continue.		Making a decision about non-medically actionable childhood conditions (Headline) *NOTE:* Interactive scale How interested are you in learning your child's genomic sequencing results for non-medically actionable childhood conditions? • Definitely not interested in these results • I'm not sure • Definitely interested in these results **[? – genomic sequencing]** **[? – 'non-medically actionable condition']**	Interactive response scale (slider); ~~Submit button~~ Next button; Q/A [?] button *Note: Joe - Default to middle point of the scale at beginning of screen.*	Capture numerical value associated with position on scale. Treat as scale ranging from 0-100, where anchor points are 0 = left-most position, Definitely not interested 50= center position, Not sure	(12) Interest inventory

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				NOTE: Example layout here file://rtints6/hserproj4/02141 32%20NEXUS%20Proj%203/Ai m%202%20Decision%20Aids/ 2.1%20DA%20Content/Final% 20Decision%20Aid%20Conten t/working/DA1_slider%20scal e%20example.docx *NOTE:* Add display window to show number associated with position of slider		100=right-most position, Definitely interested Intermedia te values captured as integers. Capture time in millisecond s spent on this screen	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.38. Couple	IF EXPERIMENT ARM & COUPLE	How interested are you in learning your child's sequencing results for carrier status? Click and drag the slider, moving it to the point on the scale that fits your answer. • We're definitely not interested in these results • We're not sure • We're definitely interested in these results When you are done, click the next button to continue.		Making a decision about carrier status (Headline) *NOTE:* Interactive scale How interested are you in learning your child's sequencing results for carrier status? • Definitely not interested in these results • We're not sure • Definitely interested in these results **[? – genomic sequencing]** **[? – 'carrier']** *NOTE: Example layout here* file://rtints6/hserproj4/0214132%20NEXUS%20Proj%203/Aim%202%20Decision%20Aids/2.1%20DA%20Content/Final%20Decision%20Aid%20Content/working/DA1_slider%20scale%20example.docx *NOTE:* Add display window to show number associated with position of slider	Interactive response scale (slider); ~~Submit button~~ Next button; Q/A [?] button *Note: Joe - Default to middle point of the scale at beginning of screen.*	Capture numerical value associated with position on scale. Treat as scale ranging from 0-100, where anchor points are 0 = left-most position, Definitely not interested 50= center position, Not sure 100=right-most position, Definitely interested Intermediate values	(12) Interest inventory

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
						captured as integers. Capture time in millisecond s spent on this screen	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.39. Couple	IF EXPERIMENT ARM & COUPLE	How interested are you in learning your child's sequencing results for *medically actionable adult onset conditions*? • We're definitely not interested in these results • We're not sure • We're definitely interested in these results When you are done, click the next button to continue.		Making a decision about medically actionable adult onset conditions (Headline) *NOTE:* Interactive scale How interested are you in learning your child's sequencing results for medically actionable adult onset conditions? • Definitely not interested in these results • We're not sure • Definitely interested in these results **[? – genomic sequencing]** **[? – 'medically actionable condition']** *NOTE: Example layout here* file://rtints6/hserproj4/0214132%20NEXUS%20Proj%203/Aim%202%20Decision%20Aids/2.1%20DA%20Content/Final%20Decision%20Aid%20Content/working/DA1_slider%20scale%20example.docx	Interactive response scale (slider); ~~Submit button~~ Next button; Q/A [?] button *Note: Joe - Default to middle point of the scale at beginning of screen.*	Capture numerical value associated with position on scale. Treat as scale ranging from 0-100, where anchor points are 0 = left-most position, Definitely not interested 50= center position, Not sure 100=right-most position, Definitely interested Intermediate values	(12) Interest inventory

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				NOTE: Add display window to show number associated with position of slider		captured as integers. Capture time in millisecond s spent on this screen	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.40. Couple	IF EXPERIMENT ARM & COUPLE	How interested are you in learning your child's sequencing results for non-*medically actionable childhood conditions*? • We're definitely not interested in these results • We're not sure • We're definitely interested in these results When you are done, click the next button to continue.		Making a decision about non-medically actionable childhood conditions (Headline) *NOTE:* Interactive scale How interested are you in learning your child's genomic sequencing results for non-medically actionable childhood conditions? • Definitely not interested in these results • We're not sure • Definitely interested in these results **[? – genomic sequencing]** **[? – 'non-medically actionable condition']** *NOTE: Example layout here* file://rtints6/hserproj4/0214132%20NEXUS%20Proj%203/Aim%202%20Decision%20Aids/2.1%20DA%20Content/Final%20Decision%20Aid%20Content/working/DA1_slider%20scale%20example.docx	Interactive response scale (slider); ~~Submit button~~ Next button; Q/A [?] button *Note: Joe - Default to middle point of the scale at beginning of screen.*	Capture numerical value associated with position on scale. Treat as scale ranging from 0-100, where anchor points are 0 = left-most position, Definitely not interested 50= center position, Not sure 100=right-most position, Definitely interested Intermediate values	(12) Interest inventory

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				NOTE: Add display window to show number associated with position of slider		captured as integers. Capture time in millisecond s spent on this screen	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.41	IF EXPERIMENT ARM	What happens next? • At your next study visit, you will meet with a genetic counselor and a doctor to discuss why you may or may not be interested in additional genomic sequencing results. • You will then be asked if you want to request your child's additional genomic sequencing results for *carrier status*, *medically actionable adult conditions*, and *non-medically actionable childhood conditions.* • At that time, you may choose to request all three kinds of your child's additional results, only one or two of them, or none of them. All of these options are up to you.		What happens next? (headline) • At your next study visit, you will meet with a genetic counselor and a doctor • Discuss if you want to request additional sequencing results: 1. Carrier status 2. Medically actionable adult onset conditions 3. Non-medically actionable childhood conditions • You may choose all three kinds of additional results, only one or two of them, or none of them **[? – genomic sequencing]** **[? – genetic counselor]** **[? – 'carrier']** **[? – 'medically actionable condition']** **[? – 'non-medically actionable condition']**	Replay button Next button; Q/A [?] button		(11) Closing

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.42	IF EXPERIMENT ARM	The work to develop this decision guide was funded by a grant from the Eunice Kennedy Shriver National Institute of Child Health and Human Development and the National Human Genome Research Institute at the National Institutes of Health.		Thank you! The work to develop this decision guide was funded by a grant from the Eunice Kennedy Shriver National Institute of Child Health and Human Development and the National Human Genome Research Institute at the National Institutes of Health. <UNC and RTI logos>	Exit/Close button		(3) General content, Text

NC NEXUS Project 3 Measures (version 9/9/15)

Refusal = Mother/couple got paper decision aid and declined to schedule a baseline/consent meeting, or attended the meeting and declined to consent to study

Intake= Mailed with paper decision aid and informed consent forms

T1 = Mother /couple consented to study (having read paper decision aid), but has not yet been randomized or completed electronic decision aid; randomization occurs after this point

T2 = Mother /couple completed electronic decision aid and either did or did not agree to NGS-NBS (e.g., because they refused it or need more time). This survey will be brief because it may occur very quickly after T1. For mother/couple who decline NGS-NBS, it is the last survey before exiting the study.

T2A = Assessment after parents in experimental group decide whether or not to get secondary results. Need to decisions about how to handle people who never get back to us with a decision (when would they complete this assessment?) and about payment for this new assessment.

T3 = Occurs after return of requested results

T4 = Occurs 3 months after T3

Construct/Variable	Measure	Citation(s)	Time	Notes/Questions
Background information – Refusers				
Demographics for refusers and reasons for refusing	Interview drafted by Niasha. Get basic demographics from the medical record.		Refusal	Page 6
Background and NGS-NGS – Participants				
Demographics for participants	Standard items: age, gender, race, ethnicity, education, marital status, income, work status, insurance status, other children, plans for children in future, parity		Intake	Page 7
Personal and Family history of genetic testing	In section called “health history”		Intake	Page 10

Knowledge about genomic sequencing	NCGENES/GeneScreen Genomic Knowledge Scale; adapted		Intake, T2, T3	Page 11
Pregnancy anxiety	Gurung et al measure	Gurung, et al (2005). JSCP, 24, 497-519; Mancuso et al. (2004). Psy Med, 66, 762-769; Parker Dominguez, et al. ABM, 29(1), 12-21; Roesch, et al. (2004). ASC, 17(1): 87-102.	Intake, T2, T2A (healthy cohort only)	Page 14
Health literacy/numeracy	SAHL-S&E	http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2910571/; PMC2910571	T1 (interview format)	
Perceived risk for getting a "positive" NGS-NBS result (showing a disease-causing variant)		See Taber et al 2014;	T2	Page 16
Perceived risk for getting a positive result in a secondary category	Base on GeneScreen items	See Taber et al 2014;	T2a	Page 17
Reasons for accepting/declining NGS-NBS	Base on GeneScreen items		T2	Page 19
Reasons for accepting/declining secondary findings	Base on GeneScreen items adapted for NGS-NBS, once general approach approved		T2A	Page 20
Relationship information				
Relationship closeness	1-item inclusion of other in self scale; called "Your Relationship with Your Partner" in the questionnaire	Aron, A., Aron E. N., & Smollan, D. (1992). Inclusion of other in the self scale and the structure of interpersonal closeness. Journal of Personality and	T1 (couples only)	Page 22

		Social Psychology, 63, 596-612.		
Perceptions of collaboration in relationship with partner	Berg "perceptions of collaboration" 3-item frequency subscale; called "decision making" in questionnaire	Berg, Schindler, Smith, Skinner, & Beveridge, Psychol Aging, 2011, 26(1), 167-73;	Intake (couples only)	Page 23
Spouse/partner support	PAIR 6 item emotional intimacy subscale	shafer & olsen	T1 (couples only)	Page 24
Spouse/partner conflict		Lepore, S. J. Social conflict, social support, and psychological distress: Evidence of cross-domain buffering effects. JPSP, 63(5), 857-867.	T1 (couples only)	Page 25
Collaborative decision making about NGS-NBS	Developed by group based on existing measures; see Cynthia's document dated 2/4/15		T2 (couples only)	Page 26
Collaborative decision making about additional findings	Subset of 5 items repeated 3 times?		T2A (couples only)	Page 28
Personal characteristics				
Attitudes/beliefs about genomic sequencing	Adapted from NCGENES and GeneScreen; covers distrust specific to our test.	See NCGENES and GeneScreen	T1	Page 29
Self-efficacy	Used in GeneScreen			

Health Care System Distrust	9 item Health Care System Distrust Scale—Revised;	J Gen Intern Med. Jun 2008; 23(6): 727–732. doi: 10.1007/s11606-008-0575-3 (Also: Rose, Peters, Shea, Armstrong. Development and Testing of the Health Care System Distrust Scale. J Gen Intern Med. 2004; 19(1): 57–63. PMC1494688; Armstrong et al., Med Care 2012;50: 381–387)	T1	Page 30 Scores range from 9 (low distrust) to 45 (high distrust). Alpha good (see Armstrong et al., 2012: Alpha=0.83 for full scale and .73 and .77 for subscales). Includes 2 subscales: competence distrust (4 items with a range of 5–20) assessing perceptions of technical competence of health care system, and values distrust (5 items with a range of 5–25) assessing values of the health care system including its honesty, motives, and equity. Items on page 13.
Information Avoidance	8-item validated measure	Howell JL et al (2014). Does lacking threat management resources increase information avoidance? A multisample, multi-method investigation. J Res Pers, 50, 102-109. See also: Tabor et al, Information avoidance tendencies, threat mgmt resources, and interest in genetic sequencing feedback. ABM. Online 1/13/2015	T1, T2	Page 31
Process Data				
Process evaluation – feedback about electronic decision aid			T2	Page 32
Process evaluation – feedback about broader process			T3, T4	Page 33
OUTCOMES				

Decisional conflict	Decisional conflict scale; 16-item	O'Connor AM. Validation of a decisional conflict scale. Med Decis Making. 1995 Jan-Mar;15(1):25-30	T2, T2A	Page 34
Decision regret			T2, T3, T4	Page 35
General depressive symptoms/anxiety	HADS		T1, T3, T4	Page 36
Concern about child's future health	From NCGENES	Ware JE Jr.(1976). Scales for measuring general health perceptions. Health Serv Res;11(4):396-415. .	T1, T2, T3, T4	Page 39
Test-related distress	MICRA -- adapted		T3, T4	Page 40
Parental bonding	.	Brockington, Fraser, Wilson, (2006). Archives of women's mental health, 9(5), 233-242 (original 25-item scale) 10 item form: Wittkowski, Williams, Wieck (2010). Br J Clin Psych, 49, 163-72. Combine 1st 9 items of Wittkowski scale with factor 3 items from Brockington scale?	T3, T4	Page 42
Communication with other family members				
Information from study or medical records				
Cohort	Diagnosed versus well-child			
Study arm	Decision vs control			

Consent for NGS-NBS	y/n			
Consent for each secondary category of information	y/n			

Measure	Intake	T1	T2	T2A	T3	T4
Demographics (5 versions: healthy cohort single, health cohort partnered moms, diagnosed cohort single, diagnosed cohort partnered moms, partners)	X					
Personal and family history of genetic testing	X					
Knowledge about genomic sequencing	X		X		X	
Pregnancy anxiety (healthy cohort only)	X		X	X		
Perceptions of collaboration in relationship with partner	X					
Relationship conflict (under consideration)	X					
Health literacy/numeracy		X				
Relationship closeness (couples only)		X				
Spouse/partner support		X				
Spouse/partner conflict		X				
Attitudes/beliefs about genetic research		X				
Health care system distrust		X				
Information avoidance		X	X			
Perceived risk for getting a “positive” NGS-NBS result (different versions for healthy and diagnosed cohort]			X			
Reasons for accepting/declining NGS-NBS			X			
Collaborative decision making about NGS-NBS			X			
Process evaluation – feedback about electronic decision aid			X			
Perceived risk for getting a “positive” additional results				X		
Reasons for accepting/declining additional results				X		
Collaborative decision making about additional findings				X		
Process evaluation – feedback about broader process					X	X

Decisional conflict			X	X		
Decision regret			X		X	X
General depressive symptoms/anxiety		X			X	X
Concern about child's future health		X	X		X	X
Test-related distress					X	X
Parental bonding					X	X

Demographics for refusers and reasons for refusing

Thank you for taking the time to review the NC NEXUS Brochure and consider joining the study. It's helpful for us to learn more about the reasons people choose not to participate. Would you be willing to help us learn more by answering a couple of questions? Your answers would be completely confidential.

1. First, what is the most important reason you don't want to join the study?

[RECORD VERBATIM]

2. Now I'm going to read some <u>other</u> reasons people choose not to join research studies. Please say <u>yes</u> or <u>no</u> to let me know if each reason was important in <u>your</u> decision not to join the study.

   A. You and your partner could not agree on whether to join
   B. You don't have enough information to want to do it
   C. You don't know enough about research, in general, to agree
   D. It's not clear to you how joining this study would help you and your family
   E. You don't have enough time
   F. You are concerned that being in the study would cause you to worry
   G. You are concerned about costs of joining the study, like time from work, travel, and other things
   H. You're not comfortable being a research participant
   I. You don't trust the health care system
   J. You object to genetic research
   K. You don't believe this kind of testing could help your child
   L. You don't want to know this kind of information about your child

3. Are there any other reasons you'd like to mention before we finish up?

   [RECORD VERBATIM]

Thank you for your time! We really appreciate your help.

INTERVIEWER: IS THIS PERSON MALE OR FEMALE? M F

ALSO NEED (AS A REQIUIREMENT OF REFERRING TO THE STUDY – GET SOME OF IT FROM REFERRING PHYSICIAN VIA REFERRAL FORM? NEED TO ADDRESS WITH LARGER GROUP):

- Cohort
- Relationship status (partner “reasonably available” yes/no)
- Sex
- Race
- ethnicity
- Age

Demographics for participants

Your Background

***1. What is your sex?** (*Check <u>one</u>*)

- ☐ 0 Male
- ☐ 1 Female

***2. How old are you?** _____Years

3. What is your child's date of birth? __ __ / __ __ / __ __ __ __
M M D D Y Y Y Y [diagnosed cohort]

3. What is your due date? __ __ / __ __ / __ __ __ __
M M D D Y Y Y Y [healthy cohort]

***4. Are you of Hispanic, Latino, or Spanish origin?** (*Check <u>one</u>*)

- ☐ 0 No
- ☐ 1 Yes

***5. What racial group(s) do you most identify with?** *(Check <u>all</u> that apply*)

- ☐ 1 White
- ☐ 2 Black or African American
- ☐ 3 American Indian or Alaska Native
- ☐ 4 Asian or Pacific Islander
- ☐ 5 Native Hawaiian
- ☐ 6 Other ➔ (What is your race? _____)

6. What is your current marital status? (*Check <u>one</u>*) [mom only]

☐1 Single/Never married
☐2 Married
☐3 Separated from my spouse
☐4 Not legally married, but in a marriage-like relationship or a domestic partnership
☐5 Divorced
☐6 Widowed
☐7 Other ➡ (Describe your current marital status: _____)

7. How long have you been in a relationship with your partner? (That is, the partner who joined the NCNEXUS study with you) [couple version only]

____ Years ___ Months

8. Who are the people who live with you in your household? (*Check <u>all</u> that apply*)

☐1 My spouse or partner
☐2 My child or children
☐3 Other family members
☐4 Other person or people who are not family or a romantic partner
☐5 I live alone
☐6 Other ➔(Who do you live with? _____)

9. How many children do you currently have (*<u>including</u> the child who will be enrolled in NCNEXUS*)?
[diagnosed cohort]

____ children

9. How many children do you currently have (*<u>including</u> your current pregnancy*)? [healthy cohort]

____ children

10. How many *<u>more</u>* children are you planning to have in the future?

____ children

***11. What is the highest level of school you completed**? (*Check <u>one</u>*)

☐1 Less Than High School Graduate
☐2 High School Graduate (or equivalent)
☐3 Partial College (at least one year)
☐4 Completed Trade School

☐5 2 year College Degree (e.g., Associate's degree)
☐6 4-year College Degree (e.g., Bachelor's degree)
☐7 Graduate or Professional Degree (e.g., MA/MS, PhD, JD, MD)

***12. As of today, what is your employment status?** (*Check <u>all</u> that apply*)

☐1 Working in paid or self-employed job 32 hours a week or more
☐2 Working in paid or self-employed job less than 32 hours a week
☐3 Homemaker or stay at home parent
☐4 Employed, but currently on medical or family leave
☐5 Unemployed and unable to work due to illness or disability
☐6 Retired
☐7 Doing unpaid or voluntary work
☐8 Other ➡(Describe your employment status: _____)

13. What was the total family income (before taxes) from all sources within your household in the last year? (*Check <u>one</u>*)

☐1 Less than $14,999
☐2 $15,000 to $29,999
☐3 $30,000 to $44,999
☐4 $45,000 to $59,999
☐5 $60,000 to $74,999
☐6 $75,000 to $89,999
☐7 $90,000 to $104,999
☐8 $105,000 to $119,999
☐9 $120,000 to $134,999

☐10 $135,000 or more

***14. What kind of health insurance do you currently have**? (*Check <u>all</u> that apply*)

☐1 Private health insurance (that you or your employer pay for, or that you got through the North Carolina exchange)
☐2 Federal insurance (such as Federal Employee Health Benefits)
☐3 Military insurance (such as TriCare)
☐4 Indian Health Services
☐5 Medicaid
☐6 Medicare
☐7 I have no health insurance
☐8 Other ➔ (Describe your health insurance _____)

15. If you have health insurance, will this insurance cover any medical expenses for your child?

☐0 No
☐1 Yes
☐2 Don't Know
☐3 I do not have health insurance

fathers fill out items with an "" only

Genetic Testing

Genetic tests provide information about a person's genetic (DNA) makeup. They are used to find out if there is a genetic cause of a condition or disease.

They may be done when there is a "family history" of a disease (that is, when many relatives have the same condition). These tests may also be done when people have a condition that is likely to have a genetic cause, even when they are the only ones in their family who have a condition. Genetic tests may also be done during a pregnancy, at the time of birth, or other times during a person's life.

1. **Are there any diseases that many people in your family have in common?** (Only include relatives related to you by blood) *(Check one)*

   ☐$_0$ No
   ☐$_1$ Yes → If "Yes", what disease(s)?_____
   ☐$_2$ Not Sure

2. **Has a genetics specialist ever asked you for a detailed family history to see if you might benefit from genetic testing**? *(Check one)*

   ☐$_0$ No
   ☐$_1$ Yes
   ☐$_2$ Not Sure

3. **Have you ever made a decision about whether or not to have a specific genetic test?** (For instance, to test a developing baby or to test for a specific disease or risk for disease in you or your family) *(Check one)*

   ☐$_0$ No

☐$_1$ Yes
☐$_2$ Not Sure

4. **Have you ever <u>had</u> a genetic test?** (Testing is most often done using a sample of blood, a cheek swab, or some other body tissue, like a tumor.) *(Check <u>one</u>)*

☐$_0$ No
☐$_1$ Yes
☐$_2$ Not Sure

Knowledge about genomic sequencing

Genes and Health [Genomic Knowledge Scale]

This section will help us learn what information NCNEXUS participants need in order to understand their child's genomic sequencing results. Before you begin, you should know that *gene variants* are genetic differences between two people.

On the next page is a list of statements. They are either **true or false**. For each statement:

- Circle (***T***) if you think it is **true**
- Circle (***F***) if you think it is **false**
- Circle (***DK***) if you are **not sure** or **don't know**.

Please answer **all** of the questions. ***Don't worry if you do not know the right answers! We do not expect you to answer all of these correctly.*** Be as honest as you can so we can develop the right educational materials for participants like you.

Information About Genes	**True**$_1$	**False**$_2$	**Not sure/ don't know**$_3$
1. Genes are made of DNA.	***T***	***F***	***DK***
2. Genes affect health by influencing the proteins our bodies make.	***T***	***F***	***DK***
3. All of a person's genetic information is called his or her "genome."	***T***	***F***	***DK***
4. A person's genes change completely every 7 years.	***T***	***F***	***DK***
5. The DNA in a gene is made of four building blocks (A, C, T, and G).	***T***	***F***	***DK***
6. Everyone has about 20,000 to 25,000 genes.	***T***	***F***	***DK***

Information About Health and Gene Variants (Variants Are Genetic Differences Between People)	**True**$_1$	**False**$_2$	**Not sure/ don't know**$_3$

7.	Gene variants can have positive effects, harmful effects, or no effects on health.	***T***	***F***	***DK***
8.	Most gene variants will affect a person's health.	***T***	***F***	***DK***
9.	Everyone who has a harmful gene variant will eventually have symptoms	***T***	***F***	***DK***
10.	Some gene variants have a large effect on health while others have a small effect.	***T***	***F***	***DK***
11.	Some gene variants decrease the chance of developing a disorder.	***T***	***F***	***DK***
12.	Two unrelated people with the same genetic variant will always have the same symptoms.	***T***	***F***	***DK***

Information About How Genes Are Inherited in Families		**True$_1$**	**False$_2$**	**Not sure/ don't know$_3$**
13.	Genetic disorders are always inherited from a parent.	***T***	***F***	***DK***
14.	If only one person in the family has a disorder it can't be genetic.	***T***	***F***	***DK***
15.	Everyone has a chance for having a child with a genetic disorder.	***T***	***F***	***DK***
16.	A girl inherits most of her genes from her mother while a boy inherits most of his genes from his father.	***T***	***F***	***DK***
17.	A mother and daughter who look alike are more genetically similar than a mother and daughter who do not look alike.	***T***	***F***	***DK***

18. If a parent has a harmful gene variant, all of his or her children will inherit it.	***T***	***F***	***DK***
19. If one of your parents has a gene variant, your brother or sister may also have it.	***T***	***F***	***DK***

Information About Genomic Sequencing in NCNEXUS	**True**$_1$	**False**$_2$	**Not sure/ don't know**$_3$
20. The genomic sequencing used in NCNEXUS will find variants in many genes at once.	***T***	***F***	***DK***
21. Only gene variants that are predicted to be harmful will be confirmed and reported in NCNEXUS	***T***	***F***	***DK***
22. Even in healthy children, genomic sequencing could unexpectedly find a mutation that causes them to be at high risk for a health condition.	***T***	***F***	***DK***
23. Genomic sequencing will find a harmful gene variant in all of the children who are tested	***T***	***F***	***DK***
24. Genomic sequencing can find harmful gene variants that cannot be found by standard newborn screening	***T***	***F***	***DK***

For experimental group only – not finalized

25. Genomic sequencing will find every harmful gene variant that can cause a genetic health condition.	***T***	***F***	***DK***
26. For recessive conditions, a carrier of a harmful gene variant would not be expected to develop the condition.	***T***	***F***	***DK***
27. If a child is a carrier, then at least one of his or her parents is probably also a carrier (T)	***T***	***F***	***DK***

28. A child who has a variant for a non-medically actionable childhood condition is certain to develop that condition (F) ***T*** ***F*** ***DK***

29. A child who has a harmful gene variant for a medically actionable adult-onset condition may have at least one parent who is also at risk for the condition. ***T*** ***F*** ***DK***

30. A harmful genetic variant for an adult onset-condition for which there are no effective medical treatments to prevent or improve the condition.

31. If a child has a harmful variant for a medically actionable adult-onset condition, there is nothing that can be done to prevent or treat the condition.

32. In NC NEXUS, parents might learn whether their child has a harmful genetic variant for an adult-onset that causes a condition for which there is no known medical treatment.

33. All the harmful gene variants found NC NEXUS are related to conditions have effective medical treatments.

Your Feelings About Pregnancy [HEALTHY COHORT ONLY]

These questions ask about how you have felt about being pregnant **in the past week, including today**.

In the past week, how often have you felt these emotions about being pregnant?	**Never**$_1$	**Rarely**$_2$	**Some-times**$_3$	**Often**$_4$	**Always**$_5$
1. Anxious	☐	☐	☐	☐	☐
2. Confident	☐	☐	☐	☐	☐
3. In conflict (you had mixed feelings)	☐	☐	☐	☐	☐
4. Lucky	☐	☐	☐	☐	☐
5. Concerned	☐	☐	☐	☐	☐
6. Excited	☐	☐	☐	☐	☐
7. Upset	☐	☐	☐	☐	☐
8. Happy	☐	☐	☐	☐	☐

9. Afraid	☐	☐	☐	☐	☐
10. Special	☐	☐	☐	☐	☐
11. Panicky	☐	☐	☐	☐	☐
12. Pleased	☐	☐	☐	☐	☐
13. Healthy	☐	☐	☐	☐	☐

Health literacy/numeracy

Use Newest Vital Sign: http://www.pfizer.com/health/literacy/public_policy_researchers/nvs_toolkit

Perceived risk for getting a "positive" NGS-NBS result (a disease-causing variant)

Note: These items (which are related to the concept of "understanding") allow us to compare joiners and decliners on perceived risk," to compare changes in perceived risk for "positive" results from pre-RoR to post-RoR for joiners, and compare perceived risk "positive" results among joiners whose child gets abnormal vs normal results.

HEALTHY COHORT

For each question, please circle the one number from 1 to 7 that best describes how likely **you** think it is that genomic sequencing in NCNEXUS would show that **your** child is at high risk for a genetic disease.

1. **How likely do you think it is that your child's genomic sequencing will show that he/she has an increased risk for a genetic disease?**

1	2	3	4	5	6	7
Extremely UNlikely						**Extremely Likely**

2. **Compared to the average child in a family like yours, how likely is it that your child's genomic sequencing will show that he/she has an increased risk for a genetic disease?**

1	2	3	4	5	6	7
Much LESS Likely Than the Average Child						**Much MORE Likely Than the Average Child**

DIAGNOSED COHORT

For each question, please circle the one number from 1 to 7 that best describes how likely **you** think it is that genomic sequencing in NCNEXUS would show that **your** child is at high risk for a genetic disease **other than the one he/she has already been diagnosed as having**.

1. **How likely do you think it is that your child's genomic sequencing will show that he/she has an increased risk for a genetic disease other than the one he/she has already been diagnosed as having?**

1	2	3	4	5	6	7
Extremely UNlikely						**Extremely Likely**

2. **Compared to the average child in a family like yours, how likely is it that your child's genomic sequencing will show that he/she has an increased risk for a genetic disease other than the one he/she has already been diagnosed as having?**

1	2	3	4	5	6	7
Much LESS Likely Than the Average Child						**Much MORE Likely Than the Average Child**

Perceived risk for getting a "positive" result in a secondary category

For each question, please circle the one number from 1 to 7 that best describes how likely **you** think it is that your child's genomic sequencing in NCNEXUS would provide the described information. These questions ask about the three categories of secondary findings: Carrier status for recessive conditions, non-medically actionable childhood conditions, and medically actionable adult conditions.

Secondary Information Category: Carrier Status

1. **How likely do you think it is that your child's genomic sequencing would show that he/she is a carrier of a gene variant that causes a health condition (if you were to ask for that category of secondary information)?**

1	2	3	4	5	6	7
Extremely UNlikely						**Extremely Likely**

2. **Compared to the average child in a family like yours, how likely is it that your child's genomic sequencing would show that he/she is a carrier of a gene variant that causes a health condition (if you were to ask for that category of secondary information)?**

1 2 3 4 5 6 7

Much LESS Likely Than the Average Child — **Much MORE Likely Than the Average Child**

Secondary Information Category: Non-medically Actionable Childhood Conditions

1. **How likely do you think it is that your child's genomic sequencing would show that he/she is at high risk for a non-medically actionable childhood condition (if you were to ask for that category of secondary information)?**

1 2 3 4 5 6 7

Extremely UNlikely — **Extremely Likely**

2. **Compared to the average child in a family like yours, how likely is it that your child's genomic sequencing would show that he/she is at high risk for a non-medically actionable childhood condition (if you were to ask for that category of secondary information)?**

1 2 3 4 5 6 7

Much LESS Likely Than the Average Child — **Much MORE Likely Than the Average Child**

Secondary Information Category: Medically Actionable Adult Conditions

1. **How likely do you think it is that your child's genomic sequencing would show that he/she is at high risk for a medically actionable adult condition (if you were to ask for that category of secondary information)?**

1	2	3	4	5	6	7
Extremely UNlikely						**Extremely Likely**

2. **Compared to the average child in a family like yours, how likely is it that your child's genomic sequencing would show that he/she is at high risk for a medically actionable adult condition that is treatable (if you were to ask for that category of secondary information)?**

1	2	3	4	5	6	7
Much LESS Likely Than the Average Child						**Much MORE Likely Than the Average Child**

Reasons for accepting/declining NGS-NBS

Note: Based on GeneScreen items

Response scale:
This **made me less interested** in genomic sequencing for my child (1)
This **did not affect my decision** about genomic sequencing for my child (2)
This **made me more interested** in genomic sequencing for my child (3)

1. How did each of these things affect your decision about genomic sequencing for your child?

Thinking about yourself and your child

- Genomic sequencing might give me information showing that my child has an increased genetic health risk
- Any results showing that my child has an increased genetic health risk will be confirmed
- I might worry while waiting for my child's genomic sequencing results
- I might worry about my child's future health if NCNEXUS finds that he/she has an increased genetic health risk
- Influence of my personal or religious beliefs
- What I think my child's doctor would want
- Any results showing that my child has an increased genetic health risk will be discussed with his/her doctor
- Any results showing that my child has an increased genetic health risk will go in his/her medical record
- How my child's privacy and confidentiality would be protected if we join NCNEXUS
- Possible future medical costs if genomic sequencing finds that my child has an increased genetic health risk
- Possible effects on long term care, disability, or life insurance if genomic sequencings finds that my child has an increased genetic health risk
- I know that the Genetic Information Nondiscrimination Act (GINA) protects against discrimination by most health insurers

Thinking about your family

- Genomic sequencing might give me information showing that family members other than my child have an increased genetic health risk
- I might worry about my family's future health if genomic sequencing finds that my child has an increased genetic health risk
- My family members' reactions if genomic sequencing finds that my child has an increased genetic health risk
- My family members' reactions to learning that they may also have an increased genetic health risk

Thinking about participating in a research study like NCNEXUS

- The NCNEXUS study is being conducted by researchers at the University of North Carolina at Chapel Hill and RTI International.
- Contributing to research
- Contributing to how genomic sequencing is used in general healthcare in the future

2. **We are in the early stages of understanding how people think of genomic sequencing for children. To help us understand reasons people do and do not decide to have genomic sequencing for their child, please let us know any other reason(s) for the decision you made.** [ALLOW OPEN-ENDED RESPONSE]

3. What was the <u>most</u> important reason in your decision about whether or not to have genomic sequencing for your child? [ALLOW OPEN-ENDED RESPONSE]

Reasons for accepting/declining additional findings –

Response scale:
This **made me less interested** in learning my child's carrier status/risk for non-medically actionable childhood conditions/risk for medically actionable adult conditions (1)
This **did not affect my decision** about learning my child's carrier status/risk for non-medically actionable childhood conditions/risk for medically actionable adult conditions (2)
This **made me more interested** in learning my child's carrier status/risk for non-medically actionable childhood conditions/risk for medically actionable adult conditions (3)

Carrier Status (see Vernooij-van Langen et al., 2013)

1. **How did each of these things affect your decision about whether or not to learn your child's carrier status?**
   - My child could use this information when deciding to have children in the future
   - I might worry about whether my child will have unhealthy children in the future
   - If my child is a carrier, I would need to tell him/her about it
   - My child has a right to know any information that comes out of his/her sequencing
   - My child cannot choose whether or not to know this information
   - The information may or may not be useful for my child in the future OR
   - My child is not certain to benefit from knowing this information
   - If my child is a carrier, my other children can be tested for the same gene variant
   - If my child is a carrier, then my partner and I could be tested for the same gene variant
   - It could give me and my partner information to help us make decisions about having more children
   - The results might give me information that could help family members other than my child
   - My child is very unlikely to have health problems because of being a carrier
   - ???

2. **Please let us know any other reason(s) for your decision about whether or not to learn your child's carrier status**. [ALLOW OPEN-ENDED RESPONSE]

3. **What was the <u>most</u> important reason in your decision about whether or not to learn your child's carrier status?** [ALLOW OPEN-ENDED RESPONSE]

Risk for non-medically actionable childhood conditions

1. **How did each of these things affect your decision about whether or not to learn your child's risk for non-medically actionable childhood conditions?**
   -

2. **Please let us know any other reason(s) for your decision about whether or not to learn your child's risk for non-medically actionable childhood conditions**. [ALLOW OPEN-ENDED RESPONSE]

3. **What was the <u>most</u> important reason in your decision about whether or not to learn your child's risk for non-medically actionable childhood conditions?** [ALLOW OPEN-ENDED RESPONSE]

Risk for medically actionable adult conditions

2. **How did each of these things affect your decision about whether or not to learn your child's risk for non-medically actionable childhood conditions?**
   -

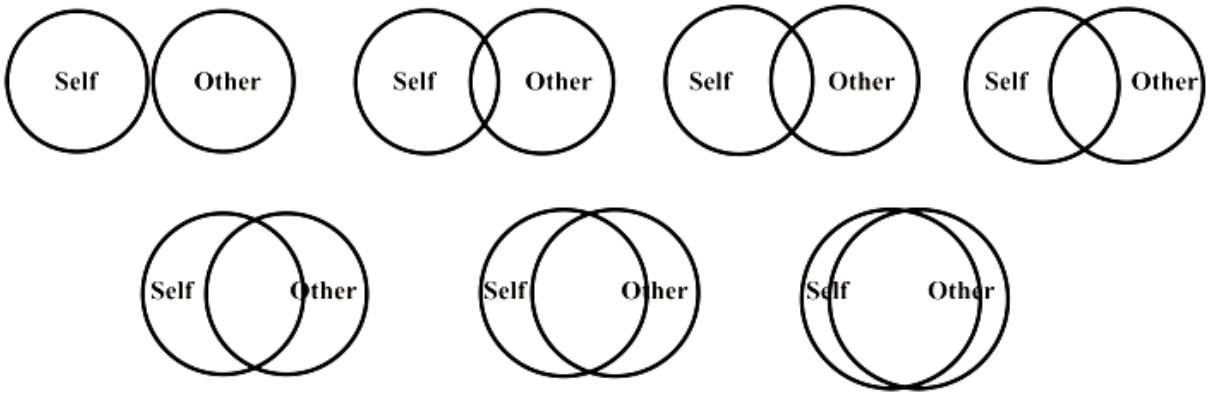
2. **Please let us know any other reason(s) for your decision about whether or not to learn your child's risk for non-medically actionable childhood conditions**. [ALLOW OPEN-ENDED RESPONSE]

3. **What was the <u>most</u> important reason in your decision about whether or not to learn your child's risk for non-medically actionable childhood conditions?** [ALLOW OPEN-ENDED RESPONSE]

Relationship closeness

Your Relationship with Your Spouse or Partner

Please circle the picture that best describes your current relationship with your spouse or partner



Perceptions of collaboration in the relationship

Decision Making

1. My partner and I always work together to deal with really important household decisions.

☐1 Strongly disagree
☐2 Disagree
☐3 Neither agree nor disagree
☐4 Agree
☐5 Strongly Agree

2. Nearly every day my partner and I work together to make decisions.

☐1 Strongly disagree
☐2 Disagree
☐3 Neither agree nor disagree
☐4 Agree
☐5 Strongly Agree

3. It is rare for my partner and I to share tasks and make decisions together.

☐1 Strongly disagree
☐2 Disagree
☐3 Neither agree nor disagree
☐4 Agree
☐5 Strongly Agree

Spouse support

Note: Changed the response scale to be the same as the “perceptions of collaboration” scale

Your Relationship

These statements are about intimacy in your relationship with your **spouse or partner**. For each statement, please place an “X” in the one box that indicates **how you feel about your relationship now**. There are no right or wrong answers.

	Strongly disagree$_0$	Disagree$_1$	Neither agree nor disagree$_2$	Agree$_3$	Strongly agree$_4$
1. My partner listens to me when I need someone to talk to.	☐	☐	☐	☐	☐
2. I can state my feelings without him/her getting defensive.	☐	☐	☐	☐	☐
3. I often feel distant from my partner.	☐	☐	☐	☐	☐
4. My partner can really understand my hurts and joys.	☐	☐	☐	☐	☐
5. I feel neglected at times by my partner.	☐	☐	☐	☐	☐
6. I sometimes feel lonely when we’re together.	☐	☐	☐	☐	☐

Spouse/partner Conflict

0 = never
1 = rarely
2 = sometimes
3 = often
4 = very often

How often in the past week have you had these things happen in your relationship with your partner?

1. You fought with your partner
2. You were upset with your partner
3. You had a disagreement with your partner
4. You felt like screaming at your partner
5. You became openly angry in your home
6. You got so angry you threw things
7. You and your partner criticized each other
8. You and your partner had to work out differences

Collaborative decision making about NGS-NBS

Areas of theory and research on shared decision-making

A. Collaborative coping/communal coping (Berg & Upchurch, 2007, Perceptions of Collaboration Questionnaire: The focus has been on the purposes of shared problem-solving in terms of compensation for inability to make decision independently and to maintain relational closeness (by reaching out to partner)

B. Values clarification (shared decision-making between patients and providers; Kriston, Scholl, Hözel, Simon, Loh, & Härter, 2010; SDM-Q-9): Were partners able to recognize different options, communicate their values with each other, weigh pros and cons with each other, and come to a shared decision?

C. Decisional conflict/satisfaction (O'Connor, 1995; Decisional Conflict/Satisfaction): Was each partner satisfied with the process of their decision-making (i.e., they felt supported by each other, were able to communicate values with each other) and with the final decision they both made?

D. Preferences for level of participation in decision-making in health care (patient preferences for involvement in health care; Degner et al., 1997; Control Preferences Scale): Was each partner's level of participation in line with his/her own preferences?

Decision Making about Genomic Sequencing

INSTRUCTIONS: These questions ask about the decision you made with your partner about whether or not to have genomic sequencing for your child. For each statement, check **one box** to indicate how much you agree or disagree.

Response scale [changed to correspond with other measures]
1=strongly agree
2=agree
3=somewhat agree
3=somewhat disagree
4=disagree
5=strongly disagree

A1. Making this decision with my partner was helpful because it would have been harder to make it by myself.
A2. We made a better decision because my partner and I decided together.

B1. My partner and I discussed good and bad things about having genomic sequencing for our child.
B2. My partner asked me what decision I prefer.
B3. My partner and I worked together to understand all the information.
B4. My partner and I made the decision together.
C1. This decision was hard for us to make together.
C2. I felt pressure from my partner when making this decision.
C3. I felt supported by my partner when making this decision.
C4. Working together helped us make a more informed decision.
C5. I am satisfied with our decision.
C6. I am satisfied with how we were able to make this decision together.
D1. I would have preferred to be more involved when making this decision with my partner.
D2. I would have preferred to be less involved when making this decision with my partner.
D3. I feel satisfied with my level of involvement in the final decision.

Collaborative decision making about additional findings

Decision Making about Additional Findings

INSTRUCTIONS: These questions ask about the decision you made with your spouse or partner about whether or not to get each category of secondary finding for your child. For each statement, check **one box** to indicate how much you agree or disagree.

Response scale [changed to correspond with other measures]
1=strongly agree
2=agree
3=somewhat agree
3=somewhat disagree
4=disagree
5=strongly disagree

B1. My partner and I discussed good and bad things about [CATEGORY].

B3. My partner and I worked together to understand all the information about [CATEGORY].

C1. It was hard for us to agree on our decision about [CATEGORY].

C4. Working together helped us make a more informed decision about [CATEGORY].

D3. I feel satisfied with my level of involvement in the final decision about [CATEGORY].

Attitudes/Beliefs about genomic sequencing – REVIEW MOST RECENT GENESCREEN ITEMS

Note: Adapted from NCGENES and GeneScreen

Notes from NCGENES data:

- Scale included 11 agree/disagree items, plus 1 item on trust in sequencing scored on a 4 point scale.
- Reliability poor for scale (alpha = .54, excluding trust item), but it was not designed to measure an underlying construct – just a checklist of positive and negative attitudes/beliefs about sequencing
- Some items had very little variability in responses in NCGENES:
  - Genetic research could be useful to cure diseases – 98% agreed
  - Genetic research could lead to improved treatment for diseases – 99% agreed
  - Minorities may be less likely than Whites to benefit from this type of research – 8% agreed (**very poor item-total correlation with other negatively worded items, .13**)
  - This type of research could reinforce racism in our society – 7% agreed
  - How much do you trust WES to give accurate information about whether or not your/your child's health concern is caused by your/his/her genes? – 91% agreed
- Other items had more variability:
  - Genetic research results could be used to discriminate against certain people – 42% agreed
  - A person could lose insurance coverage as a result of being in a genetic study – 24% agreed
  - Genetic research could reduce racial differences in disease – 59% agreed
  - People could lose their privacy as a result of being in a genetic study – 20% agreed
  - The government cannot be trusted to regulate the use of genetic information – 34% agreed
  - This kind of research could cause insurance companies to charge some people higher premiums – 44% agreed
  - This type of research should not be done until we know how the information will be used – 18% agreed (**very poor item-total correlation with other negatively worded items, .07**)

Although nearly everyone agreed with the items about curing/treating diseases, perhaps we can keep something like these for NCNEXUS, which is a very different population and these views might be quite different in the healthy and diagnosed cohort. However, those two items are very similar, and perhaps we can change them to a single item.

If we want 6-7 items (with an approximately equal number of positively and negatively worded items), here's what I propose (CR).

Response scale
1=strongly agree
2=agree
3=somewhat agree

3=somewhat disagree
4=disagree
5=strongly disagree

These questions are about how you think genomic sequencing for children may affect society. Please tell us how much you agree or disagree with each statement.

1. Research on genomic sequencing could be used to prevent future health problems in children
2. Research on genomic sequencing could reduce racial differences in childhood disease
3. Genetic research could lead to better treatments for childhood diseases by helping us understand them
4. Using genomic sequencing to look for health problems before they occur could lead to harms like unnecessary testing and treatment
5. Results from research on genomic sequencing could be used to discriminate against certain children
6. Results from research on genomic sequencing could cause insurance companies to charge higher premiums for some children
7. The government cannot be trusted to prevent children's genomic information from being misused

Health Care System Distrust

Note: From Shea et al., 2008

PMC full text: J Gen Intern Med. 2008 Jun; 23(6): 727–732.
Published online 2008 Mar 28. doi: 10.1007/s11606-008-0575-3

Table 3

Rotated Factor Loadings Overall and by Race

		Overall		Black		White	
Full scale		C	V	C	V	C	V
1. The Health Care System does its best to make patients' health better*	C	.77	.21	.76	.17	.78	.21
2. The Health Care System covers up its mistakes	V	.32	.63	.37	.65	.38	.68
3. Patients receive high quality medical care from the Health Care System*	C	.80	.15	.86	−.04	.84	.22
4. The Health Care System makes too many mistakes	C	.42	.57	.50	.44	.40	.67
5. The Health Care System puts making money above patients' needs	V	.38	.48	.61	.47	.05	.66
6. The Health Care System gives excellent medical care*	C	.80	.20	.79	.09	.80	.22
7. Patients get the same medical treatment from the Health Care System, no matter what the patient's race or ethnicity*#	V	.64	.31	.52	.34	.60	.43
8. The Health Care System lies to make money	V	.29	.76	.18	.78	.48	.63
9. The Health Care System experiments on patients without them knowing	V	.05	.83	−.14	.80	.24	.77

* *Item is reverse scored*

Item added after factor analysis

Items are answered on a scale where 1 = strongly disagree and 5 = strongly agree

C competence, V values

Information avoidance

Your Information Preferences

Instructions: Parents have different preferences for how much information they want to receive about their child's NGS-NBS results. Some want all the information they can get, and others prefer to know only the basics. Below is a list of things parents sometimes say about getting information from NGS-NBS. For each statement, write an "X" in the box that best describes how much you agree or disagree.

Response scale
1=strongly agree
2=agree
3=somewhat agree
3=somewhat disagree
4=disagree
5=strongly disagree

1. I would rather know just the basics of what genomic sequencing results mean for my child, me, and my family.
2. I would avoid learning details I do not really need to know about my child's genomic sequencing results and their meaning
3. Even if it will upset me, I want to know everything I can about what the genomic sequencing results mean for my child, me, and my family.
4. When it comes to knowing the details about what my child's genomic sequencing results mean, sometimes ignorance is bliss
5. I want to know everything I can about what the genomic sequencing results mean for my child, me, and my family.

6. I can think of situations in which I would rather not know details about what my child's genomic sequencing results mean.
7. It is important to know every bit of information that the genomic sequencing results can provide for my child, me, and my family.
8. I would want to know everything about what the genomic sequencing results mean for my child, me, and my family, immediately.

Process Evaluation – Feedback on Electronic Aid

Note: Randall Teal (CHAI Core) offered these items used by Lixin Song.

Response scale
1=strongly agree
2=agree
3=somewhat agree
3=somewhat disagree
4=disagree
5=strongly disagree

Please let us know how much you agree with each of the following statements.

General
1. I thought the website was easy to use.
2.The website has a very attractive presentation.
3.The website is interesting and engaging.

Content
4. The content on the website is written in clear and simple language.
5. The content is easy to understand and follow.
6. The content is of high quality.
7. The content is highly relevant to me.

Navigation
8. I found what I was looking for quickly and easily.
9. I found the website too complicated. .
10. The website didn't always do what I expected it to do.
11.I did not know how to find what I was looking for.
12.I felt that I had to click too many times to go through the website.
13.The website responds quickly.
14.Using the website is frustrating.

Process Evaluation – Feedback on Broader Process

Note: Developed for GeneScreen – adapt some of these

*Important note: Qs 7-9 are for joiners only.

How much do you agree or disagree with each statement?	Strongly Disagree	Disagree	Slightly Disagree	Slightly Agree	Agree	Strongly Agree
1. I was satisfied with going to the website and learning about the study.	1	2	3	4	5	6
2. I know how to get more information about the study if I need it.	1	2	3	4	5	6
3. I understand the types of conditions the NCNEXUS looks for	1	2	3	4	5	6
4. The information provided on the website was enough for me to make a decision about whether or not to join the study	1	2	3	4	5	6
5. It was easy for me to complete this questionnaire online	1	2	3	4	5	6
6. This questionnaire took too long to fill out	1	2	3	4	5	6
~~7.~~ *If my child gets a positive test result, I am comfortable with it going into my UNC medical record	~~1~~	~~2~~	~~3~~	4	~~5~~	~~6~~

~~8.~~ What is the most important thing you would like to tell us to make the NCNEXUS study better?

~~**1**~~ ~~**2**~~ ~~**3**~~ ~~**4**~~ ~~**5**~~ ~~**6**~~

Decisional conflict

Traditional Decisional Conflict Scale (DCS) – Statement Format: 16 item 5 response categories

This is our most tested version. Many people like the personal response format. However, it is more difficult to respond to than questions in those with limited reading and response skills.
Note: We always precede the DCS with an option preference question, which is not included in scoring.
[See item 'A' below].

My difficulty in making this choice

A. Which [insert treatment/screening] option do you prefer? Please check ☑ one.

- ☐ [Option 1]
- ☐ [Option 2]
- ☐ [Option 3]
- ☐ Unsure

B. Considering the option you prefer, please answer the following questions:

	Strongly Agree [0]	Agree [1]	Neither Agree Nor Disagree [2]	Disagree [3]	Strongly Disagree [4]
1. I know which options are available to me.	☐	☐	☐	☐	☐
2. I know the benefits of each option.	☐	☐	☐	☐	☐
3. I know the risks and side effects of each option.	☐	☐	☐	☐	☐
4. I am clear about which benefits matter most to me.	☐	☐	☐	☐	☐
5. I am clear about which risks and side effects matter most to me.	☐	☐	☐	☐	☐
6. I am clear about which is more important to me (the benefits or the risks and side effects).	☐	☐	☐	☐	☐
7. I have enough support from others to make a choice.	☐	☐	☐	☐	☐
8. I am choosing without pressure from others.	☐	☐	☐	☐	☐
9. I have enough advice to make a choice.	☐	☐	☐	☐	☐
10. I am clear about the best choice for me.	☐	☐	☐	☐	☐

Decision Regret

Note: O'Connor's measure (The Decision Regret Scale)

Please think about the decision you made to get [genomic sequencing for your child/additional information from your child's genomic sequencing). Then, show how you feel about these statements by circling a number from 1 (strongly agree) to 5 (strongly disagree)

	Strongly agree	Agree	Neither agree nor disagree	Disagree	Strongly disagree
1. It was the right decision	1	2	3	4	5
2. I regret the choice that was made	1	2	3	4	5
3. I would go for the same choice if I had to do it over again	1	2	3	4	5
4. The choice did me a lot of harm	1	2	3	4	5
5. The decision was a wise one	1	2	3	4	5

Additional questions for T3 and T4 assessments, only for experimental/decision group members:

Think about the different types of additional information you got from your child's genomic sequencing. How much do you regret or not regret getting each type?

1. Carrier status for autosomal recessive conditions
    A. I regret getting this type of additional information a lot
    B. I regret getting this type of additional information a little
    C. I do not regret getting this type of additional information at all
    D. I did not get this type of additional information

2. Non-medically actionable childhood conditions
    A. I regret getting this type of additional information **a lot**
    B. I regret getting this type of additional information **a little**
    C. I **do not regret** getting this type of additional information at all
    D. I **did not get** this type of additional information

3. Medically actionable adult conditions
    A. I regret getting this type of additional information **a lot**
    B. I regret getting this type of additional information **a little**
    C. I **do not regret** getting this type of additional information at all
    D. I **did not get** this type of additional information

General depressive symptoms/anxiety symptoms

Note: will need to be adapted from interview format

Next I'll read some statements about things that people sometimes feel, and I'd like you to use Card D to tell me how much you feel each of these things right now. You'll notice that each of these 14 questions has a different response scale. I'll read the question number along with the question to make it easy for you to respond. You should give an immediate response. Don't think too long about your answers.

1. I feel tense or 'wound up':

MOST OF THE TIME 1
A LOT OF THE TIME 2
FROM TIME TO TIME, OCCASIONALLY 3
NOT AT ALL 4

2. I still enjoy the things I used to enjoy:

DEFINITELY AS MUCH 1
NOT QUITE SO MUCH 2
ONLY A LITTLE 3
HARDLY AT ALL 4

3. I get a sort of frightened feeling as if something awful is about to happen:

VERY DEFINITELY AND QUITE BADLY 1
YES, BUT NOT TOO BADLY 2
A LITTLE, BUT IT DOESN'T WORRY ME 3
NOT AT ALL 4

4. I can laugh and see the funny side of things:

AS MUCH AS I ALWAYS COULD 1
NOT QUITE SO MUCH NOW 2
DEFINITELY NOT SO MUCH NOW 3
NOT AT ALL 4

5. Worrying thoughts go through my mind:

A GREAT DEAL OF THE TIME 1
A LOT OF THE TIME 2
FROM TIME TO TIME, BUT NOT TOO OFTEN 3
ONLY OCCASIONALLY 4

6. I feel cheerful:

NOT AT ALL 1
NOT OFTEN 2
SOMETIMES 3
MOST OF THE TIME 4

7. I can sit at ease and feel relaxed:

DEFINITELY 1
USUALLY 2
NOT OFTEN 3
NOT AT ALL 4

8. I feel as if I am slowed down:

NEARLY ALL THE TIME 1
VERY OFTEN 2
SOMETIMES 3
NOT AT ALL 4

9 I get a sort of frightened feeling like 'butterflies' in the stomach:

NOT AT ALL 1
OCCASIONALLY 2
QUITE OFTEN 3
VERY OFTEN 4

10. I have lost interest in my appearance:

DEFINITELY 1
I DON'T TAKE AS MUCH CARE AS I SHOULD 2
I MAY NOT TAKE QUITE AS MUCH CARE 3
I TAKE JUST AS MUCH CARE AS EVER 4

11. I feel restless as I have to be on the move:

VERY MUCH INDEED 1
QUITE A LOT 2
NOT VERY MUCH 3
NOT AT ALL 4

12. I look forward with enjoyment to things:

AS MUCH AS I EVER DID 1
RATHER LESS THAN I USED TO 2
DEFINITELY LESS THAN I USED TO 2
HARDLY AT ALL 4

13. I get sudden feelings of panic:

VERY OFTEN INDEED 1
QUITE OFTEN 2
NOT VERY OFTEN 3
NOT AT ALL 4

14. I can enjoy a good book or radio or TV program:

OFTEN 1
SOMETIMES 2
NOT OFTEN 3
VERY SELDOM 4

Concern about child's future health

Note: used in NCGENES

Your Child's Health Outlook

INSTRUCTIONS: Please read each of the following statements and then check *one* of the boxes to indicate how true or false the statement is **for your child who is receiving NGS-NBS**. There are no right or wrong answers. Some of the statements may look or seem like others. But each statement is different, and should be rated by itself.

	Definitely false$_1$	**Mostly false**$_2$	**Don't know**$_3$	**Mostly true**$_4$	**Definitely true**$_5$
1. My child will probably be sick a lot in the future	☐	☐	☐	☐	☐
2. In the future, I expect my child to have better health than other children I know	☐	☐	☐	☐	☐
3. I think my child's health will become worse over time	☐	☐	☐	☐	☐
4. I expect my child to have a very healthy life	☐	☐	☐	☐	☐

Test-related distress

Note: MICRA, adapted for GeneScreen (current as of 2/19/15); Cella D, Hughes C, Lerman C, et al. A brief assessment of concerns associated with genetic testing for cancer: The multidimensional impact of cancer risk assessment (MICRA) questionnaire. Health Psychology. 2002;21(6):564-572. Available from: PsycARTICLES, Ipswich, MA. Accessed February 10, 2015. This measure will be completed online in the follow-up/exit survey for people who do not receive positive result, and in a paper and pencil version for people who receive a positive result.

Now I'd like you to think about the information you have received from your child's genomic screening in NCNEXUS. Right now, how much are you experiencing each of these things about that information?

		Never	Rarely	Sometimes	Often
1	Feeling relieved about your child's genomic sequencing result	0	1	3	5
2	Feeling upset about your child's genomic sequencing result	0	1	3	5
3	Feeling happy about your child's genomic sequencing result	0	1	3	5
4	Feeling sad about your child's genomic sequencing result	0	1	3	5
5	Feeling anxious or nervous about your child's genomic sequencing result	0	1	3	5
6	Feeling like you've done something important for your child and/or your family	0	1	3	5
7	Feeling worried about your child's risk for having health problems in the future	0	1	3	5
8	Having problems enjoying life because of your child's genomic sequencing result	0	1	3	5
9	Feeling guilty about your child's te genomic sequencing st result	0	1	3	5
10	Feeling a loss of control because of your child's genomic sequencing result	0	1	3	5
11	Being uncertain about what your child's genomic sequencing result means for his/her future health	0	1	3	5
12	Being uncertain about what your child's genomic sequencing result means for his/her future medical care	0	1	3	5

13	Being uncertain about what your child's genomic sequencing result means for your child's and your family's risk for disease	0	1	3	5
14	Having difficulty talking about your child's genomic sequencing results with family members	0	1	3	5
15	Thinking about your child's genomic sequencing result has affected your work or family life	0	1	3	5
16	Feeling that your family has been supportive during the genomic sequencing process	0	1	3	5
17	Worry that the genomic sequencing process has caused conflict in your family	0	1	3	5

Original items for the MICRA

The Multidimensional Impact of Cancer Risk Assessment (MICRA) Questionnaire

The questions below are about some specific responses you may have had after receiving your genetic test results. Please answer every question in Section 1, regardless of whether you were given a positive or negative test result. Please indicate whether you have experienced each statement *never, rarely, sometimes*, or *often* in the past week, by circling the corresponding number.

Section 1	Never	Rarely	Sometimes	Often
1. Feeling upset about my test result	0	1	3	5
2. Feeling sad about my test result	0	1	3	5
3. Feeling anxious or nervous about my test result	0	1	3	5
4. Feeling guilty about my test result	0	1	3	5
5. Feeling relieved about my test result	0	1	3	5
6. Feeling happy about my test result	0	1	3	5
7. Feeling a loss of control	0	1	3	5
8. Having problems enjoying life because of my test result	0	1	3	5
9. Worrying about my risk of getting cancer [or getting cancer again if you have ever been diagnosed with cancer]	0	1	3	5
10. Being uncertain about what my test result means about my cancer risk	0	1	3	5
11. Being uncertain about what my test result means for my child(ren) and/or family's cancer risk	0	1	3	5
12. Having difficulty making decisions about cancer screening or prevention (e.g., having preventive surgery or getting medical tests done)	0	1	3	5
13. Understanding clearly my choices for cancer prevention or early detection	0	1	3	5
14. Feeling frustrated that there are no definite cancer prevention guidelines for me	0	1	3	5
15. Thinking about my test results has affected my work or family life.	0	1	3	5
16. Feeling concerned about how my test results will affect my insurance status	0	1	3	5
17. Having difficulty talking about my test results with family members	0	1	3	5
18. Feeling that my family has been supportive during the genetic counseling and testing process	0	1	3	5
19. Feeling satisfied with family communication about my genetic test result	0	1	3	5
20. Worrying that the genetic counseling and testing process has brought about conflict within my family	0	1	3	5
21. Feeling regret about getting my test results	0	1	3	5

Section 2. *If you have children, regardless of your test result, please answer Questions 22 and 23. Otherwise, please go to Section 3.*

	Never	Rarely	Sometimes	Often
22. Worrying about the possibility of my children getting cancer	0	1	3	5
23. Feeling guilty about possibly passing on the disease risk to my child(ren)	0	1	3	5

Section 3. *If you currently have cancer, or have had it in the past, please answer Questions 24 and 25. Otherwise, please check this box* ☐. *You are finished with this questionnaire.*

Parental bonding

Please indicate how often the following things are true of you. There are no right or wrong answers. Choose the answer that seems right in your recent experience.

Item	Scale statement	Corrected item total correlation	α if item deleted
1	I feel close to my baby	0.499	0.612
2	I wish the old days when I had no baby would come back	0.420	0.625
3	I feel distant from my baby	0.401	0.631
4	I love to cuddle my baby	0.432	0.626
5	I wish that I had never had this baby	0.308	0.653
6	I feel happy when my baby looks at me	0.303	0.656
7	My baby cries too much	0.313	0.674
8	I love my baby with all my heart	0.341	0.653
9	My baby annoys me	0.454	0.612
10	I feel confident when changing my baby′s diapers	0.175	0.666

Note: the above measure has poor reliability (<.70). Perhaps add back in some of the original items (next page) and take out the one about changing diapers?

Perhaps add in original **factor 3** items left out of this brief measure? They include: My baby makes me feel anxious, I am afraid of my baby, I feel confident when caring for my baby, My baby is easily comforted.

Appendix 1

Post Partum Bonding Questionnaire

Please indicate how often the following ae true for you.

There are no 'right' or 'wrong' answers. Choose the answer which seems right in your recent experience.

Factor	Scoring	Statement	Always	Very often	Quite often	Some-times	Rarely	Never
1	0 → 5	I feel close to my baby						
1	5 → 0	I wish the old days when I had no baby would come back						
2	5 → 0	I feel distant from my baby						
2	0 → 5	I love to cuddle my baby						
2	5 → 0	I regret having this baby						
1	5 → 0	The baby does not seem to be mine						
1	5 → 0	My baby winds me up						
1	0 → 5	I love my baby to bits						
1	0 → 5	I feel happy when my baby smiles or laughs						
1	5 → 0	My baby irritates me						
2	0 → 5	I enjoy playing with my baby						
1	5 → 0	My baby cries too much						
1	5 → 0	I feel trapped as a mother						
2	5 → 0	I feel angry with my baby						
1	5 → 0	I resent my baby						
1	0 → 5	My baby is the most beautiful baby in the world						
1	5 → 0	I wish my baby would somehow go away						
4	5 → 0	I have done harmful things to my baby						
3	5 → 0	My baby makes me feel anxious						
3	5 → 0	I am afraid of my baby						
2	5 → 0	My baby annoys me						
3	0 → 5	I feel confident when caring for my baby						
2	5 → 0	I feel the only solution is for someone else to look after my baby						
4	5 → 0	I feel like hurting my baby						
3	0 → 5	My baby is easily comforted						

University of North Carolina-Chapel Hill
Consent to Include NGS-NBS Results in the Electronic Medical Record

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Study Title: NC NEXUS: North Carolina Newborn Exome Sequencing for Universal Screening
Principal Investigator: Cynthia Powell, M.D.
Consent Form Version Date: 09/12/2015
Co-Investigators: Jonathan S. Berg, Karen Weck, Kirk Wilhelmsen, and Christine Rini
Study Contact telephone number: 919-537-3795

As part of the NC NEXUS study, your child had a type of genetic evaluation called next-generation sequencing newborn screening (**NGS-NBS**). The CLIA-certified, Molecular Genetics Lab at UNC Hospitals has confirmed the clinically significant variants. A genetic counselor and/or medical geneticist on the research team has/have discussed the results with you and you have received a copy of them.

You may request that these results be entered into your child's UNC electronic medical record. If you wish to have them included, they will be labeled with your child's name. All of your child's UNC healthcare providers would be able to see the results. The results would have the same privacy protection as any other lab results or clinic visit notes.

I have read the information above and have asked all the questions I have at this time.

Please initial one:
I do _____
I do not _____

wish for these **NGS-NBS results** to be included in my child's UNC electronic medical record.

_____ _____
Signature of Participant's Parent Date

_____
Printed Name of Participant's Parent and of Child Participant

_____ _____
Signature of Research Team Member Obtaining Consent Date

_____
Printed Name of Research Team Member Obtaining Consent

University of North Carolina-Chapel Hill
Consent to Include <u>Additional</u> Results in the Electronic Medical Record

---

Study Title: NC NEXUS: North Carolina Newborn Exome Sequencing for Universal Screening
Principal Investigator: Cynthia Powell, M.D.
Consent Form Version Date: 09/26/2015
Co-Investigators: Jonathan S. Berg, Karen Weck, Kirk Wilhelmsen, and Christine Rini
Study Contact telephone number: 919-537-3795

As part of the NC NEXUS study, your child had a type of genetic evaluation called next-generation sequencing newborn screening (**NGS-NBS**). You decided to learn about one or more **additional** categories of genomic information that can be found by this test. The CLIA-certified, Molecular Genetics Lab at UNC Hospitals has confirmed the clinically significant variants. A genetic counselor and/or medical geneticist on the research team discussed the results with you and you have received a copy of them.

You may request that one or more categories of results be included in your child's UNC electronic medical record. If you wish to have them included, they will be labeled with your child's name. All of your child's UNC healthcare providers would be able to see the results. The results would have the same privacy protection as any other lab results or clinic visit notes.

I have read the information above and have asked all the questions I have at this time.

I. Category: Adult-onset, medically actionable conditions **Initials:** _____

Circle: [N/A] **OR**

[I do] / [I do not] wish to include these results in my child's electronic medical record.

II. Category: Childhood onset NON-medically actionable conditions **Initials:** _____

Circle: [N/A] **OR**

I [do] / I [do not] wish to include these result in my child's electronic medical record.

III. Category: Carrier status for recessive conditions **Initials:** _____

Circle: [N/A] **OR**

I [do] / [do not] wish to include these results in my child's electronic medical record.

_____ _____

Signature of Participant's Parent Date

_____

Printed Name of Participant's Parent and of Child Participant

_____ _____

Signature of Research Team Member Obtaining Consent Date

_____

Printed Name of Research Team Member Obtaining Consent

List of Appendices

Volume 4

DON BAILEY

Summary of Professional Experience

Don Bailey is a Distinguished Fellow at RTI International, where he serves as chair of the RTI Fellows program (http://www.rti.org/page.cfm/Fellow_Program) and a member of the RTI Executive Leadership Team. For 27 years, he was on the faculty of the University of North Carolina at Chapel Hill, where he was a W.R. Kenan, Jr. Distinguished Professor and for 14 years Director of the Frank Porter Graham Child Development Institute. Dr. Bailey's research addresses early identification and early intervention for children with disabilities, as well as family adaptation to disability. For the past 20 years, much of his work has focused on children with fragile X syndrome (FXS), the leading inherited cause of intellectual impairment, and their families. He has an extensive record of publications, with more than 220 peer-reviewed articles, chapters, and books on a wide variety of topics related to early education, early intervention, disability, and family support. Currently, he directs several projects funded by the National Institutes of Health on various aspects of fragile X and broader issues surrounding the ethical, legal, and social consequences of genetic discoveries and the disclosure of genetic information to families, including newborn screening. He also serves as RTI's lead partner with UNC-CH on NC TraCS, the North Carolina Clinical and Translational Sciences Institute. In 2006, he received the Career Research Scientist Award from the Academy on Mental Retardation. From 2006 to 2009 he served as President of the Board of Directors of the National Fragile X Foundation (www.fragileX.org). Currently he is serving a 6-year term as an appointed member of the DHHS Secretary's Advisory Committee on Heritable Disorders in Newborns and Children.

Education

PhD, Early Childhood Special Education, University of Washington, 1979.
MEd, Early Childhood Special Education, University of North Carolina at Chapel Hill, 1973.
BA, Psychology, Davidson College, 1971.

Professional Experience

2013 to date	Chair, RTI Fellows Program
2005 to date	Distinguished Fellow, Social and Statistical Sciences, RTI International, Research Triangle Park, NC.
2006 to date	Research Professor, School of Education, University of North Carolina at Chapel Hill.
2002 to 2006	W.R. Kenan Distinguished Professor, School of Education, University of North Carolina at Chapel Hill.
1999 to 2006	Professor, School of Education, University of North Carolina at Chapel Hill.

1994 to 1999	Professor, Medical Allied Health and Research Professor, Education, University of North Carolina at Chapel Hill.
1992 to 2006	Director, Frank Porter Graham Child Development Institute, Chapel Hill, NC.
1990 to 1994	Associate Professor, Medical Allied Health, University of North Carolina at Chapel Hill.
1986 to 1994	Clinical Associate Professor, Education, University of North Carolina at Chapel Hill.
1984 to 1992	Director of Early Childhood Research, Frank Porter Graham Child Development Center, Chapel Hill, NC.
1979 to 1986	Clinical Assistant Professor, Division of Special Education, University of North Carolina at Chapel Hill.
1976 to 1979	Research Assistant/Teaching Assistant, Special Education, University of Washington.
1973 to 1976	Preschool and K-1 teacher for children with disabilities, Chapel Hill-Carrboro City Schools, Chapel Hill, NC.
1971 to 1972	Psychological Technician, Mental Retardation Unit, Central State Hospital, Milledgeville, Georgia.

Awards

Research Career Scientist Award, Academy on Mental Retardation, May 2006
Fellow, Academy of Mental Retardation, 2005
Rosen Research Award, National Fragile X Foundation, 2004
American Association on Mental Retardation Research Award, 2001
James E. Favell Excellence in Research Award, NC AAMR, 2000
Division for Early Childhood (Council for Exceptional Children) Service to the Field Award, 1994

Current Grants and Contracts

Fragile X Caregiver Survey: Canada, Portugal, and United Kingdom (8/1/2012 – 12/31/2015), Novartis Pharmaceuticals, AFQ056B/5006, Principal Investigator.

Decisional Capacity and Informed Consent in Fragile X syndrome (9/26/2012 – 6/30/2017), National Institute of Child Health & Human Development, 1R01HD071987, Principal Investigator.

NC NEXUS, North Carolina Newborn Exome Sequencing for Universal Screening (9/5/2013 – 8/31/2018), National Institute of Child Health & Human Development, 1U19HD077632, Project 3 Principal Investigator (J. Berg and C. Powell, UNC-Chapel Hill, Principal Investigators).

North Carolina Translational and Clinical Sciences Institute (NC TraCS) (9/26/2013 – 4/30/2018), National Center for Advancing Translational Sciences, 1UL1TR001111, Deputy Director (J. Buse and T. Carey, UNC-Chapel Hill, Principal Investigators).

Tier 2 Voluntary Newborn Screening: Planning Proposal (9/30/14 – 9/30/17), The John Merck Fund, Principal Investigator.

SCID Pilot Implementation Study (10/1/15 – 9/30/17). Centers for Disease Control, Principal Investigator, 1U88EH001312-01, Principal Investigator.

Newborn screening for Mucopolysaccharidosis (MPSI) Pilot Study (10/1/15 – 3/31/17). Eunice Kennedy Shriver National Institute of Child Health and Human Development, HHSN27000001, Principal Investigator

Peer-Reviewed Journal Articles

Lewis, M.A., Paquin, R., Roche, M., Furberg, R.D., Rini, C., Berg, J.S., Powell, C.M., & Bailey, D.B. (in press). Parental decisions about genomic sequencing for newborn screening: The NC NEXUS decision aid. *Pediatrics*.

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Roberts, J.E., Tonnsen, B.L., McCary, L.M., Ford, A.L., Golden, R.N., & Bailey, D.B. (2015). Trajectory and predictors of depression and anxiety disorders in mothers with the *FMR1* premutation. *Biological Psychiatry*. Jul 31. pii: S0006-3223(15)00609-5. doi: 10.1016/j.biopsych.2015.07.015. [Epub ahead of print].

Hartley, S.L., Wheeler, A.C., Mailick, M.R., Raspa, M., Mihaila, I., Bishop, E., & Bailey, D.B. (2015). Autism symptoms across adulthood in men with fragile X syndrome: A cross-sectional analysis. *Journal of Autism and Developmental Disorders*. 2015 Jun 28. [Epub ahead of print].

Bailey, D.B., Wheeler, A.C., Berry-Kravis, E., Hagerman, R., Tassone, F., Powell, C.M., Roche, M., Gane, L.W., & Sideris, J. (2015). Maternal consequences of the detection of fragile X carriers in newborn children. *Pediatrics, 136*, e433-3440.

Klusek, J., Hunt, A.W., Mirrett, P.L., Hatton, D. D., Hooper, S.R., Roberts, J.E., & Bailey, D.B. (2015). Reading and phonological skills in boys with fragile X syndrome. *Journal of Autism and Developmental Disorders, 45*, 1699-1711.

Bailey, D.B., & Gehtland, L. (2015). Newborn screening: Evolving challenges in an era of rapid discovery. *Journal of the American Medical Association, 313*, 1511-1512.

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Wheeler, A., Sideris, J., Hagerman, R., Berry-Kravis, E., Tassone, F., & Bailey, D.B. (under review). Developmental profiles of infants with an *FMR1* premutation.

Bailey, D.B., Berry-Kravis, E., Gane, L.W., Guarda, S., Hagerman, R., Powell, C.M., Tassone, F., & Wheeler, A. (under review). Fragile X newborn screening: Lessons learned from a multi-site screening study. *Pediatrics*.

Raspa, M., Edwards, A., Wheeler, A.C., Bishop, E., & Bailey, D.B. (under review). Family communication and cascade testing in fragile X syndrome.

Bailey, D.B., Berry-Kravis, E., Wheeler, A., Raspa, M., Merrien, F., Ricart, J., Koumaras, B., Rosenkranz, G., Tomlinson, M., von Raison, F., & Apostol, G. (under review). Mavoglurant in adolescents with Fragile X syndrome: Analysis of Clinical Global Impression-Improvement source data from a double-blind therapeutic study followed by an open-label, long-term extension study.

Selected Recent Presentations

Supporting children and families in early intervention and preschool programs: Four essential components. Invited presentation, National Institute of Education, Nanyang Technical University, Singapore, October 7, 2015.

Comparative and longitudinal perspectives on mood and anxiety disorders in mothers with the FMR1 premutation. 2nd International Conference on FMR1 Premutation: Basic Mechanisms and Clinical Involvement, Sitges, Spain, October 2, 2015.

Fragile X newborn screening pilot: Summary findings and future directions. 17th International Workshop on Fragile X and other early onset cognitive disorders, Strasbourg, France, September 29, 2015.

Early Check: Rationale and progress towards a voluntary newborn screening program. Invited presentation, Newborn Screening Translational Research Network Meeting, Bethesda, MD, September 11, 2015.

Early Check: Expanded voluntary screening for newborns. Invited presentation, Pre-Competitive Industry Meeting, Cure SMA Conference, Kansas City, MO, June 17, 2015.

Early Check: Expanded voluntary health screening for newborns. Invited presentation, The John Merck Fund Program Grantees and Executive Board Meeting, New York, NY, June 10, 2015.

NC NEXUS Project 3: Understanding and supporting parent decisions. Invited presentation, Genomes of Newborns: Medicine, Pharmacogenomics, and Ethics Conference, Mercy Children's Hospital, Kansas City, MO, April 9, 2015.

Evolving concepts of treatment and benefit in newborn screening. Invited presentation, RUSP Roundtable Meeting, Rockville, MD, August 26, 2015.

Tier 2 newborn screening. Invited presentation, North Carolina Newborn Screening Advisory Committee, Raleigh, NC, February 20, 2015.

Why we need a "Tier 2" newborn screening option and what we need to do to get there. Invited presentation, National Center on Birth Defects and Developmental Disability, Centers for Disease Control and Prevention, Atlanta, GA, December 8, 2014.

How might family outcomes be incorporated in early childhood programs? Invited plenary speaker, Early Years Conference 2014, Vancouver, BC, Canada, January 31, 2014.

Assessing family outcomes in the U.S., Japan, and Singapore. Symposium chair and presenter, 3rd International Association for the Scientific Study of Intellectual Disability Asia-Pacific Regional Conference, Tokyo, Japan, August 23, 2013.

Fragile X research at RTI International: Current activities, future possibilities. Invited presentation, Novartis Pharmaceuticals, Basel, Switzerland, July 2, 2013.

The fragile X newborn screening pilot study: Lessons learned from the detection of FMR1 premutation carrier infants. Invited presentation, 1st International Conference on the *FMR1* Premutation: Basic Mechanisms and Clinical Involvement, Perugia, Italy, June 26, 2013.

Fragile X Newborn screening pilot study: Lessons learned. Symposium chair and presenter, American Public Health Laboratories Newborn Screening and Genetic Testing Symposium, Atlanta, GA, May 8, 2013.

Early intervention in fragile X syndrome. Invited panel presentation, International Congress on Fragile X Syndrome, Evora, Portugal, April 13, 2013.

Past, present, and future of research and intervention in fragile X syndrome. Invited plenary presentation, International Congress on Fragile X Syndrome, Evora, Portugal, April 12, 2013.

Challenges and opportunities associated with carrier detection in newborn screening for fragile X syndrome. Poster presentation, American College of Medical Genetics and Genomics Annual Clinical Genetics Meeting, Phoenix, AZ, April 8, 2013.

Newborn screening for fragile X: Reflections on a multi-year pilot investigation. Invited keynote presentation, 46th Annual Gatlinburg Conference on Research and Theory in Intellectual and Developmental Disabilities, San Antonio, TX, March 7, 2013.

Fragile X newborn screening: Controversies and consequences. Acibadem Maslak Hospital, Istanbul, Turkey, September 26, 2012.

Using survey research to understand the nature and consequences of fragile X. Acibadem Maslak Hospital, Istanbul, Turkey, September 26, 2012.

Fragile X newborn screening: Pilot project. Centers for Disease Control and Prevention, Atlanta, GA, August 3, 2012.

Caregiver burden in fragile X syndrome. 13th International Fragile X Conference, Miami, FL, July 28, 2012.

Family perspectives on fragile X newborn screening. 13th International Fragile X Conference, Miami, FL, July 26, 2012.

How would FXS fare today if submitted for newborn screening evidence review? 13th International Fragile X Conference, Miami, FL, July 26, 2012.

Families and fragile X syndrome. Invited presentation, Nanyang Technical University, Singapore, June 13, 2012.

Family outcomes in early intervention. Invited presentation, Nanyang Technical University, Singapore, June 12, 2012.

How newborn screening for fragile X syndrome might inform decisions about Alpha-1 screening. Invited presentation, Alpha-1 Testing Ethical, Legal, and Social Issues Workshop, Miami, FL, February 25, 2012.

Selected National Service Activities

Currently serve on the editorial boards of four journals (*American Journal on Intellectual and Developmental Disabilities*, *Intellectual and Developmental Disabilities Research Reviews*, *Topics in Early Childhood Special Education*, *Journal of Early Intervention*) and regularly serve as guest reviewer for other journals.

2011–2017: Appointed member of the DHHS Advisory Committee on Heritable Disorders in Newborns and Children.

2009: Committee to review and evaluate the Intellectual and Developmental Disabilities Branch of the National Institute on Child Health and Human Development.

2008: Trans-NIH Fragile X and Associated Disorders Research Coordinating Group. Congress directed the NIH to develop a cross-institute plan for research on FXS and related disorders. Chaired the working group on fragile X syndrome and led the writing of the FXS section of the report (http://www.nichd.nih.gov/publications/pubs/upload/NIH_Research_Plan_on_Fragile_X_and_Assoc_Disorders-06-2009.pdf).

2007–2009: President, Board of Directors, National Fragile X Foundation (www.fragilex.org).

2006–2008: Chair, University Committee on Tissue Repositories and DNA Banking, University of North Carolina at Chapel Hill. Led the writing of a report submitted to the Vice Chancellor for Research.

2004–2006: 2-year term as an appointed member of the Secretary's Advisory Committee on Head Start Accountability and Educational Performance Measures, U.S. Department of Health and Human Services.

1998–present: Executive Planning Committee, Gatlinburg Conference on Research and Theory in Intellectual and Developmental Disabilities.

JONATHAN S. BERG
CURRICULUM VITAE
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Education:

Postdoctoral Research, Baylor College of Medicine, April 2009, Stem Cells and Epigenetics

Residency, Baylor College of Medicine, May 2007, Clinical Genetics

MD, The University of North Carolina at Chapel Hill, May 2003

PhD, The University of North Carolina at Chapel Hill, December 2001, Neuroscience

BS, Emory University, May 1994, Biology

Professional Experience – Employment History

Associate Professor
Department of Genetics
The University of North Carolina at Chapel Hill
7/1/2015 – present

Assistant Professor
Department of Genetics
The University of North Carolina at Chapel Hill
7/1/2009 – 6/30/2015

Adjunct Appointment
Department of Medicine, Division of Hematology/Oncology, UNC-CH
7/2009 – present

Member
Lineberger Comprehensive Cancer Center, UNC-CH
7/2009 – present

Associate Director
Carolina Center for Genome Sciences, UNC-CH
6/2011 – 5/2013

Member
Curriculum in Genetics and Molecular Biology, UNC-CH
2011 – present

Clinical Assistant Professor (non-tenure track)
Department of Molecular and Human Genetics
Baylor College of Medicine
7/2007 – 6/2009

Honors and Awards

Richard King Trainee Award for Best Publication in Genetics in Medicine, 2009
Alpha Omega Alpha, UNC Chapel Hill, 2002
Phi Beta Kappa, Emory University, 1993

Bibliography

http://www.ncbi.nlm.nih.gov/sites/myncbi/jonathan.berg.1/bibliography/47903854/public/?sort=date&direction=ascending

Refereed Papers/Articles

Original research

1. **Berg JS**, Foreman AKM, O'Daniel JM, Booker JK, Boshe L, Carey T, Crooks KR, Jensen BC, Juengst ET, Lee K, Nelson DK, Powell BC, Powell CM, Roche MI, Skrzynia C, Strande NT, Weck KE, Wilhelmsen KC, Evans JP. 2015. A semi-quantitative metric for evaluating clinical actionability of incidental or secondary findings from genome-scale sequencing. Genet Med. (Accepted).
2. Couser NL, Masood MM, Strande NT, Foreman AK, Crooks K, Weck KE, Lu M, Wilhelmsen KC, Roche M, Evans JP, **Berg JS**, Powell CM. 2015. The phenotype of multiple congenital anomalies-hypotonia-seizures syndrome 1: Report and review. Am J Med Genet A. doi: 10.1002/ajmg.a.37129. [Epub ahead of print]
3. Lee K, **Berg JS**, Milko L, Crooks K, Lu M, Bizon C, Owen P, Wilhelmsen KC, Weck KE, Evans JP, Garg S. 2015. High Diagnostic Yield of Whole Exome Sequencing in Participants With Retinal Dystrophies in a Clinical Ophthalmology Setting. Am J Ophthalmol. doi: 10.1016/j.ajo.2015.04.026. [Epub ahead of print]
4. Amendola LM, Dorschner MO, Robertson PD, Salama JS, Hart R, Shirts BH, Murray ML, Tokita MJ, Gallego CJ, Kim DS, Bennett JT, Crosslin DR, Ranchalis J, Jones KL, Rosenthal EA, Jarvik ER, Itsara A, Turner EH, Herman DS, Schleit J, Burt A, Jamal SM, Abrudan JL, Johnson AD, Conlin LK, Dulik MC, Santani A, Metterville DR, Kelly M, Foreman AK, Lee K, Taylor KD, Guo X, Crooks K, Kiedrowski LA, Raffel LJ, Gordon O, Machini K, Desnick RJ, Biesecker LG, Lubitz SA, Mulchandani S, Cooper GM, Joffe S, Richards CS, Yang Y, Rotter JI, Rich SS, O'Donnell CJ, **Berg JS**, Spinner NB, Evans JP, Fullerton SM, Leppig KA, Bennett RL, Bird T, Sybert VP, Grady WM, Tabor HK, Kim JH, Bamshad MJ, Wilfond B, Motulsky AG, Scott CR, Pritchard CC, Walsh TD, Burke W, Raskind WH, Byers P, Hisama FM, Rehm H, Nickerson DA, Jarvik GP. 2015. Actionable exomic

incidental findings in 6503 participants: challenges of variant classification. Genome Res. 25(3):305-15.

5. Ritter DI, Haines K, Cheung H, Davis CF, Lau CC, **Berg JS**, Brown CW, Thompson PA, Gibbs R, Wheeler DA, Plon SE. 2015. Identifying gene disruptions in novel balanced de novo constitutional translocations in childhood cancer patients by whole-genome sequencing. Genet Med. doi: 10.1038/gim.2014.189. [Epub ahead of print]
6. Challen GA, Sun D, Mayle A, Jeong M, Luo M, Rodriguez B, Mallaney C, Celik H, Yang L, Xia Z, Cullen S, **Berg J**, Zheng Y, Darlington GJ, Li W, Goodell MA. 2014. Dnmt3a and Dnmt3b Have Overlapping and Distinct Functions in Hematopoietic Stem Cells. Cell Stem Cell. 15(3):350-64.
7. Anders C, Deal AM, Abramson V, Liu MC, Storniolo AM, Carpenter JT, Puhalla S, Nanda R, Melhem-Bertrandt A, Lin NU, Kelly Marcom P, Van Poznak C, Stearns V, Melisko M, Smith JK, Karginova O, Parker J, **Berg J**, Winer EP, Peterman A, Prat A, Perou CM, Wolff AC, Carey LA. 2014. TBCRC 018: phase II study of iniparib in combination with irinotecan to treat progressive triple negative breast cancer brain metastases. Breast Cancer Res Treat. 146(3):557-66.
8. Hill DA, Horick NK, Isaacs C, Domchek SM, Tomlinson GE, Lowery JT, Kinney AY, **Berg JS**, Edwards KL, Moorman PG, Plon SE, Strong LC, Ziogas A, Griffin CA, Kasten CH, Finkelstein DM. 2014. Long-term risk of medical conditions associated with breast cancer treatment. Breast Cancer Res Treat. 145(1):233-43.
9. Fan Z, Greenwood R, Felix AC, Shiloh-Malawsky Y, Tennison M, Roche M, Crooks K, Weck K, Wilhelmsen K, **Berg J**, Evans J. 2014. GCH1 heterozygous mutation identified by whole-exome sequencing as a treatable condition in a patient presenting with progressive spastic paraplegia. J Neurol. 261(3):622-4.
10. **Berg JS**, Amendola LM, Eng C, Allen EV, Gray SW, Wagle N, Rehm HL, Dechene ET, Dulik MC, Hisama FM, Burke W, Spinner NB, Garraway L, Green RC, Plon S, Evans JP, Jarvik GP. 2013. Processes and preliminary outputs for identification of actionable genes as incidental findings in genomic sequence data in the Clinical Sequencing Exploratory Research Consortium. Genet Med. 15(11):860-7.
11. Goddard KA, Whitlock EP, **Berg JS**, Williams MS, Webber EM, Webster JA, Lin JS, Schrader KA, Campos-Outcalt D, Offit K, Feigelson HS, Hollombe C. 2013. Description and pilot results from a novel method for evaluating return of incidental findings from next-generation sequencing technologies. Genet Med. 15(9):721-8.
12. Knowles MR, Leigh MW, Ostrowski LE, Huang L, Carson JL, Hazucha MJ, Yin W, **Berg JS**, Davis SD, Dell SD, Ferkol TW, Rosenfeld M, Sagel SD, Milla CE, Olivier KN, Turner EH, Lewis AP, Bamshad MJ, Nickerson DA, Shendure J, Zariwala MA; Genetic Disorders of Mucociliary Clearance Consortium. 2013. Exome sequencing identifies mutations in CCDC114 as a cause of primary ciliary dyskinesia. Am J Hum Genet. 92(1):99-106.
13. Cancer Genome Atlas Network (357 authors). 2012. Comprehensive molecular portraits of human breast tumours. Nature 490(7418):61-70.
14. **Berg JS**, Adams M, Nassar N, Bizon C, Lee K, Schmitt CP, Wilhelmsen KC, Evans JP. 2013. An informatics approach to analyzing the incidentalome. Genet Med. 15(1):36-44.
15. Green RC, **Berg JS**, Biesecker L, Dimmock D, Evans JP, Grody WE, Hegde M, Kalia S, Korf BR, Krantz I, McGuire AL, Miller D, Murray M, Nussbaum R, Plon S, Rehm

H, Jacob HJ. 2012. Expert concordance and discordance for return of incidental findings from whole genome sequencing. Genet Med. 14(4):405-10.
16. Challen GA, Sun D*, Jeong M*, Luo M*, Jelenik J*, **Berg JS***, Bock C, Vasanthakumar A, Gu H, Xi Y, Ling S, Lu Y, Darlington GJ, Meissner A, Issa JP, Godley LA, Li W, Goodell MA. 2012. Dnmt3a is essential for hematopoietic stem cell differentiation. Nat Gen. 44(1): 23-31. (* equal contributors)
17. **Berg JS***, Lin KK*, Sonnet C, Boles NC, Weksberg DC, Nguyen H, Holt LJ, Rickwood D, Daly RJ, Goodell MA. 2011. Imprinted genes that regulate early mammalian growth are coexpressed in somatic stem cells. PLoS One. 6(10): e26410. (* equal contributors)
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19. Liu P, Erez A, Nagamani SCS, Bi W, Carvalho CMB, Simmons AD, Wiszniewska J, Fang P, Eng PA, Cooper ML, Sutton VR, Roeder ER, Bodensteiner JB, Delgado MR, Prakash SK, Belmont JW, Stankiewicz P, **Berg JS**, Shinawi M, Patel A, Cheung SW, Lupski JR. 2011. Copy number gain at Xp22.31 includes complex duplication rearrangements and recurrent triplications. Hum Molec Genet. 20(10): 1975-1988.
20. **Berg JS**, Evans JP, Leigh MW, Omran H, Bizon C, Mane K, Knowles MR, Weck KE, Zariwala MA. 2011. Next generation massively parallel sequencing of targeted exomes to identify genetic mutations in primary ciliary dyskinesia: Implications for application to clinical testing. Genet Med. 13(3): 218-229.
21. Dhar SU, del Gaudio D, German JR, Peters SU, Ou Z, Bader PI, **Berg JS**, Blazo M, Brown CW, Graham BH, Grebe TA, Lalani S, Irons M, Sparagana S, Williams M, Phillips JA 3rd, Beaudet AL, Stankiewicz P, Patel A, Cheung SW, Sahoo T. 2010. 22q13.3 deletion syndrome: clinical and molecular analysis using array CGH. Am J Med Genet A. 152A: 573-581.
22. Brunetti-Pierri N*, **Berg JS***, Scaglia F, Belmont J, Bacino CA, Sahoo T, Lalani SR, Graham B, Lee B, Shinawi M, Shen J, Kang SH, Pursley A, Lotze T, Kennedy G, Lansky-Shafer S, Weaver C, Roeder ER, Grebe TA, Arnold GL, Hutchison T, Reimschisel T, Amato S, Geragthy MT, Innis JW, Obersztyn E, Nowakowska B, Rosengren SS, Bader PI, Grange DK, Naqvi S, Garnica AD, Bernes SM, Fong CT, Summers A, Walters WD, Lupski JR, Stankiewicz P, Cheung SW, Patel A. 2008. Recurrent reciprocal 1q21.1 deletions and duplications associated with microcephaly or macrocephaly and developmental and behavioral abnormalities. Nat Genet. 40(12): 1466-1471. (** equal contributors)*
23. Ou Z*, **Berg JS***, Yonath H*, Enciso VB, Miller DT, Picker J, Spence E, Brasington C, Lenzi T, Keegan CE, Sutton VR, Belmont J, Chinault AC, Lupski JR, Cheung SW, Roeder E, Patel A. 2008. Array-CGH detects typical and atypical duplications at 22q11.2, a genomic disorder that is frequently inherited and associated with a variable phenotype. Genet Med. 10(4): 267-277. (** equal contributors*)
24. Ben-Shachar S, Ou Z, Shaw C, Belmont J, Patel MS, Hummel M, Amato S, Tartaglia N, **Berg J**, Sutton VR, Lalani S, Chinault AC, Cheung SW, Lupski JR, Patel A. 2008. 22q11.2 Distal Deletion: A Recurrent Genomic Disorder Distinct from DiGeorge Syndrome and Velocardiofacial Syndrome. Am J Hum Genet. 82(1): 214–221.
25. **Berg JS***, Brunetti-Pierri N*, Peters SU, Kang S-H, Fong C-T, Salamone J, Freedenberg D, Hannig VL, Albers Prock L, Miller DT, Raffalli P, Harris DJ, Peiffer DA, Gunderson KL, Sahoo T, Patel A, Clark GD, Lupski JR, Beaudet AL,

Cheung SW. 2007. Speech delay and autism-spectrum behaviors are frequently associated with duplication of the 7q11.23 Williams-Beuren syndrome region. Genet Med. 9(7): 427-441. (** equal contributors*)

26. Smyk M*, **Berg JS***, Pursley A, Curtis FK, Fernandez BA, Bien-Willner GA, Lupski JR, Cheung SW, Stankiewicz P. 2007. Male-to-female sex reversal due to an ~250 Kb deletion upstream of NR0B1 (DAX1). Hum Genet. 122(1): 63-70. (* *equal contributors*)
27. Thimgan MS, **Berg JS**, Stuart AE. 2006. Comparative sequence analysis and tissue localization of members of the SLC6 family of transporters in adult Drosophila melanogaster. J Exp Biol. 209(Pt 17): 3383-3404.
28. Sousa A, **Berg JS**, Robertson BW, Meeker RB, Cheney RE. 2006. Myo10 in brain: developmental regulation, identification of a headless isoform and dynamics in neurons. J Cell Sci. 119(Pt 1): 184-194.
29. Weber KL, Sokac AM, **Berg JS**, Cheney RE, Bement WM. 2004. A microtubule-binding myosin required for nuclear anchoring and spindle assembly. Nature. 431(7006): 325-329.
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35. **Berg JS**, Farel PB. 2000. Developmental regulation of sensory neuron number and limb innervation in the mouse. Brain Res Dev Brain Res. 125(1-2), 21-30.

<u>Other peer reviewed articles (including reviews)</u>

1. Roche MI and **Berg JS**. 2015. Incidental Findings with Genomic Testing: Implications for Genetic Counseling Practice. Current Genetic Medicine Reports (Accepted).
2. Botkin JR, Belmont JW, **Berg JS**, Berkman BE, Bombard Y, Holm IA, Levy HP, Ormond KE, Saal HM, Spinner NB, Wilfond BS, McInerney JD; ASHG Workgroup on Pediatric Genetic and Genomic Testing. 2015. Points to Consider: Ethical, Legal, and Psychosocial Implications of Genetic Testing in Children and Adolescents. Am J Hum Genet. 97:6-21.
3. Rehm HL, **Berg JS**, Brooks LD, Bustamante CD, Evans JP, Landrum MJ, Ledbetter DH, Maglott DR, Martin CL, Nussbaum RL, Plon SE, Ramos EM, Sherry ST, Watson MS; ClinGen. 2015 ClinGen--the Clinical Genome Resource. N Engl J Med. 372(23):2235-42.
4. Adams MC, **Berg JS**, Pearlman MD, Vora NL. 2015. Look before you leap: genomic screening in obstetrics and gynecology. Obstet Gynecol. 125(6):1299-305.

5. Skrzynia C, **Berg JS**, Willis MS, Jensen BC. 2015. Genetics and Heart Failure: A Concise Guide for the Clinician. Curr Cardiol Rev. 11(1):10-17.
6. Khan CM, Rini C, Bernhardt BA, Roberts JS, Christensen KD, Evans JP, Brothers KB, Roche MI, **Berg JS**, Henderson GE. 2014. How Can Psychological Science Inform Research About Genetic Counseling for Clinical Genomic Sequencing? J Genet Couns. 24(2):193-204.
7. Prince AE, **Berg JS**, Evans JP, Jonas DE, Henderson G. 2014. Genomic screening of the general adult population: key concepts for assessing net benefit with systematic evidence reviews. Genet Med. 17(6):441-3.
8. Jarvik GP, Amendola LM, **Berg JS**, Brothers K, Clayton EW, Chung W, Evans BJ, Evans JP, Fullerton SM, Gallego CJ, Garrison NA, Gray SW, Holm IA, Kullo IJ, Lehmann LS, McCarty C, Prows CA, Rehm HL, Sharp RR, Salama J, Sanderson S, Van Driest SL, Williams MS, Wolf SM, Wolf WA; eMERGE Act-ROR Committee and CERC Committee; CSER Act-ROR Working Group, Burke W. 2014. Return of genomic results to research participants: the floor, the ceiling, and the choices in between. Am J Hum Genet. 94(6):818-26.
9. Ramos EM, Din-Lovinescu C, **Berg JS**, Brooks LD, Duncanson A, Dunn M, Good P, Hubbard TJ, Jarvik GP, O'Donnell C, Sherry ST, Aronson N, Biesecker LG, Blumberg B, Calonge N, Colhoun HM, Epstein RS, Flicek P, Gordon ES, Green ED, Green RC, Hurles M, Kawamoto K, Knaus W, Ledbetter DH, Levy HP, Lyon E, Maglott D, McLeod HL, Rahman N, Randhawa G, Wicklund C, Manolio TA, Chisholm RL, Williams MS. 2014 Characterizing genetic variants for clinical action. Am J Med Genet C Semin Med Genet. 166(1):93-104.
10. Krantz MS, **Berg JS**. 2013. Crowdsourcing to define the clinical actionability of incidental findings of genetic testing. N C Med J. 74(6):501-2.
11. Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group. 2014. Recommendations from the EGAPP Working Group: does PCA3 testing for the diagnosis and management of prostate cancer improve patient health outcomes? Genet Med. 16(4):338-46.
12. Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group. 2014. The EGAPP initiative: lessons learned. Genet Med. 16(3):217-24.
13. Rehm HL, Bale SJ, Bayrak-Toydemir P, **Berg JS**, Brown KK, Deignan JL, Friez MJ, Funke BH, Hegde MR, Lyon E; Working Group of the American College of Medical Genetics and Genomics Laboratory Quality Assurance Commitee. 2013. ACMG clinical laboratory standards for next-generation sequencing. Genet Med. 15(9):733-47.
14. Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group. 2013. Recommendations from the EGAPP Working Group: does genomic profiling to assess type 2 diabetes risk improve health outcomes? Genet Med. 15(8):612-7.
15. Green RC, **Berg JS**, Grody WW, Kalia SS, Korf BR, Martin CL, McGuire AL, Nussbaum RL, O'Daniel JM, Ormond KE, Rehm HL, Watson MS, Williams MS, Biesecker LG. 2013. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. Genet Med. 15(7):565-74.
16. Evans JP, **Berg JS**, Olshan AF, Magnuson T, Rimer BK. 2013. We screen newborns, don't we?: realizing the promise of public health genomics. Genet Med. 15(5):332-4.
17. Evans JP, **Berg JS.** 2011. Next-Generation DNA Sequencing, Regulation, and the Limits of Paternalism: The Next Challenge. JAMA. 306(21): 2376-2377.

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Editorials or letters

1. **Berg JS**. 2014. Genome-scale sequencing in clinical care: establishing molecular diagnoses and measuring value. JAMA. 312(18):1865-7.
2. **Berg JS**. 2013. Response to Lindor et al. Genet Med. 15(5):409-10.
3. **Berg JS**, Goodell MA. 2008. An argument against a role for Oct4 in somatic stem cells. Cell Stem Cell. 1(4): 359-360.

Book chapters

1. **Berg JS** and Powell CM. Potential Uses and Inherent Challenges of Using Genome-Scale Sequencing to Augment Current Newborn Screening. In Molecular Approaches to Reproductive and Newborn Medicine, CSH Perspectives. (In Press)
2. Haskell GT and **Berg JS**. Whole Genome Sequencing in the Molecular Pathology Laboratory. In Diagnostic Molecular Pathology, First Edition. (In Press)

Invited Presentations

1. **Berg JS.** 2014. "Exploring the yield of exome sequencing in a broad range of genetic conditions: The first 300+ cases in the NCGENES study." Cold Spring Harbor Laboratory; Personal Genomes: Discovery, Treatment and Outcomes.
2. **Berg JS.** 2014. "Translation of genome-scale sequencing into clinical care and public health." Duke University; Human Genetics Evening.
3. **Berg JS**. 2014. "Interpretation can't happen in isolation." American College of Medical Genetics Annual Meeting; Highlights Plenary Session.
4. **Berg JS**. 2014. "Application of a semi-quantitative metric to assess medical actionability of genomic findings." Institute of Medicine Roundtable on

Translating Genomic-Based Research for Health; Assessing Genomic Sequencing Information for Health Care Decision Making.

5. **Berg JS**. 2013. "ClinGen: Curating the Data." Human Genome Variation Society Annual Scientific Meeting.
6. **Berg JS**. 2013. "Clinical Data: CSER Perspective." NHGRI Sequencing Network Meeting; Genome Sequencing Informatics Tools Consortium.
7. **Berg JS**. 2013. "The Clinical Genomic Resource: A Knowledge Base for Clinically Relevant Genes and Variants." 2nd Annual Biomedical Informatics Symposium, Georgetown University Medical Center.
8. **Berg JS**. 2013. "Navigating the Genome: UNC's Experience with Clinical Genome Sequencing." UNC Institute for Personalized and Individualized Therapy.
9. **Berg JS**, Evans JP. 2013. "Navigating the Genome: UNC's Experience with Clinical Genome Sequencing." Genomic and Personalized Medicine Forum, Duke University Institute for Genome Sciences and Policy.
10. **Berg JS**. 2013. "Binning the genome: practical management of genomic incidental findings in a clinical context." Advances in Genome Biology and Biotechnology.
11. **Berg JS**. 2012. "Managing incidental findings from genome-scale sequencing tests: Maximizing benefits and minimizing harm." North Carolina Medical Genetics Association Meeting.
12. **Berg JS**. 2012. **"**Clinical analysis of genome-scale sequencing tests: Maximizing benefits and minimizing harm." Wellcome Trust Genomic Disorders 2012: The Genomics of Rare Diseases.
13. **Berg JS**. 2011. Moderator, Plenary Panel Debate "Owning the Genome: Gene Patenting and Licensing and Their Impact on Medical Genetics." International Congress of Human Genetics.
14. **Berg JS**. 2011. "Categorizing variants after whole genome sequencing: Implementation of "binning" -- a structured algorithm for the identification of clinically relevant incidental findings." NHGRI/Wellcome Trust Workshop.

Other oral presentations and/or abstracts

1. **Berg JS**. 2013. "NCGENES: One lesson learned." NHGRI Clinical Sequencing Exploratory Research Consortium Steering Committee Meeting.
2. **Berg JS**. 2013. "The diagnostic odyssey: Lessons learned along the way." NHGRI Clinical Sequencing Exploratory Research Consortium Steering Committee Meeting.
3. **Berg JS**. 2011. "A structured clinical analysis of 81 whole genome sequences." UNC Genetics Departmental Retreat.
4. **Berg JS**. 2011. "Moving forward on a framework for analysis of diagnostic and incidental results of whole exome / genome sequencing." Webinar, Illumina.
5. **Berg JS**. 2011. "Next-generation sequencing for germline variants: research and clinical applications." 2nd Annual UNC Next-generation Sequencing Symposium.

Teaching Record

Lectures:

2014:

MS4 Integration Selective "Science of Medicine"
Course Director: Dr. Michael Meyers
2 lectures: Clinical medicine in an age of personal genomics

Lecture given for PATH 723: 1 contact hour
Course Organizer: Bill Coleman
- 1/28/2013: "Introduction to genomics"

Lecture given for PHYI 703: 1 contact hour
Course Organizer: Michael Goy
- 1/23/2013: "Cancer genetics, genomics, and personalized medicine"

2013:

Obstetrics and Gynecology Residency Genetics Curriculum Seminar: 1 contact hour
Course Organizer: Neeta Vora
- 10/16/2013: "Clinical cancer genetics"

Pathology Residency Molecular Diagnostics and Cytogenetics Course: 1 contact hour
Course Organizer: Margaret Gulley
- 10/14/2013: "Next-generation sequencing: From DNA to data to diagnosis"

Dermatology Residency Lecture Series: 1 contact hour
Course Organizer: Christopher Sayed
- 4/26/2013: "Strange skin lesions and hereditary cancer syndromes"

Lecture given for GNET 647: 1 contact hour
Course Organizer: Karen Mohlke
- 4/25/2013: "Medical genetics"

Lecture given for Medical Genetics course: 1 contact hour
Course Organizer: Cynthia Powell
- 4/15/2013: "Clinical analysis of whole exome/whole genome sequencing data"

Lecture given for Molecular Pathology course: 1 contact hour
Course Organizer:
- 2/13/2013: "From DNA to data to diagnosis"

Lecture given for PATH 723: 1 contact hour
Course Organizer: William Coleman
- 1/28/2013: "Introduction to Genomics"

Lecture given for PHYI 703: 1 contact hour
Course Organizer: Michael Goy

- 1/25/2013: "Personalized Medicine – cancer phenotyping and modern genetic diagnostic technologies"

MS1 Molecules to Cells
Course Director: Dr. Gwen Sancar
6 lectures
- Chromosomes
- X-linked disorders
- Autosomal Dominant disorders
- Epigenetics
- Common Disease I
- Common Disease II

3 case presentations
3 small group sessions

MS4 Integration Selective "Science of Medicine"
Course Director: Dr. Michael Meyers
6 lectures: Clinical medicine in an age of personal genomics

2012:

MS1 Molecules to Cells
Course Director: Dr. Gwen Sancar
6 lectures
- Chromosomes
- X-linked disorders
- Autosomal Dominant disorders
- Epigenetics
- Common Disease I
- Common Disease II

3 case presentations
4 small group sessions

MS4 Integration Selective "Science of Medicine"
Course Director: Dr. Michael Meyers
6 lectures: Clinical medicine in an age of personal genomics

2011:

MS1 Molecules to Cells
Course Director: Dr. Gwen Sancar
5 lectures
- Chromosomes
- X-linked disorders
- Epigenetics
- Common Disease I
- Common Disease II

3 case presentations
4 small group sessions

MS4 Integration Selective "Science of Medicine"

Course Director: Dr. Michael Meyers
6 lectures: Clinical medicine in an age of personal genomics

2010:

Dermatology Residency Lecture Series
Conference Organizer: Donna Culton
- 2/12/2010: "Strange skin lesions and hereditary cancer syndromes"

MS1 Molecules to Cells
Course Director: Dr. Gwen Sancar
4 lectures
- Chromosomes
- X-linked disorders
- Epigenetics
- Common Disease

1 case presentation
4 small group sessions

MS4 Integration Selective "Science of Medicine"
Course Director: Dr. Michael Meyers
4 lectures: Clinical medicine in an age of personal genomics

2009:

MS1 Molecules to Cells
Course Director: Dr. Gwen Sancar
4 lectures
- Chromosomes
- X-linked disorders
- Epigenetics
- Common Disease

3 small group sessions

Grand Rounds:

2014:

Department of Genetics Seminar Series
Conference Organizer: Jason Whitmire
- 3/12/2014: "150+ exomes sequenced in a diagnostic setting: the NCGENES experience"

Medicine and Pediatrics Endocrine Research Conference
Conference Organizer: Ali Calikoglu
- 2/27/2014: "Exome sequencing in a diagnostic setting: the NCGENES experience"

2011:

Department of Genetics Seminar Series

Conference Organizer: Sandee English

- 6/1/2011: "Harnessing the power of next-generation sequencing in medical genetics"

MD/PhD Program Seminar Series
Conference Organizer: Alison Regan

- 3/7/2011: "Next-generation sequencing for novel gene discovery in families with apparently hereditary cancer susceptibility"

2010:

Department of Medicine Grand Rounds
Conference Organizer: Sarah L. Perry

- 5/5/2010: "Clinical Genetics and Medicine in an Era of Personal Genomics"

2009:

Duke University "Genomes @ 4" Seminar Series
Conference Organizer: Shandra L. Robertson

- 10/14/2009: "On the brink of personal genomics: Seven ways in which the sequencing revolution could bend or break established norms of genetic testing"

Continuing Education Seminars:

2014:

Current Topics in Medical and Human Genetics
Conference Organizer: Dr. Arthur Aylsworth

- 3/13/2014: "150+ exomes sequenced in a diagnostic setting: the NCGENES experience"

2013:

Foundation for Genetic Technology 2013 Southeast Regional Genetics Conference (Invited)
Conference Organizer: Catherine Rehder (Duke University Health System Clinical Laboratories, Durham, NC)

- 10/13/2013: "Frameworks for Diagnostic and Incidental Findings in Clinical Genome-Scale Sequencing"

Summer Course in Translational Research in Genomic Medicine
Teresa R. Parker (Program Coordinator, Epidemiology Department, Emory University Rollins School of Public Health, Atlanta GA)

- 8/15/2013: "Defining the scenario: what is to be tested in whom and for what purpose?"

Raleigh Academy of Medicine (Invited)
Conference Organizer: James Coxe (Program Chair, Raleigh Academy of Medicine)

- 4/25/2013: "Genomic Medicine – Where Are We 10 Years After Sequencing The Human Genome?"

City of Hope "Genomics Bootcamp" (Invited)
Conference Organizer: Jeffrey Weitzel (Chief, Division of Clinical Cancer Genetics, City of Hope Comprehensive Cancer Center, Duarte, CA)

- 3/19/2013: "Medical Genomics: Opportunities and Challenges of Germline Genomics"

Current Topics in Medical and Human Genetics
Conference Organizer: Dr. Arthur Aylsworth

- 2/14/2013: "Mosaicism and risk for disease"

2012:

Current Topics in Medical and Human Genetics
Conference Organizer: Dr. Arthur Aylsworth

- 9/27/2012: "Population allele frequencies and assessment of variants of uncertain clinical significance"
- 2/9/2012: "Mystery patient presentations"

2011:

Current Topics in Medical and Human Genetics
Conference Organizer: Dr. Arthur Aylsworth

- 12/15/2011: "Context matters: Using Bayes to guide the reporting of variants"
- 11/10/2011: "An unusual cause of hyperammonemia in an adult"
- 6/30/2011: "Chondrodysplasia punctata - another 'blast from the past'"
- 4/14/2011: "A family with an autosomal dominant autoinflammatory disorder?"
- 2/17/2011: "NCGENES (North Carolina Clinical Genome Evaluation using Next-generation Exome Sequencing): A proposal for clinical sequencing exploratory research at UNC"
- 1/27/2011: "A fatal familial disorder"

2010:

Current Topics in Medical and Human Genetics
Conference Organizer: Dr. Arthur Aylsworth

- 10/14/2010: "A mystery patient"
- 9/16/2010: "Expression of imprinted genes in the mouse brain… can imprinting get even MORE complicated!?!?!?"
- 6/24/2010: "An adult with bone dysplasia – differential diagnosis"
- 5/27/2010: "Synthetic Life! Science or science-fiction?"
- 4/15/2010: "An adult with an undiagnosed childhood-onset genetic disorder"
- 2/4/2010: "When should genetic testing be considered in patients with pheochromocytoma or paraganglioma?"

2009:

Current Topics in Medical and Human Genetics

Conference Organizer: Dr. Arthur Aylsworth

- 12/17/2009: "Case Report: Ataxia"
- 10/29/2009: "Case Report: 39-year-old man with adult-onset neuro/GI decompensation"
- 9/24/2009: "What do medical students learn and retain about genetics?"
- 6/4/2009: "Copy number as a novel cancer risk factor"

Lab Mentoring:

2014-2015:

Gloria Haskell (Postdoctoral fellow)
Natasha Strande (Postdoctoral fellow)
Bryce Seifert (Postdoctoral fellow)
Alison Homstad (Graduate student)
Katie Bolling (GS1 graduate research rotation)
Kristen Dougherty (Master's student)
Daniel Marchuk (Informatics technician)
Christian Tilley (Lab technician)
Michael Adams (MS3-MS4 Holderness Medical Research Fellow)
Linran Zhou (Senior undergraduate research)
Krunal Amin (Junior undergraduate research)

2013-2014:

Peter Noone (MS1-MS2 medical student summer research rotation)
Bianca Harris (SURE undergraduate summer research rotation)
Daniel Marchuk (Informatics technician)
Gloria Haskell (Postdoctoral fellow)
Linran Zhou (Junior undergraduate research)

2012-2013:

Maren Ettinger (GS1 graduate research rotation)
Jonathan Mathew (MS3-MS4 independent research year)
Linran Zhou (Sophomore undergraduate research)

2011:

Michael Adams (MS1-MS2 summer research rotation)
David DeWeese (MS1-MS2 summer research rotation)
Linran Zhou, (Freshman undergraduate research)

2010:

Jonathan Mathew (MS1-MS2 summer research project)

ACMG Summer Scholars Program (MS1-MS2 summer experience in medical genetics):

2013: Matthew Krantz
2012: Michelle Brown
2011: Elizabeth Blyth

Other Clinical Teaching:

Genetic Counseling interns rotating in Cancer and Adult Genetics
Hematology-Oncology Fellows rotating in Cancer Genetics

Dissertation Committees:

Megan Schertzer (Genetics and Molecular Biology)
Doug Ball (Health Policy and Management)
Alexander Raines (Curriculum in Neurobiology)

Mentoring Committees:

Martilias Farrell (K01 committee)

Grants

Active Grants

U01 HG007437-01 Berg, Evans, Watson, Ledbetter (Co-PIs) 09/23/2013 – 07/31/2017
NHGRI (32%)
A Knowledge Base for Clinically Relevant Genes and Variants
This grant is part of a consortium project entitled the "Clinical Genomics Resource (ClinGen)" that aims to establish an evidence-based resource for the assessment of the clinical relevance of genes and variants. This knowledge base is critical for confident, efficient analysis and interpretation of genome-scale sequence data. The objective is to provide a publicly available consensus summary of evidence regarding the genes and variants that are implicated in human health and disease.
Role: Co-PI (Contact PI),

U19 HD077632-01 Powell (Co-PI) 09/05/2013 – 08/31/2018
NICHD/NHGRI (20.7%)
NC NEXUS, North Carolina Newborn Exome Sequencing for Universal Screening
This project explores the use of whole exome sequencing in a newborn screening context, evaluating the ability of this new technology to augment current biochemical screening and extend the types of conditions that can be effectively screened for. The study will also focus on the social and ethical implications of such screening and the nature of informed consent and parental decision-making.
Role: Co-PI

U01 HG006487-01 Evans (PI) 12/05/2011 – 11/30/2015
NHGRI (12%)
NC GENES: North Carolina Clinical Genomic Evaluation by NextGen Exome Sequencing
This large multidisciplinary project will examine the utility of whole exome sequencing as a diagnostic test in diverse patient populations, the discovery and impact of clinically relevant incidental findings, and ultimately provide insight into the best practices for genomic medicine.

Role: Co-PI

P50 HG004488-06 Henderson (PI) 09/27/2007 – 05/31/2018
NHGRI (3.3%)
Center for Genomics and Society
This project addresses NIH research priorities related to genomic health care by conducting research to address how evolving genomic technologies can be practically and ethically used in general medical care. The identification of asymptomatic individuals in the population at large who are at high risk for preventable disease represents an important opportunity as genomics is increasingly applied to the general population.
Role: Investigator

Completed Grants

550KR61305 Berg (PI) 02/01/2014 – 1/31/2015
NC TraCS (0%) $50,000
Validation of Whole Exome Sequencing-Identified Genomic Variants in Cardiac Disease
This project will support detailed follow-up of novel genetic variants identified through exome sequencing in the NCGENES project, which are considered to be potential candidates to explain the cardiac phenotypes in research participants. This study will include family segregation analysis, histological analysis of existing pathology specimens, and *in vitro* experiments.
Role: PI

Clinical Translational Cancer Research Award 08/01/2011 – 07/31/2012
Lineberger Comprehensive Cancer Center $25,000
A Next-Generation Diagnostic and Research Platform for Hereditary Cancer Susceptibility
This project supports a pilot study of young women with breast cancer who are undergoing genetic testing for possible hereditary breast cancer susceptibility. We are investigating the performance of whole exome sequencing as a possible diagnostic test in these individuals, compared to BRCA1/BRCA2 testing by protein truncation testing.
Role: PI (0% effort, reagents and supplies only)

UCRF Keystone Project 07/01/2010 - 06/30/2011
Lineberger Comprehensive Cancer Center $300,000
Harnessing the power of genetics in whole genome analysis of hereditary cancer susceptibility
This project supports whole-genome sequencing in multiple families with likely Mendelian cancer susceptibility in order to comprehensively identify candidate disease-causing mutations. We will then confirm candidate genes by sequencing in other unrelated probands with possible hereditary cancer susceptibility.
Role: Co-PI (0% effort, reagents and supplies only)

3-42066 04/15/10 – 07/14/11
NC TraCS $50,000
Harnessing the power of next-generation sequencing to identify novel disease genes
This project supported whole-genome sequencing in one proband with likely Mendelian breast cancer susceptibility in order to comprehensively identify candidate disease-causing mutations.
Role: PI

U24-CA078157 06/01/06 – 03/31/11
NCI
Cancer Genetics Network
This was an NCI contract for a multicenter project aimed at collecting a registry of patients with cancer or at risk for cancer because of a family history. 1087 participants were enrolled through UNC and our team continued to contact these individuals for periodic follow-up interviews until the registry was centralized.
Role: PI for the UNC/Emory registry

F32 HL086223-01 11/01/06 – 10/31/08
NHLBI
Epigenetic control of gene expression in hematopoiesis
This was an individual postdoctoral research grant to examine the role of chromatin in modulating hematopoietic stem cell gene expression.
Role: PI (Advisor: Dr. Margaret Goodell)

Professional Service

Within Discipline

- American Society for Human Genetics: Junior faculty representative to the AAMC College of Faculty in Arts and Sciences (CFAS)
  (2013 – present)

- American Society for Human Genetics: work group on "Pediatric Genetic Testing"
  (2013 – 2015)

- American College of Medical Genetics: work group on "Secondary Findings in Whole Exome/Genome Sequencing"
  (2012 - 2013)

- CDC Office of Public Health Genomics: "Evaluation of Genomic Applications in Practice and Prevention (EGAPP)" working group
  (2012 – present)

- American College of Medical Genetics: taskforce on "Clinical Laboratory Standards for Next Generation Sequencing"
  (2011 - 2013)

- American Board of Medical Genetics: development of examination questions
  (2011 - present)

- NCI Cancer Genetics Network and Rare Cancer Registry Steering Committee (2009 – present)

Within UNC School of Medicine

- Department of Pediatrics Search Committee: Division Chief, Pediatric Genetics & Metabolism (member, 2013 – 2014)
- Medical School Curriculum Task Force (member, 2012 – 2013)
- Strategic Planning for Sequencing Bioinformatics (member, 2012)
- MD-PhD Program Advisory Committee (member, 2009 – present)
- NC Cancer Hospital Transition Team (member, 2009 – 2010)

Memberships

- Member, The American Society for Human Genetics (2003 – present)
- Fellow, The American College of Medical Genetics (2011 – present)

Peer Review Activities

- Journals (alphabetical):
  - *American Journal of Human Genetics*
  - *American Journal of Medical Genetics Part A*
  - *Annals of Human Genetics*
  - *Clinical Genetics*
  - *Genetics in Medicine*
  - *Genome Research*
  - *JAMA*
  - *Molecular Systems Biology*
  - *Nature Biotechnology*
  - *North Carolina Medical Journal*
  - *Orphanet Journal of Rare Diseases*

- Grants:
  - Genome Canada, Genomics Applications Partnership Program (2015)
  - NHGRI Special Emphasis Panel on Non-Coding Variation 2014/10 ZHG1 HGR-M (O1) (7/10/2014)
  - NHGRI Special Emphasis Panel on Genomic Medicine 2014/01 ZHG1 HGR-M (J4) (11/14/2013)

- Site Visitor: Johns Hopkins University, "Online Mendelian Inheritance in Man" (6/19/2013)

- NHGRI Special Emphasis Panel on Genomic Medicine 2013/01 ZHG1 HGR-P (J2) (1/08/2013)

- Genome Canada, Large-Scale Applied Research Project Competition in Genomics and Personalized Health (2012)

- Wellcome Trust, Strategic Awards (2012)

Clinical Activities

UNC Cancer Genetics Clinic

- Multidisciplinary Breast Oncology Clinic
- Outpatient Cancer Genetics Clinic

UNC Adult Genetics Clinic

- Outpatient Adult Genetics Clinic

All clinical activities are carried out in conjunction with Genetic Counselors. From 2009-2013, clinical activities represented ~50% of my total effort. From 2014-present, clinical activities represented ~30% of my total effort.

Reflective Statement

As a clinician and researcher, I am interested in the development and application of genetic tests in patients and their families. The recent revolution in genetic sequencing technology has led to an unprecedented opportunity to investigate the underlying etiology in families with genetic conditions, both in the research arena and in the clinic. I am board certified in Clinical Genetics, and my primary clinical efforts are in the Adult and Cancer Genetics clinics at UNC, as part of a team of MD geneticists and genetic counselors evaluating individuals and families with Mendelian disorders. My clinic responsibilities currently account for ~30% of my effort. I also respond to inpatient consults for adult and cancer genetics.

My main research efforts extend directly from my clinical activities. First, I am spearheading a gene discovery project in collaboration with members of the clinical cancer genetics team, in which we are enrolling probands with a strong family history of cancer but negative results on clinically available genetic tests. We hypothesize that these individuals harbor rare deleterious mutations in potentially novel cancer susceptibility genes and we are utilizing high-throughput sequencing technology to comprehensively identify candidate disease-causing mutations in these probands.

I am also integrally involved in the planning and execution of a translational research project, called "NCGENES," which is evaluates the utility of whole exome sequencing in a

clinical context. As part of this effort, I helped to conceive an *a priori* structured clinical analysis paradigm that we are testing in collaboration with colleagues in the Center for Genomics and Society. This project, which was funded as a U01 grant from the NHGRI, examines the impact of incidental findings discovered during the course of whole exome sequencing, including the requirements for pre-test counseling and informed consent, computational methods for determining the likely clinical relevance of variants, best practices for return of incidental findings to patients, and the impact of these findings on patients and their families. The "binning" process that we conceived (Berg, 2011) has now been implemented in a computational algorithm by a student in my lab (Berg, 2012) so that we can begin to understand the burden of incidental findings that will be uncovered in an average individual and fine-tune the necessary computational analyses.

More recently, I helped to assemble a team of investigators to develop a translational research project aimed at examining the use of next-generation sequencing to augment traditional newborn screening methods. This project, called "NC NEXUS," was funded by NICHD/NHGRI as a U19 award. As co-PI, I am responsible for the design of the overall study and the implementation of the genomic sequencing and analysis pipelines. In particular, I am leading the process of delineating categories of genomic information that will be provided to parents either as part of a "next-generation newborn screening" panel of results, or as several succinct categories of "non-medically actionable" findings.

I am also a co-investigator on the Center for Genomics and Society's P50 center project that was recently awarded by NHGRI. This project is addressing the question of how genomic technologies might be deployed in a public health setting in order to identify rare individuals with highly actionable adult-onset conditions.

Finally, I am the contact PI on a multi-site collaborative U01 project aimed at producing a publically available resource for clinical relevant genes and variants. This project, called "ClinGen," brings together experts from three funded awards (two U01 grants and a U41 grant) and the National Center for Biotechnology Information. The goals of the consortium are to facilitate deposition of variant assessments in the ClinVar database by clinical laboratories, to define standard procedures for curating the clinical validity and clinical actionability of gene-phenotype pairs and the clinical significance of variants in those genes, and to develop a computational infrastructure to support expert curation groups and computational analysis. The ultimate goal of this project is to promote genomic medicine by providing open access to a carefully curated knowledge base.

My long-term goals are to contribute meaningfully to the implementation of genomic medicine, by studying the most fruitful applications of next-generation sequencing, understanding the impact on patients and their family members, and developing best practices for the clinical application of genome-wide sequence information. On the way, I will seek opportunities to make new discoveries about the genetic causation of human disease, with an emphasis on hereditary cancer susceptibility. The University of North Carolina and the School of Medicine have provided a fertile environment for exploring these avenues for translational research.

Teaching Statement

My clinical and research activities are bolstered by my involvement with teaching in the School of Medicine. It is well appreciated that many physicians are underprepared for an age of genomic medicine, even those who have recently completed their training. My approach to teaching medical students is to provide them with the broad view needed to appreciate the impact of genetics in their chosen fields, not to necessarily teach them to become clinical geneticists, but to provide them with the resources needed to recognize the rare clinical scenarios in which genetic evaluation might be important.

The lectures I gave in the MS1 "Molecules to Cells" course covered broad topics of importance to the students' basic understanding of genetics as it relates to human disease. These sessions were mostly didactic in nature but I also engaged the students with questions to consider in my syllabi and lecture materials. Interspersed in the lectures were short case presentations that were naturally more amenable to posing questions for the students to consider, and I used an audience response system to poll them on questions related to the cases, including ethical/legal/social implications. My participation in the student small group sessions typically involved moving between the groups, answering questions raised by the students, and prompting them to explore certain aspects of the case presentation.

I use a very different approach in the MS4 Integration Selective. In this course, students meet in groups of <20, sometimes as few as 5-10 students, which lends itself much more to a discussion format. My goal in this selective is to prompt the students to participate by posing questions for them to answer amongst themselves. We cover a range of topics loosely organized around "genetic testing" – the purposes of genetic testing, the different types of genetic tests, and the disparate phases of life in which testing is done. The seminar ends with a consideration of cutting edge genome-wide association studies and their implications (or lack thereof) for routine medical care, and the impending use of whole genome sequencing in a clinical context, which will reveal all different kinds of genetic findings with their myriad implications. The goal is for the students to realize that genetics touches on almost all specialties, and to recognize the implications and limitations of genetic testing.

The restructuring of the medical school curriculum in 2014 led to substantial changes in the lecture pattern for genetics topics. The course director responsible for genetics is still making assignments for the new lectures and small groups, and I anticipate continuing my involvement in medical student teaching in whatever manner I am able.

In addition to teaching within the medical school curriculum, I have recently joined the Curriculum in Genetics and Molecular Biology, where I have begun contributing to graduate level courses in the areas of medical genetics and the use of next-generation sequencing. I also teach in several other lecture series in the graduate school and medical school. In 2015, I will organize a "Genomic Medicine Colloquium" for post-doctoral fellows who have interests in clinical genetics and/or genomic medicine research, in order to build relationships and further their development.

Finally, I initiated the development of a T32 institutional training grant for postdoctoral training in Genomic Medicine, which I believe would greatly enhance the ongoing programs in medical genetics and research at UNC. The initial proposal was scored but not funded. This proposal was revised and resubmitted.

Bradford Powell, MD, PhD

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Last revision: November 10, 2015

Professional Experience

University of North Carolina, Chapel Hill, NC **8/2013-present**
Clinical Assistant Professor, Genetics

Education

Baylor College of Medicine, Houston, TX **7/2007-8/2013**
Postdoctoral Fellowship 7/2013-8/2013
Clinical Genetics Academic Research Fellowship, completed 6/2013 7/2012-6/2013
Medical Genetics Residency, completed 6/2102 7/2010-6/2012

University of Florida/Shands Hospital, Gainesville, FL **7/2007-6/2010**
Categorical Pediatric Residency, completed 6/2010

University of North Carolina School of Medicine, Chapel Hill, NC **8/1998-8/2000, 9/2005-5/2007**
MD, 5/2007

University of North Carolina at Chapel Hill, Chapel Hill, NC **8/2000-9/2005**
PhD in Genetics and Molecular Biology, 12/2005

Georgia Institute of Technology, Atlanta, GA **9/1994-6/1998**
BS in Applied Biology with Highest Honor, 6/1998

Honors and Awards

- Resident/Fellow Teaching Award, Baylor College of Medicine Department of Human and Molecular Genetics, 2012
- Customer Service is Key award, Shands Hospital, 2010

Bibliography

Refereed Articles:

1. Berg JS, Foreman AK, O'Daniel JM, Booker JK, Boshe L, Carey T, Crooks KR, Jensen BC, Juengst ET, Lee K, Nelson DK, **Powell BC**, Powell CM, Roche MI, Skrzynia C, Strande NT, Weck KE, Wilhelmsen KC, Evans JP. *A semi-quantitative metric for evaluating clinical actionability of incidental or secondary findings from genome-scale sequencing.* Genetics in Medicine, 13 August 2015, doi: 10.1038/gim.2015.104
2. **Powell BC**, Jiang L, Muzny DM, Treviño LR, Dreyer ZE, Strong LC, Wheeler DA, Gibbs RA, Plon SE. *Identification of TP53 as an Acute Lymphocytic Leukemia Susceptibility Gene Through Exome Sequencing*. Pediatric Blood and Cancer, December 2012, DOI: 10.1002/pbc.24417
3. Crayton ME 3rd*, **Powell BC***, Vision TJ, Giddings MC. *Tracking the evolution of alternatively spliced exons within the Dscam family*. BMC Evolutionary Biology, February 2006, Volume: 6, Pages: 16 (* Joint first-authors)

4. **Powell BC** and Hutchison CA 3rd. *Similarity-based gene detection: using COGs to find evolutionarily-conserved ORFs*. BMC Bioinformatics, January 2006, Volume: 7, Page: 31
5. Benders GA, **Powell BC**, Hutchison CA 3rd. *Transcriptional analysis of the conserved* ftsZ *cluster in* Mycoplasma genitalium *and* Mycoplasma pneumonia. Journal of Bacteriology, July 2005, Volume: 187, Pages: 4542-4551
6. Berg JS, **Powell BC**, Cheney RE. *A millennial myosin census*. Molecular Biology of the Cell, April 2001, Volume: 12, Pages: 780-794

Presentations

1. **Powell BC** and Sittler A. *Costs and Consent for Genetic Testing – Who Pays and How Much do Families and Health Care Providers Need To Know?* Oral Presentation at American Academy of Pediatrics National Conference and Exhibition. October 24, 2015.
2. **Powell BC**, O'Daniel, JM, Strande NT, Foreman KM, Lee, K, *Cardio, Coags, Cancer, Oh My: Factors that impact whether to report secondary, medically-actionable findings.* Webinar presentation. Genomics Case Conference of the American College of Medical Genetics and Genomics. February 18, 2015.
3. **Powell BC,** Foreman AKM, O'Daniel JM, Lee K, Boshe L, Crooks KR, Lu M, Booker JK, Weck KE, Evans JP, Berg JS. *Look before you leap, and list before you look: the use of* a priori *curated gene lists to guide exome analysis*. Platform talk 372. American Society of Human Genetics Annual Meeting. October 21, 2014.
4. **Powell BC**, representing the Clinical Sequencing Exploratory Research (CSER) Consortium Sequencing Standards Working Group. *Communicating Sequencing Standards to Clinicians and Patients.* CSER Steering Committee Meeting. October 9, 2014.
5. **Powell BC.** *Clinical Genomics Research at UNC – Opportunities for Collaboration with Hematology/Oncology Faculty*. Lineberger Comprehensive Cancer Center Annual Retreat. September 5, 2014.
6. **Powell BC**. *VARITAS: Variant analysis with rapid incorporation of tabular annotation sources*. (3601F) Poster presentation at the 67th Annual Meeting of the American Society of Human Genetics, November 9, 2012. San Francisco, CA.
7. **Powell BC,** Peddibhotla S, Cheung H, Ritter D, Strong LC, Wheeler DA, Gibbs RA, Plon SE. *Whole-exome sequencing to identify candidate genes for Li Fraumeni syndrome and Genomic instability disorders*. Oral presentation at 2012 Ataxia-Telangiectasia and Genomic Instability Workshop. November 6, 2012. San Francisco, CA.
8. **Powell BC**, Ritter D, Cheung H, Strong LC, Wheeler DA, Gibbs RA, Plon SE. *Identifying novel cancer susceptibility genes through exome sequencing and copy number analysis of individuals with Li-Fraumeni-like cancer phenotypes.* Poster presented at Annual Meeting of the Cancer Prevention and Research Institute of Texas, October 24, 2012. Austin, TX
9. **Powell BC.** *Sequence-based approaches to structural variant detection.* Oral presentation for Baylor College of Medicine Clinical Genetics Conference. March 26, 2012. Houston, TX
10. **Powell BC**, Delario M, Jiang L, Trevino L, Zabriskie R, Kimmel M, Strong LC, Wheeler DA, Gibbs RA, Plon SE, *Use of Whole Exome Sequencing to Identify the*

Molecular Basis of Susceptibility to Lymphoid Malignancies in Childhood;(1205F). Poster presentation at the 12th International Congress of Human Genetics/61st Annual Meeting of The American Society of Human Genetics, October 14, 2011, Montreal, Canada.

11. **Powell BC** and Hutchison CA 3rd. *Improving gene annotation: detecting errors using sequence homology*, Poster session at General Meeting of American Society of Microbiology, July 2005
12. **Powell, BC**. *Dabbling with Piddles: numerical computation in Perl*, Oral presentation to the Raleigh Perl Users' Group (Raleigh NC), September 2001
13. **Powell BC**, Lawerence D and Seillier-Moiseiwitsch F. *Identification of sequence characteristics of the HIV envelope that correspond with viral neutralization*. Statistics in functional genomics conference of AMS/IMS/SIAM, June 2001
14. **Powell, BC**. *Just enough DBI to be dangerous: an introduction to using databases with Perl*. Oral presentation to the Raleigh Perl Users' Group; http://raleigh.pm.org/dbi, September 2000

Software

1. VARITAS (Variant Analysis with Rapid Incorporation of Tabular Annotation Sources) https://github.com/bpow/varitas (available under MIT-style license)

Teaching Record

2013-present

University of North Carolina **Clinical Mentor**

As an attending physician in the Adult and Cancer Genetics program, I directly supervise required clinical rotations for Clinical Genetics Fellows, and elective rotations for Hematology/Oncology Fellows, Medicine Residents, and Medical Students in Adult Genetics and Cancer Genetics clinics. I also serve on the UNC medical genetics residency's Clinical Competency Committee.

University of North Carolina **Didactic Lectures**

Pediatric Cancer Predisposition. Lecture to Pediatric Hematology/Oncology Fellows. October 12, 2015. (one contact hour).

Genetics of Hemoglobinopathies and Thrombophilias: Molecular mechanisms, pedigrees and populations. Lecture in 1st year Hematology course, School of Medicine. MTEC101.HEM. October 9, 2015. (one contact hour)

Behavioral Traits, Complex Traits and Epigenetics. Lecture in 2nd year Human Behavior and Development course, School of Medicine. MTEC103.HBHD. August 7, 2015. (one contact hour)

University of North Carolina **Online modules**

Genetics of Muscular Dystrophies. Online module for 2nd year Neurology course, School of Medicine. MTEC102.NEU.April 28, 2014. (0.5 contact hour)

2012

Baylor College of Medicine **Research Mentor**
Supervised undergraduate student during summer research program and rotating graduate student in projects involving molecular confirmation of variants associated with inherited cancer predisposition

2010, 2011, 2012

Baylor College of Medicine **Facilitator**
Facilitated small-group discussions for ethics cases and patient/family interactions for first and second-year medical students.

6/2002 - 8/2002

University of North Carolina **Teaching Assistant**
Developed assignments and written materials for a graduate bioinformatics course--"Methods in Biological Sequence Analysis"

8/2001 - 12/2001

University of North Carolina **Teaching Assistant**
Taught weekly recitation for graduate course dealing with design of genetic experiments: "Principles of Genetic Analysis"

8/1999 - 6/2000

UNC School of Medicine **Teaching Assistant**
Assisted with course in Pre-clinical informatics (course for first-year medical students). Provided computer support for First and second-year medical students

Professional Service

8/2008 – 6/2010

Shands Healthcare **Clinical Content Committee**
Resident representative to committee providing input to decisions regarding implementation of an electronic medical record for outpatient clinics

Current Certification/Licensure

- North Carolina Medical License 2013-01999
- Diplomate of the American Board of Pediatrics (10/18/2010)
- Diplomate of the American Board of Medical Genetics, certified in Clinical Genetics (9/1/2013)

CURRICULUM VITAE

Personal Information:

Cynthia Marion Powell

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Work Address:	Campus Box #7487 Medical School Wing E, room 117 University of North Carolina at Chapel Hill Chapel Hill, NC 27599-7487
Home Phone:	(919) 933-6046
Work Phone	(919) 966-4202

Education:

1993	American Board of Medical Genetics: Clinical Genetics, recertified 2003, 2005, 2007, 2010 #93238 Clinical Cytogenetics, recertified 2003, 2005, 2007, 2010 #93238
1993	Board of Medical Examiners, State of North Carolina, license #0093-00571 (expires 7/18/2016)
7/1990 – 6/1993	Fellowship, Clinical Genetics and Cytogenetics, Interinstitute Genetics Program, Children's National Medical Center, Washington, DC and National Institutes of Health, Bethesda, MD
1990	American Board of Pediatrics, recertified 1997, 2004, 2011 #045388
1989	Board of Medical Examiners, District of Columbia, 1989, license #18141 (inactive)
1988	National Board of Medical Examiners, certificate #337614
7/1987 – 6/1990	Pediatric Residency, Children's National Medical Center, Washington, DC
5/1987	M.D., Medical College of Virginia, Richmond, VA
1982	American Board of Medical Genetics, Genetic Counseling #1388 (permanent certificate)
5/1978	M.S. in Human Genetics, Sarah Lawrence College, Bronxville, NY

5/1976	A.B. in Biology, with concentration in Genetics, Cornell University, College of Arts and Sciences, Ithaca, NY

Professional Experience – Employment History:

11/2012 - present	Professor of Pediatrics with tenure
11/2012 – present	Research Professor of Genetics
7/2004 – 11/2014	Chief, Division of Genetics and Metabolism, Department of Pediatrics, University of North Carolina at Chapel Hill
11/2001 – 10/2012	Research Associate Professor, Department of Genetics, University of North Carolina at Chapel Hill
7/2001 – present	Director, Medical Genetics Residency Training Program, Department of Genetics, University of North Carolina at Chapel Hill
9/2000 – 10/2012	Associate Professor of Pediatrics with tenure, Division of Genetics and Metabolism, University of North Carolina at Chapel Hill
7/1996 - present	Medical Director, Cytogenetics Laboratory, UNC Hospitals University of North Carolina at Chapel Hill
9/1993 – 8/2000	Assistant Professor of Pediatrics, Division of Genetics and Metabolism, University of North Carolina at Chapel Hill
9/1993 - present	Medical Staff, UNC Hospitals, Chapel Hill, NC
7/1990 – 8/1993	Medical Staff, Children's National Medical Center, Washington, DC
8/1978 – 7/1983	Genetic Counselor, Department of Clinical Genetics, Children's Hospital National Medical Center, Washington, DC

Honors:

Best Doctors in America, 2015-2016 database

RTI University Scholar Program 2015-16: provides support for distinguished academic researchers to spend scholarly leave time at RTI International

WR Kenan Jr. Senior Faculty Research and Scholarly Leave, UNC-CH, Academic Year 2015-16.

Best Doctors in America, 2014 database

Newborn Screening Translational Research Network Newsletter Spotlight Newborn Screening Researcher, July 2014

Best Doctors in America, 2013 database

Bridges Academic Leadership for Women Program, UNC-CH September – November 2009.

Elizabeth J. Harbison Memorial Award in Pediatrics, Medical College of Virginia; May, 1987.

Summer Research Fellowship, Masonic Medical Research Laboratory, Utica, NY; 1976.

Bibliography:

Books and Chapters:

Berg JS and **Powell CM**: Potential uses and inherent challenges of using genome-scale sequencing to augment current newborn screening. In Molecular Approaches to Reproductive and Newborn Medicine. Cold Spring Harbor Perspectives in Medicine Collection, DW. Bianchi and ER Norwitz, eds., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2015.

Powell CM: Sex chromosomes, sex chromosome disorders, and disorders of sex development. In The Principles of Clinical Cytogenetics, third edition, SL Gersen and MB Keagle, eds., Springer, New York, 2013.

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Powell CM: Achondroplasia. In Essence of Office Pediatrics JA Stockman and JA Lohr eds., WB Saunders Co. Philadelphia, PA, p 3, 2001.

Powell CM: The current state of prenatal genetic testing in the U.S. In Prenatal Genetic Testing and the Disability Rights Critique, E Parens and A Asch, eds., Georgetown University Press, Washington, D.C. 2000, pp 44-53.

Parens E, Asch A, **Powell CM**: Reproduction, ethics, prenatal genetic testing, and the disability rights critique. In Encyclopedia of Biotechnology: Ethical, Legal, and Policy Issues, MJ Mehlman and TH Murray, eds., John Wiley and Sons, 2000, pp 957-969.

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Refereed Papers/Articles:

Berg JS, Foreman AK, O'Daniel JM, Booker JK, Boshe L, Carey T, Crooks KR, Jensen BC, Juengst ET, Lee K, Nelson DK, Powell BC, **Powell, CM**, Roche MI, Skrzynia C, Strande NT, Weck KE, Wilhelmsen KC, Evans JP: A semi-quantitative metric for evaluating clinical actionability of incidental or secondary findings from genome-scale sequencing. Genet Med 2015 Aug 13 Epub ahead of print. PMID: 26270767

Bailey DB, Wheeler A, Berry-Kravis E, Hagerman R, Tassone F, **Powell CM**, Roche M, Gane LW, Sideris J: Maternal Consequences of the Detection of Fragile X Carriers in Newborn Screening. Pediatrics. Epub ahead of print. July 13, 2015. PMID: 26169437

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Chico, C, Heyrman K, Ibrahim J, Janssens S, Keena BA, Llano-Rivas O, Leppig K, McDonald M, Misra VK, Mulbury J, Narayanan V, Orenstein N, Galvin-Parton P, Pedro H, Pivnick EK , Powell CM, Randolph L, Raskin S, Rubin K, Seashore M, Schaaf CP, Scheuerle A, Schultz M, Schorry E, Schnur R, Siqveland E, Tkachuk A, Tonsgard J, Upadhyaya M, Verma IC, Wallace S, Williams C, Zackai E, Zonana J, Lazaro C, Claes K, Korf B, Martin Y, Legius E, Messiaen L: High incidence of Noonan syndrome features including short stature and pulmonic stenosis in patients carrying NF1 missense mutations affecting p.Arg1809: genotype-phenotype correlation. Hum Mutat Jul 14, 2015. Epub ahead of print. PMID: 26178382

Couser NL, Masood MM, Strande NT, Foreman AK, Crooks K, Weck KE, Lu M, Wilhelmsen KC, Roche M, Evans JP, Berg JS, **Powell CM**: The phenotype of multiple congenital anomalies-hypotonia-seizures syndrome 1: Report and review. Am J Med Genet A. 2015 Apr 29. Epub ahead of print.PMID: 25920937.

Blatt J, **Powell CM**, Burkhart CN, Stavas J, Aylsworth AS: Genetics of hemangiomas, vascular malformations, and primary lymphedema J Pediatr Hematol Oncol. 2014; 36:587-93. PMID: 25222064

Lindhurst MJ, Wang JA, Bloomhardt HM, Witkowski AM, Singh LN, Bick DP, Gambello MJ, **Powell CM**, Lee CC, Darling TN, Biesecker LG: AKT1 gene mutation levels are correlated with the type of dermatologic lesions in patients with Proteus syndrome. J. Invest. Derm. 2014, Feb;134(2):543-6. PMCID: PMC3868633 PMID: 23884311

Horstick EJ, Linsley JW, Dowling JJ, Hauser MA, McDonald KK, Ashley-Koch A, Saint-Amant L, Satish A, Cui WW, Zhou W, Sprague SM, Stamm DS, **Powell CM**, Speer MC, Franzini-Armstrong C, Hirata H, Kuwada JY: Stac3 is a component of the excitation-contraction coupling machinery and mutated in Native American myopathy. Nat. Commun. 4:1952 doi:10.1038/ncomms2952 (2013). Online publication, 4 Jun 2013. PMID: 23736855

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Bailey DB Jr, Lewis MA, Harris SL, Grant T, Bann C, Bishop E, Roche M, Guarda S, Barnum L, **Powell C**, Therrell BL Jr: Design and evaluation of a decision aid for inviting parents to participate in a fragile X newborn screening pilot study. J Genet Couns. 2013, 22:108-117.

Traylor RN, Dobyns WB, Rosenfeld JA, Wheeler P, Spence JE, Bandholz AM, Bawle EV, Carmany EP, **Powell CM**, Hudson B, Schultz RA, Shaffer LG, Ballif BC: Investigation of *TBR1* hemizygosity: four individuals with 2q24 microdeletions. Mol. Syndromol. 2012, 3:102-112.

Rosenfeld JA, 1q21.1 Study Group*, Traylor RN, Schaefer GB, McPherson EW, Ballif BC, Klopocki E, Mundlos S, Shaffer LG, Alysworth AS
*****1q21.1 Study Group Contributors
The 1q21.1 Study Group includes the following: Ilse Anderson, Brad Angle, Holly Ardinger, Alexander Asamoah, Joan F. Atkin, Judith Axelrod, Patricia Bader, Carrie Blout, Campbell Brasington, Lauren C. Briere, Pamela L. Brock, Barbara Burton, David Chitayat, Lisa J. Cushman, Dawn L. Earl, Dima El-Khechen, Luis F. Escobar, Aline Hamati, David J. Harris, Gail Herman, Jacqueline Hoover, Kelly Jackson, Elizabeth A. Jenkins, Lawrence C. Kaplan, Abigail Klemsz, Yves Lacassie, Roger Ladda, Helen Lyon, Gregory P. MacDonald, Suneeta Madan-Khetarpal, Michael Marble, Paul R. Mark, Laura S. Martin, Nicole Martin, Juliann S. McConnell, Elizabeth McCracken, Marie McDonald, Marianne McGuire, Roberto Mendoza-Londono, Amanda N. Miller, John B. Moeschler, Amanda G. Noyes, Kathryn Platky, **Cynthia M. Powell**, Abbey Putnam, Vickie Roberts, Warren G. Sanger, Susan Sell, Yasmin Senturias, Vandana Shashi, Natasha Shur, Komudi Siriwardena, Annemarie Sommer, J. Edward Spence, James Stavropoulos, Cathy A. Stevens, Mark Tarnopolsky, Ellen Thomas, Matthew J. Thomas, Amy Venter, David D. Weaver, William G. Wilson
Proximal microdeletions and microduplications of 1q21.1 contribute to variable abnormal phenotypes; Eur J Hum Genet, Feb. 2012, 1-8, online publication.

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Kimani JW, Buchman, CA, Booker JK, Huang BY, Castillo M, **Powell CM**, Weck KE: Sensorineural hearing loss in a pediatric population: association of congenital cytomegalovirus infection with intracranial abnormalities. Arch Otolaryngol Head Neck Surg. 2010 Oct;136(10):999-1004.

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Rampersaud E, Bassuk AG, Enterline DS, George TM, Siegel DG, Melvin EC, Aben J, Allen J, Aylsworth A, Brei T, Bodurtha J, Buran C, Floyd LE, Hammock P, Iskandar B, Ito J, Kessler JA, Lasarsky N, Mack P, Mackey J, McLone D, Meeropol E, Mehltretter L, Mitchell LE, Oakes WJ, Nye JS, **Powell C**, Sawin K, Stevenson R, Walker M, West SG, Worley G, Gilbert JR, Speer MC. Whole genomewide linkage screen for neural tube defects reveals regions of interest on chromosomes 7 and 10. J Med Genet. 42(12):940-6, 2005.

Ravassipour DB, **Powell CM**, Phillips CL, Hart PS, Hart TC, Boyd C, Wright JT: Variation in dental and skeletal open bite malocclusion in humans with amelogenesis imperfecta. Arch Oral Biol 50(7):611-23, 2005.

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Mah ML, Wallace, DK, **Powell CM**: Ophthalmic manifestations of Angelman syndrome. Journal of the American Association for Pediatric Ophthalmology and Strabismus 4(4):248-249, 2000.

Ashley-Koch A, Wolpert CM, Menold MM, Zaeem L, Basu S, Donnelly SL, Ravan SA, **Powell CM**, Qumsiyeh MB, Aylsworth AS, Vance JM, Gilbert JR, Wright HH, Abramson RK, DeLong GR, Cuccaro, Pericak-Vance MA: Genetic studies of autistic disorder and chromosome 7. Genomics 61:227-236, 1999.

Powell CM, Michaelis RC: Townes-Brocks syndrome. Journal of Medical Genetics 36:89-93, 1999.

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Other Peer-Reviewed Articles:

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Published Abstracts:

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Basu S, Ashley-Koch A, Wolpert CM, Menold MM, Matsumoto N, Powell CM, Qumsiyeh MB, Cuccaro ML, Ledbetter DH, Green ED, Vance JM, Pericak-Vance MA, Gilbert JR: Autistic disorder and chromosome 7: analysis of an inversion breakpoint in a multiplex family. American Journal of Human Genetics 65(4):A264, 1999.

Lamb AN, Lytle CH, Aylsworth AS, Powell CM, Rao KW, Hendrickson M, Carey JC, Opitz JM, Viskochi DH, Leonard CO, Brothman AR, Stephan M, Bartley JA, Hackbarth M, McCarthy D, Proffitt J: Low proportion of subtelomeric rearrangements in a population of patients with mental retardation and dysmorphic features. American Journal of Human Genetics 65(4):A169, 1999.

Kaiser-Rogers KA, Rao KW, Roche MI, Lese CM, Powell CM: A rare terminal deletion involving the distal short arm of chromosome 12. American Journal of Human Genetics 65(4):A166, 1999.

Ashley-Koch A, Wolpert CM, Menold MM, Zaeem L, Basu S, Donnelly SL, Ravan SA, Powell CM, Qumsiyeh MB, Aylsworth AS, Vance JM, Gilbert JG, Wright HH, Abramson RK, DeLong GR, Cuccaro ML, Pericak-Vance MA: Evidence for a paternal effect on chromosome 7 in autistic disorder. American Journal of Human Genetics 65(4):A60, 1999.

Powell CM, Aylsworth AS, Kaiser-Rogers KA, Rao KW: Duplication 17p11.2 in two patients: clinical features and molecular cytogenetic findings. Genetics in Medicine 1:60, 1999.

Nandi KN, Kaiser-Rogers KA, Powell CM, Phillips KK, Callanan NP, Rao KW: A small interstitial deletion of chromosome 11p in a child with dysmorphic features and Duane anomaly. American Journal of Human Genetics 63(4):A114, 1998.

Phillips KK, Kaiser-Rogers KA, Powell CM, Nandi KN, Rao KW: A paracentric inversion X chromosome found in four apparently unrelated families: is inv(X)(q26q28) a recurring rearrangement? American Journal of Human Genetics 63(4):A147, 1998.

Del Vecchio MA, Matika GL, Grebe TA, Butler MG, Powell CM, Smith ACM, Curry CJR, Stevenson RE, Greenberg F, Bay CA: Food foraging behaviors: a phenotypic overlap between Smith-Magenis and Prader-Willi syndromes. American Journal of Human Genetics 63(4): A101, 1998.

Pericak-Vance, MA, Wolpert CM, Menold MM, Bass MP, Hauser ER, Donnelly SL, Ravan SA, Powell CM, Qumsiyeh M, Aylsworth AS, Vance JM, DeLong GR, Wright HH, Abramson RK, Gilbert JG, Cuccaro ML: Chromosome 7 and autistic disorder (AD). American Journal of Human Genetics 63(4):A16, 1998.

Reitnauer PJ, Powell CM: Delineation of Williams syndrome phenotype in an African American female and her mother. Are the facial features of Williams syndrome less obvious in African Americans? Proceedings of the Greenwood Genetic Center 16:234, 1997.

Powell CM, Michaelis RC: Two new microdeletion syndromes: Townes-Brocks "Plus" and DEFECT 11. Proceedings of the Greenwood Genetic Center 16:214, 1997.

Powell CM: Fetal valproate syndrome: case report and review. Proceedings of the Greenwood Genetic Center 16:208, 1997.

Kaiser-Rogers KA, Powell CM, Callanan NP, Ledbetter DH, Teplin S, Rao KW: The usefulness and limitations of using FISH to characterize two partially cryptic complex chromosome rearrangements. American Journal of Human Genetics 61(4):A129, 1997.

Michaelis RC, Kaiser-Rogers KA, Reitnauer PJ, Rao KW, Powell CM: Refinement of the critical region for Townes-Brocks syndrome. American Journal of Human Genetics 59(4):A228, 1996.

Kaiser-Rogers KA, Rao KW, Callanan NP, Powell CM: A rare interstitial deletion of chromosome 12: del(12)(q15q21.1). American Journal of Human Genetics 59(4):A121, 1996.

Endrigkeit US, Doron MW, Aylsworth AS, Rao KW, Powell CM: Tetrasomy 9p: case report and delineation of the syndrome. American Journal of Human Genetics 59(4):A117, 1996.

Powell CM, Reitnauer PJ, Kaiser-Rogers KA, Rao KW: Anus, hand, and ear anomalies in a patient with a paracentric inversion of 16q: further evidence for a Townes-Brocks syndrome gene at 16q12.1. Proceedings of the Greenwood Genetic Center 15:192, 1996.

Reitnauer PJ, Albright SG, Aylsworth AS, Powell CM, Kaiser-Rogers KA, Rao KW: A subtle familial translocation 11;22 associated with macrocephaly in "balanced" carriers. American Journal of Human Genetics, 57(4):A124, 1995.

Kaiser-Rogers KA, Davenport ML, Powell CM, Rao KW: A recombinant X chromosome with an atypical centromere observed in a child with Turner Syndrome. American Journal of Human Genetics, 57(4):A118, 1995.

Powell CM, Reitnauer PJ, Kaiser-Rogers KA, Rao KW: A paracentric inversion of 16q in a patient with anus, hand, and ear anomalies: further evicence for a Townes-Brocks syndrome gene at 16q12.1. American Journal of Human Genetics, 57(4):A100, 1995.

Powell CM, Saal HM: Oblique facial clefts: review of cases and etiology: Proceedings of the Greenwood Genetic Center 14:42, 1995.

Reitnauer PJ, Rao KW, Tepperberg JH, Aylsworth AS, Powell CM: Malformation/dysplasia syndrome (neural tube defect, hypospadias, neuroblastoma) associated with an extra dicentric marker chromosome 15 ("inversion duplication 15"). American Journal of Human Genetics 55(3):A321, 1994.
Tepperberg JH, Rao KW, Albright SG, Kaiser-Rogers K, Powell CM: Deletion 6(p25.1) in a child with mild dysmorphic features and absence of major eye malformations: implications for the location of genes involved in ocular development. American Journal of Human Genetics 55(3):A119, 1994.

Powell CM, Saal HM: Congenital heart defects in incontinentia pigmenti. Proceedings of the Greenwood Genetic Center 13:66, 1994.

Powell CM, Taggart RT, Drumheller TC, Wangsa D, Qian C, Nelson LM, White BJ: Molecular and cytogenetic studies of an X;autosome translocation in a patient with premature ovarian failure and review of other cases: is there a POF2 gene? American Journal of Human Genetics 53(3): Abstract 1215, 1993.

Powell CM, Schapiro MB, Rapoport SI, White BJ: Nucleolus organizing regions in Alzheimer's disease. American Journal of Human Genetics 51(4):A316, 1992.

Powell CM, Ellingham TJ, Rosenbaum KN, Stanley WS: Unbalanced 15;18 translocation in a Prader-Willi patient mosaic for a normal cell line. American Journal of Human Genetics 49(4):261, 1991.

Powell CM, Saal HM, Chandra RS: PHEVR syndrome: an autosomal recessive syndrome of pterygia, congenital heart anomalies, ear anomalies, vertebral defects and radial dysplasia. American Journal of Human Genetics 49(4):157, 1991.

In Press/Submitted:

Oral Presentations:

Powell, CM: Next Generation Newborn Screening, Invited Speaker, Mayo Clinic Individualizing Medicine Conference, Session 4B September 22, 2015.

Powell, CM: NSIGHT Projects Update (Newborn Sequencing In Genomic medicine and public HealTh). Invited speaker, Newborn Screening Translational Research Network Annual Meeting, North Bethesda, MD September 10, 2015.

Powell, CM: Genomics and Newborn Screening. Southeast Regional Newborn Screening and Genetics Collaborative 33rd Annual Meeting of the Southeastern Regional Genetics Group (SERGG), July 16, 2015, Asheville, North Carolina. Invited keynote speaker.

Powell, CM: Next Generation Newborn Screening. American Academy of Pediatrics 86th Perinatal & Developmental Medicine Symposium, *Perinatal Genomics*, June 5, 2015, Aspen, Colorado. Invited speaker. Lectures "Next Generation Newborn Screening" and "From Sideshows to Mudminnows to Newborns: the Career Path of a Medical Geneticist/Dysmorphologist".

Powell, CM: Next Generation Newborn Screening: The NC NEXUS Project. Newborn Genomics Conference, Children's Mercy Hospital Kansas City, Missouri, April 9, 2015. NSIGHT participant.

Powell, CM: NC NEXUS. Newborn Screening Translational Research Network Clinical Integration Group meeting presentation. February 2-3, 2015. Bethesda, Maryland.

Powell, CM: North Carolina Newborn Exome Sequencing for Universal Screening (NC NEXUS). Platform presentation at the Association of Public Health Laboratories Newborn Screening and Genetic Testing Symposium, Anaheim, California, October 27, 2014. (solicited)

Powell, CM: Next Generation Newborn Screening. North Carolina Medical Genetics Association Meeting, Duke University, Durham, North Carolina. October 10, 2014. Invited speaker.

Powell, CM: Next Generation Newborn Screening. The 15th International Conference on Human Genome Variation and Complex Genome Analysis (HGV2014), 17th - 19th September 2014, Belfast, Northern Ireland. Invited speaker.

Powell, CM: Next Generation Newborn Screening. Carolina Institute for Developmental Disabilities Investigator Forum, September 9, 2014. Invited speaker.

Powell, CM: The Future of Newborn Screening and Genomics. Texas Department of State Health Services Newborn Screening Conference, August 2, 2014, Dallas, Texas. Invited speaker.

Powell, CM: Next Generation Newborn Screening. National Academy of Sciences Committee on Science, Technology, and Law 27th Meeting, June 16, 2014. Invited speaker.

Powell, CM: The Narrowing Gap Between Screening and Diagnosis: Next Generation Newborn Screening. The Joint Garrod and Canadian Newborn and Child Screening Symposium. May 30, 2014. Invited speaker.

Powell CM, Eric J. HorstickEJ, Linsley JW, Dowling JJ, Aylsworth AS, Keelean-Fuller D, Hayden MA, Thorne LB, Stamm DS, Hauser MA, Ashley-Koch A, Hirata H, Franzini-Armstrong C, Satish A, Saint-Amant L, Gibson KM, Kuwada JY: Native American Myopathy is an arthrogryposis syndrome with susceptibility to malignant hyperthermia caused by mutation in *STAC3.* Platform presentation at the American College of Genetics and Genomics Annual Meeting, Nashville, Tennessee, March 27, 2014.

Powell CM, Roche M, Skinner D, Wheeler A, Bailey D: Newborn Screening for Fragile X Syndrome – Lessons Learned, presented at the Southeastern Regional Genetics Group annual meeting, Asheville, NC, July 20, 2013.

Powell, CM: Association of Public Health Laboratories Annual Meeting, Newborn Screening, Atlanta, GA, May 8, 2013: "Newborn Screening for Fragile X – Diagnosis, Counseling and Follow-Up." Platform presentation.

Powell, CM: Common Trisomies and Sex Chromosome Variations, National Birth Defects Prevention Network 15th Annual Meeting, February 28, 2012, Crystal City, Arlington, Virginia. (invited)

Powell C, Roche M: Assessment of Parental Attitudes about Genetics and Congenital Hearing Loss, presented at the 2011 ELSI Congress, April 13, 2011, UNC-Chapel Hill. (solicited/invited)

Powell C: Townes-Brocks syndrome. Cardinal Signs Symposium. American College of Medical Genetics Annual Clinical Genetics Meeting, Vancouver, British Columbia, Canada March 19, 2011. (invited)

Roche M, Skinner D, Choudhury S, Powell C, Bailey D: Parents' decision to accept or decline newborn FMR1 screening. American College of Medical Genetics Annual Clinical Genetics Meeting, Albuquerque, New Mexico. March 24-28, 2010. (solicited)

Powell CM: Sakoda complex with prenatal lamotrigine exposure: possible clue to etiology? Presented at the 30th Annual David W. Smith Workshop on Morphogenesis and Malformations, Philadelphia, Pennsylvania, August 8, 2009. (solicited)
Davis A, Powell C, Henderson GE, King NMP, Whitmarsh I, Manickam M: More Than You Bargained For: When Genetic Testing Finds "Other" Variations, American Society for Bioethics and Humanities. 10th Annual Meeting, Cleveland, Ohio, October 23-26, 2008. (solicited)

Powell CM: Newborn Screening Overview. UNC Center for Genomics and Society 2008 Policy Forum on Newborn Screening. May 5, 2008, UNC-CH. (invited)

Powell CM: Increasing Family Needs for Genetic Information and Counseling in Expanded Newborn Screening. International ELSI Congress, Cleveland, Ohio, May 2, 2008. (invited)

Powell CM, Roche MI: A survey assessing parental attitudes and genetic services for early-onset hearing loss. Abstract 45. Platform presentation at the 2008 American College of Medical Genetics Annual Clinical Genetics Meeting, Phoenix, Arizona, March 14, 2008. (solicited)

Manickam K, Gucsavas-Calikoglu M, Moldenhauer J, Vargo D, Crowe C, Powell C, Aylsworth: Chondrodysplasia punctata and maternal mixed connective tissue disease. Abstract 24. Platform presentation (Manickam) at the 2008 American College of Medical Genetics Annual Clinical Genetics Meeting, Phoenix, Arizona, March 14, 2008. (solicited)

Powell CM, Mayo R, Aylsworth AS: Native American (Lumbee) Myopathy: Cleft Palate, Congenital Contractures and Malignant Hyperthermia. Presented at the XXI David W. Smith Workshop on Morphogenesis and Malformations, La Jolla, California, August 3, 2000. (solicited)

Powell CM: Discordant Expression of Oculoauriculofrontonasal Malformation in Monozygous Twins. Presented at the Eighteenth Annual David W. Smith Workshop on Morphogenesis and Malformations, Litchfield Beach, South Carolina, August 16, 1997. (solicited)

Powell CM: Prenatal Testing: What is Currently Being Offered, How Did It Come About, What Will Be Available in the Future? Presented at the Hastings Center, Garrison, New York, June 3, 1997. (invited)

Michaelis RC, Kaiser-Rogers KA, Reitnauer PJ, Rao KW, Powell CM: Refinement of the Critical Region for Townes-Brocks Syndrome. Presented (Dr. Michaelis) at the Southern Genetics Group Summer Meeting, Fort Walton Beach, Florida, July 1996. (solicited)

Endrigkeit US, Doron MW, Aylsworth AS, Rao KW, Powell CM: Tetrasomy 9p: Case Report and Delineation of the Syndrome. Presented (Dr. Endrigkeit) at the Boat Evening of Scholarship, Department of Pediatrics, UNC-Chapel Hill, May 1996. (solicited)

Powell CM, Saal HM: Oblique Facial Clefts: Review of Cases and Etiology. Presented at the Fifteenth Annual David W. Smith Workshop on Morphogenesis and Malformations, Tampa, Florida, August 5, 1994. (solicited)

Powell CM, Saal HM: Congenital Heart Defects in Incontinentia Pigmenti. Presented at the Fourteenth Annual David W Smith Workshop on Morphogenesis and Malformations, Quebec, Canada, August 13, 1993. (solicited)

Gorelick MH, Powell CM, Rosenbaum KN, Saal HM, Conry J, Fitz CR: Progressive Cerebrovascular Occlusive Disease in a Patient with Neurofibromatosis Type 1. Presented at the Annual National Neurofibromatosis Foundation Clinical Case Symposium; November, 1990. (solicited)

Posters:

Powell CM, Roche M, Foreman KM, Weck-Taylor K, Strande T, Lu M, Wilhelmsen KC , Berg JS, Evans JP: *PIGN* mutations in a child with severe developmental disability, epilepsy, and dysmorphic features: multiple congenital anomalies-hypotonia-seizures syndrome. Presented at 2015 American College of Medical Genetics and Genomics Meeting, Salt Lake City, UT March 26, 2015.

Kaiser-Rogers K, Keelean-Fuller D, Hudson B, Powell C: Two cases with combined uniparental isodisomy and a supernumerary marker chromosome. Presented at 2014 American College of Medical Genetics and Genomiocs Meeting, Nashville, TN March 28, 2014.

Powell CM, Aylsworth AS, Turcott CM, Spector E: Mild achondroplasia with acanthosis nigricans, normal development and unique FGFR3 mutation. Presented at 2013 American College of Medical Genetics and Genomics Meeting, Phoenix, AZ, March 22, 2013.

Powell CM, Kaiser-Rogers K: Chromosome microarray analysis in patients with seizures and epilepsy. 2012 American College of Medical Genetics and Genomics meeting, Charlotte, NC March 27-31, 2012.

Kaiser-Rogers K, Tepperberg J, Blatt J, Powell C: Further evidence of an association between Diamond-Blackfin anemia and a reduced number of functional RPS17 genes. American College of Medical Genetics Annual Clinical Genetics Meeting, Vancouver, BC, CA March 16-20, 2011.

Roche M, Powell C, Gane L: Genetic counseling experience of families of newborns screening positive for FMR1 expansion. American College of Medical Genetics Annual Clinical Genetics Meeting, Vancouver, BC, Canada, March 16-20, 2011.

Aylsworth AS, Rosenfeld J, McPherson EW, Powell CM, Asamoah A, Mundlos S, Shaffer LG, and the 1q21.1 Study Group: Phenotypic variability in patients with chromosomal microdeletions at 1q21.1, including deletions confined to the proximal TAR region. 2009 American College of Medical Genetics Annual Clinical Genetics Meeting, Tampa, FL.

Powell CM, Kimani J, Booker J, Buchman C, Weck K: Etiology in congenital and early onset hearing loss. 2009 American College of Medical Genetics Annual Clinical Genetics Meeting, Tampa, FL.

Humberson JB, Powell CM, Schmitt C: Laryngotracheal atresia and pulmonary agenesis in a newborn with 22q11.2 deletion. Abstract 187. 2008 American College of Medical Genetics Annual Clinical Genetics Meeting, Phoenix, AZ.

Schmitt C, Moldenhauer J, Powell C, Wolfe H: Longest known survival in prenatally diagnosed duplication of the complete arm of chromosome 1. Abstract 149. 2008 American College of Medical Genetics Annual Clinical Genetics Meeting, Phoenix, AZ.

Kaiser-Rogeers K, Henderson F, Azam A, Powell CM: An atypical patient with immunodeficiency, centromeric instability and facial dysmorphism (ICF) syndrome. Abstract 94. 2008 American College of Medical Genetics Annual Clinical Genetics Meeting, Phoenix, AZ.

Powell CM, Kaiser-Rogers K, Rao KW: Chromosome rearrangements found in patients referred with a diagnosis of autism. Abstract 231. 2006 American College of Medical Genetics Annual Clinical Genetics Meeting, San Diego, CA.

Brailey LL, Powell CM, Carey JC, Rope A, Lenglet P, Lin AE, Battaglia A, Pober BR: Non-compaction of the ventricular myocardium in patients with 1p36 deletion. Abstract 126. 2006 American College of Medical Genetics Annual Clinical Genetics Meeting, San Diego, CA.

Fan Z, Fisher A, Powell CM: Study of 56 patients with Prader-Willi syndrome: higher incidence of seizures in deletion group. Abstract 199. 2006 American College of Medical Genetics Annual Clinical Genetics Meeting, San Diego, CA.

Stamm D, Powell C, Kahler S, Aylsworth A, Deak K, West S, Craig D, Lince D, Stephan D, Gilbert J, Speer M, Genome-wide SNP chip homozygosity mapping defines critical region for Native American Myopathy. 2005 American Society of Human Genetics Meeting.

Martin CL, Ilkin Y, Powell C, Rao K, Whichello A, Cook E: Breakpoint mapping of a de novo 15p;16p translocation reveals a candidate gene for autism, 2003 American Society of Human Genetics Meeting.

Powell CM, Fordham LA: Hajdu-Cheney syndrome with unusual presentation in infancy. 23rd Annual David W. Smith Workshop on Malformations and Morphogenesis, Furman University, Greenville, South Carolina, August 9, 2002.

Powell CM, Rao KW: Congenital diaphragmatic hernia in two patients with Y chromosome abnormalities: clue to etiology. XXII Annual David W. Smith Workshop on Malformations and Morphogenesis, Lake Arrowhead, California, September 2001.

Powell CM, Kaiser-Rogers KA, Langstaff EE, Rao KW: Distal duplication 10q in a father and son. Presented at the Annual Clinical Genetics Meeting, Miami, Florida, March 4, 2001.

Powell CM, Meira LB, Friedberg EC: Mutation in the CSB gene in a patient with cerebro-oculo-facio-skeletal syndrome. Presented at the Annual Clinical Genetics Meeting, Palm Springs, California, March 10, 2000.

Reitnauer PJ, Powell CM: Delineation of Williams syndrome phenotype in an African American female and her mother. Are the facial features of Williams syndrome less obvious in African Americans? Presented (Dr. Reitnauer) at the 17th Annual David W. Smith Workshop on Malformations and Morphogenesis, Lake Arrowhead, California, September 1996.

Powell CM, Michaelis RC: Two new microdeletion syndromes: Townes-Brocks "Plus" and Defect 11. 17th

Annual David W. Smith Workshop on Malformations and Morphogenesis, Lake Arrowhead, California, September 1996.

Powell CM, Reitnauer PJ, Kaiser-Rogers KA, Rao KW: A paracentric inversion of 16q in a patient with anus, hand, and ear anomalies: further evidence for a Townes-Brocks syndrome gene at 16q12.1. 16th David W. Smith Workshop on Malformations and Morphogenesis, August 1, 1995 and 45th Annual Meeting American Society of Human Genetics, October 28, 1995.

Tepperberg JH, Rao KW, Albright SG, Kaiser-Rogers K, Powell CM: Deletion 6(p25.1) in a child with mild dysmorphic features and absence of major eye malformations: implications for the location of genes involved in ocular development. Presented (Dr. Tepperberg) at the 44th Annual Meeting of the American Society of Human Genetics, Montreal, Canada, October 1994.

Powell CM, Taggart RT, Drumheller TC, Wangsa D, Qian C, Nelson LM, White BJ: Molecular and cytogenetic studies of an X autosome translocation in a patient with premature ovarian failure and review of other cases: is there a POF2 gene? Presented at the 43rd Annual Meeting of the American Society of Human Genetics, October 8, 1993.

Lapuk S, Lewis D, Powell C, Stern H, Saal H, Chandra R, Kapur S, Quivers E, Tifft C: Pompe's disease in three unrelated children of African descent. Presented (Dr. Lapuk) at the Children's Research Institute, 7th Annual Educational and Scientific Forum, Washington, DC, May 20, 1993

Powell CM, Stanley WS, Devine GC, Ellingham T, Samango-Sprouse CA, Vaught DR, Murphy BA, Rosenbaum KR: Mosaic Tetrasomy 5p: A New Mosaic Segmental Aneusomy Syndrome Confirmed by FISH. Presented at the 24th Annual March of Dimes Clinical Genetics Conference, Stanford University, Palo Alto, California, July 1992.

Powell CM, Ellingham TJ, Rosenbaum KN, Stanley WS: Unbalanced 15;18 translocation in a Prader-Willi patient mosaic for a normal cell line, presented (by Ms. Ellingham) at the 8th International Congress of Human Genetics, Washington, DC, Oct. 1991.

Powell CM, Saal HM, Chandra RS: PHEVR Syndrome: An Autosomal Recessive Syndrome of Pterygia, Congenital Heart Anomalies, Ear Anomalies, Vertebral Defects and Radial Dysplasia. Presented at the Twelfth Annual David W Smith Workshop on Morphogenesis and Malformations, Lake Arrowhead-UCLA Conference Center, Sept-Oct 1991 and at the 8th International Congress of Human Genetics, Washington DC, Oct. 1991.

Panels:

"The Career Path of a Medical Geneticist/Dysmorphologist/Genomicist", Alpha Epsilon Delta UNC Pre-Health Student Honor Society Meeting September 24, 2015.

RUSP Roundtable Meeting (expert panel to discuss the Recommended Uniform Screening Panel parameters) Rockville, MD August 26, 2015.

Genomic Medicine Meeting VIII: NHGRI's Genomic Medicine Portfolio (GM8), Hilton Washington D.C./Rockville Hotel & Executive Meeting Center, Rockville, MD, June 8-9, 2015.

Newborn Screening Research Meeting to discuss the Newborn Screening Saves Lives Act and consent for research on dried blood spots. National Institutes of Health, March 9, 2015.

Association of Public Health Laboratories Newborn Screening Conference, Roundtable discussion: NSIGHT Projects, October 28, 2014.

"The Ethics of Prenatal Testing", The Hastings Center, October 21-22, 1996; February 12-13, 1997; June 2-3, 1997; October 23-24, 1997; May 11-12, 1998.

"Neurofibromatosis", Public Service Information Program, WDCU radio, 1983.

"Genetic Counseling", Workshop for Public Health Personnel of the District of Columbia, Washington, D.C., April 1983.

"Support Groups", National Symposium of the National Society of Genetic Counselors, Birmingham, Alabama, June 1982.

"A Neurofibromatosis Support Group", National Symposium of The National Society of Genetic Counselors, San Diego, California, June 1981.

Teaching Activities:

Course Director

Medical Genetics Course for Medical Genetics Residents and Clinical Genetics Fellows, 2009-2011, 2012-2013, 2015-.

APSM 404-13: Advanced Practice Selective for Fourth Year UNC Medical Students in Genetics, 2009-present.

PED 443: Elective for fourth year UNC Medical Students in Genetics, 2004-present

UNC-CH School of Medicine, Block 9, Reproductive Medicine and Genetics, Second Year Curriculum Block Committee Member, 2004 – 2005.

UNC-CH School of Medicine, MEDI 226: Second year medical student genetics course. Course co-director, 2002- 2003.

Lectures and Seminars (select representation)

"Native American Myopathy", UNC Pediatric Dentistry Maternal and Child Health Seminar Series September 18, 2015.

"Patterns of Inheritance and Pedigree Analysis", TEC Curriculum, First year UNC medical student curriculum lecture, August 7, 2015.

Ethical, Legal and Social Issues in Genetics, Block 9, Second Year Medical Student Curriculum lecture, March 20, 2015.

Genetic Aspects of Deveolpment and Birth Defects, Block 9, Second Year Medical Student Curriculum lecture March 19, 2015.

Pedigree Analysis – Block 9, Second Year Medical Student Curriculum lecture. March 16, 2015.

Small Group (30 students) Second Year Medical Students, Block 9 Reproductive Medicine and Genetics, 4 contact hours March 17-20

"NC NEXUS", Center for Genomics and Society seminar series presentation, February 12, 2015.

"Genetics and Craniofacial Dysmorphology", ORT Lecture Series, UNC Dental School, January 15, 2015.

Newborn Screening Exome Sequencing Project Overview, Cytogenetics Laboratory Continuing Education seminar, UNC Hospitals, November 4, 2014.

"Chromosome Abnormalities" Pediatric Resident Lecture, UNC Hospitals, October 14, 2014.

"Patterns of Inheritance and Pedigree Analysis", Pediatric Resident Lecture, UNC Hospitals, October 13, 2014.

"Patterns of Inheritance and Pedigree Analysis", TEC Curriculum, First year UNC medical student curriculum lecture, August 18, 2014.

"Diagnostic Result through NCGENES", Current Topics in Medical and Human Genetics, UNC April 10, 2014.

"Overview of Genetic Syndromes: Etiology, Physical and Behavioral Phenotypes", LEND trainees lecture, Carolina Institute for Developmental Disabilities, October 30, 2013.

"Native American Myopathy", Current Topics in Medical and Human Genetics, UNC CME Conference October 17, 2013.

"NC NEXUS: North Carolina Newborn Exome Sequencing as Universal Screening", Current Topics in Medical and Human Genetics, UNC CME Conference, April 11, 2013.

"Dermatologic Disorders in Genetics aka: the genodermatoses", UNC Dermatology residents lecture, March 14, 2013.

"Epigenetics: Lessons from the Tasmanian Devil", Current Topics in Medical and Human Genetics, UNC, January 17, 2013.

"Clinical Genetics: Patients with Speech and Language Problems" Speech Pathology Class, School of Allied Health, UNC-CH, November 20, 2012.

"Myopathy with muscle spindle excess and mutations in HRAS gene; Coffin Siris Syndrome?" Current Topics in Medical and Human Genetics, UNC, Clinical Genetics Conference, November 3, 2011.

"Giving Bad News". Current Topics in Medical and Human Genetics Conference, UNC, October 20, 2011.

Multiple Circumferential Skin Rings "Michelin Tire Baby Syndrome" Current Topics in Medical and Human Genetics June 16, 2011.

"What's in a Name: change in use of the term 'mental retardation' to 'intellectual disability.'"Current Topics in Medical and Human Genetics May 12, 2011.

Case Presentation (Interesting Syndromes), Current Topics in Medical and Human Genetics, January 13, 2011.

"Review of Townes-Brocks syndrome; Clinical and Molecular Findings". Current Topics in Medical and Human Genetics, UNC-CH, December 2, 2010.

"Simpson-Golabi-Behmel Syndrome and Unknown Syndrome", Current Topics in Medical and Human Genetics, UNC-CH, September 23, 2010

Vascular Malformation Conference, UNC Hospitals: "RASA1 mutations" June 4, 2010.

"Vascular Malformations", Current Topics in Medical and Human Genetics, UNC-CH, May 27, 2010.

"CMV and Hearing Loss", Current Topics in Medical and Human Genetics Conference, UNC-CH, May 13, 2010.

"Sensorineural Hearing Loss and Cytomegalovirus" UNC-CH: Lecture to Audiology Students and Faculty, Center for Development and Learning, March 22, 2010.

"Angelman Syndrome", Current Topics in Medical and Human Genetics Conference, UNC-CH, February 18, 2010.

"Update on the Rise and Fall of the Y Chromosome (Maybe Not Falling as Fast as Predicted)". Presented at Currrent Topics in Medical and Human Genetics Conference, UNC-CH. January 21, 2010.

Native American Myopathy. Morbidity and Mortality Conference, Department of Pediatrics, UNC: September 17, 2009.

Genetics and Craniofacial Dysmorphology. Orthodontics Seminar, ORT 808. UNC School of Dentistry. January 14, 2010.

"Parry-Romberg Syndrome; Neonatal Transient Diabetes". Presented at Currrent Topics in Medical and Human Genetics, Clinical Genetics Conference, UNC-CH, November 12, 2009.

"Cleft Palate and Craniofacial Disorders: Diagnosis and Management". Dental School Seminar, Pediatric Dentistry, UNC-CH, November 6, 2009.

PKU Case. Small Group Activity for First Year UNC Medical Students, September 17, 2009.

"Genetic Discrimination". UNC-Greensboro Genetic Counseling Student Course, Greensboro, NC, yearly lecture 2009 - present.

"Craniofacial Disorders". Annual lecture presented at UNC-Greensboro Genetic Counseling Student Lecture, Greensboro, NC, 2009 - present.

Cytogenetics Conference Seminars, 1-2 per year, for Cytogenetics Laboratory staff and trainees 2006-present.

Sakoda Complex with Prenatal Lamotrigine Exposure: Possible Clue to Etiology? Platform Presentation at David W. Smith30th Annual Workshop on Malformations and Morphogenesis. Philadelphia, PA, August 6, 2009.

Medical Management of Prader-Willi Syndrome. Presented at Prader-Willi Syndrome Day, Chapel Hill, NC, July 18, 2009.

UNC-CH School of Medicine, Block 9, Reproductive Medicine and Genetics, Second Year Medical Student Curriculum, three lectures per year: Family History and Pedigree Analysis, Genetic Aspects of Development and Birth Defects, Ethical Issues in Genetics and small group leader (2004 – 2011)

UNC-CH School of Medicine, MEDI 226: Second year medical student genetics course.
Course co-director, 2002 - 2003
Three lectures including Introduction to Medical Genetics, Autosomal Recessive and X-Linked Inheritance, and Non-Traditional Patterns of Inheritance, 9 hours per course of small group conference teaching. 1996 – 2003

UNC-CH School of Medicine, Genetics Course for First Year Students, (Molecules to Cells) small group leader, 2003 – 2010.

UNC Hospitals, Pediatric Resident Seminars: Approach to the Dysmorphic Child, Chromosome Abnormalities, Craniofacial Disorders (2-3 lectures per year)

UNC Hospitals, Molecular Diagnostics and Cytogenetics Course for Pathology Residents. "Cytogenetics: Usefulness in Clinical Genetics. Yearly lecture, 2005-present.

UNC School of Nursing, Genomics and Society Course, lecture on "Prenatal Testing and the Disability Rights Critique" September 2010.

UNC-CH School of Medicine: Monthly seminars for third year medical student pediatric clerkship 1995 - 2005

UNC-CH Dental School: ORT 208, 4 hours of lectures on genetics and dysmorphology each year to second year dental students and graduate students in orthodontics and pedodontics (2005-2012)

UNC Hospitals: Seminars for Neurology and Psychiatry residents and fellows 1-2 lectures per year, 2007 - present

"Newborn Screening: Historical Perspectives" Center for Genomics and Society, Seminar, October 21, 2008.

Visiting Lectureships/Invited Speaking Engagements

"Newborn Screening and Genomics: Fast Forward Into the Future" Texas Dept. of State Health Services Genetics Conference 2015 "Genetics: The Future is Now", August 8, 2015) invited speaker.

Guest Interview, The Measure of Everyday Life with Brian Southwell, PhD, WNCU Radio Program, January 16, 2015, with Megan Lewis, PhD and Julianne O'Daniel, MS, about NC NEXUS project.

NSIGHT Meeting, Bethesda, MD September 29, 2014 Presentation summarizing four projects.

The 15th International Conference on Human Genome Variation and Complex Genome Analysis (HGV2014), 17th - 19th September 2014, Belfast, Northern Ireland: Next Generation Newborn Screening. Invited speaker.

Next-Generation Newborn Screening, Invited speaker Carolina Institute for Developmental Disabilities Seminar series, UNC, September 9, 2014

Texas Genetics Conference: Newborn Screening - Tales from the Crib. Dallas, Texas August 2, 2014: "Next-Generation Newborn Screening". Invited speaker.

Meeting of the Committee on Science, Technology and Law, 27th Meeting, National Academy of Sciences, June 16, 2014: "Next Generation Newborn Screening". Invited meeting speaker.

The Joint Garrod and Canadian Newborn and Child Screening Symposium, Ottawa, Ontario, Canada, May 30, 2014: "The Narrowing Gap Between Screening and Diagnosis: Next Generation Newborn Screening".

The Future of Fragile X: CDC's Public Health Research Agenda Meeting, CDC Campus, Atlanta, GA May 19-20, 2014. Invited meeting participant.

Radio Program Interview with Philip Clark, 666 ABC Canberra, Australia February 10, 2014, about NY Times article on Newborn Screening/Whole Exome Sequencing project.

North Carolina State Newborn Screening Laboratory Seminar, Raleigh, NC, December 17, 2013: "Next Generation Newborn Screening"

Guest, Radio In Vivo Program, WCOM-FM Carrboro, NC, November 27, 2013: Use of next-generation sequencing for newborn screening.

Pinehurst Pediatric Symposium, Pinehurst, NC, November 16, 2013: "Native American Myopathy"

Association of Public Health Laboratories Annual Meeting, Newborn Screening, Atlanta, GA, May 8, 2013: "Newborn Screening for Fragile X – Diagnosis, Counseling and Follow-Up."

Pediatric Grand Rounds, Rex Hospital, April 25, 2012: "Dysmorphic Features in the Newborn"

Pediatric Grand Rounds, WakeMed, July 15, 2009: "Genetic Evaluation for Children with Autism"

"Diagnosis and Findings with Oral-Facial Implications", UNC School of Dentistry, February 20, 2009

Village Elders, Chapel Hill Seymore Senior Center, Invited Lecturer: "DNA Testing – Crystal Ball or Weather Forecast?" February 19, 2009.

UNC Dental Research and Review Day, Lunch and Learn Session, "Craniofacial Genetics", February 18, 2009.

Guest on The State of Things, host Frank Stasio, WUNC Radio, to discuss DNA testing, February 17, 2009.

Moses Cone Pediatric Grand Rounds, September 10, 2008: "FISH and Chips: Update on Genetic Testing, What Every Pediatrician Should Know"

Pediatric AHEC, New Hanover Regional Medical Center, October 4, 2005: "Update and Future in Genetics".

North Carolina Council of Child and Adolescent Psychiatry, North Carolina Psychiatric Association 2005 Annual Meeting , Wilmington, NC September 24, 2005: "Current Concepts in Pediatric Genetics.

Advances in Clinical Perinatal Medicine, 31st Annual Regional Perinatal Symposium, Update in Clinical Genetics, SUNY Upstate Medical University, Crouse Hospital, Syracuse, NY: Margaret Williams Lecture, October 22, 2004: "Dysmorphology: Diagnostic Clues in the Newborn, 'It's not F.L.K. Anymore' " and "Further Diagnostic Clues".

Moutain AHEC, Asheville, NC, April 29, 2004: "Craniofacial Genetics"

American College of Medical Genetics, conference for high school students: "Why I Like Genetics", March 2004.

North Carolina Medical Genetics Association meeting, Wake Forest University School of Medicine, Winston-Salem, NC, April 26, 2002: "Incontinentia Pigmenti"

Twelfth Annual Leo M. Croghan Conference, Raleigh, NC, December 4, 2000: "Cleft Lip and Palate: Pediatric, Genetic and Developmental Perspectives"

Institute of Government Summer Internship Program, Peace College, Raleigh, NC, July 11, 2000: "Ethical Issues of Prenatal Testing"

Neurofibromatosis, Inc. Mid-Atlantic Meeting, Rex Hospital, Raleigh, NC, November 6, 1999: "Genetics and NF Issues"

Fayetteville AHEC visiting professor, October 21, 1998: "Evaluation of a Patient with Mental Retardation"

Association of Genetic Technologists Southeastern Regional Annual Conference, Research Triangle Park, September 24, 1998: "Townes-Brocks Syndrome: Localizing the Gene by Cytogenetic and Molecular Methods"

Ninth Annual Leo M. Croghan Conference, Raleigh, NC, December 2, 1997: "Current Genetic Update: Microdeletion Syndromes and Syndromes with Hypotonia"

Seventh Annual Leo M. Croghan Conference, Raleigh, NC, December 5, 1995: "Recent Advances in Genetics"

Southeastern Regional ACT Meeting, Chapel Hill, NC, October 6, 1995: "Clinical Importance of FISHing for Microdeletion Syndromes"

Clinical Staff Conference, National Institutes of Health, Bethesda, Maryland, May 25, 1994: "X Chromosomal Loci for Premature Ovarian Failure"

Georgetown University School of Dentistry, Washington, DC, November, 1982: "Clinical Genetics"

Advanced Neonatology Nursing Course, Children's Hospital National Medical Center, February 1979: "Principles of Genetic Counseling"

Clinical Teaching (appendix available on request)

Medical Genetics, Pediatrics, Maternal Fetal Medicine and Dental Residents rotating in Pediatric Genetics Clinics. 1993-present.

UNC-CH and visiting third and fourth year Medical Students rotating in Pediatric Genetics and Metabolism Clinics 1993-present.

UNC-Greensboro Genetic Counseling Students rotating in Pediatric Genetics Clinics 2001-present.

Third year Medical Students: daily teaching during one month of general attending or Teach attending service 1994 - 2003

Pediatric Resident Elective in Genetics: daily or weekly teaching of resident in clinics and on consult service, 1993 – present.

Pediatric Dental Resident rotation: weekly Genetics and Metabolism Clinic and Craniofacial Center Clinic, 1993-2009.

Clinical Genetics and Clinical Cytogenetics and Molecular Fellows, didactic teaching, 1993-present.

Continuing Education Lectures, Seminars, Conferences

Grand Rounds presentations, including Pediatrics, Neurology, and Reproductive Endocrinology at UNC, and Pediatrics at Wake, Moses Cone, Rex, and New Hanover Hospitals 1993 – present

In-service lectures to staff of Developmental Evaluation Centers in the state, 1995 – 2007.

Seminars for Neonatology fellows, Center for Development and Learning staff, and Pediatric Psychiatry staff 1993 – present

Continuing education conference 4-6 times per year for the UNC Cytogenetics Laboratory staff 2001-2010

Host for the Visiting Clinician Program in the Medical School, 1997

Attending on Clinical Service

Attending on consultation service for Pediatric Genetics 6 months each year 1993-present

Attending 2-4 clinics per week Pediatric Genetics and Metabolism UNC Hospitals/North Carolina Children's Hospital 1993-present

Attending/consultant Craniofacial Center Clinic 1993 - present

Genetics Satellite Clinics one-two days per month 1993 – present

Medical Director, Prader-Willi Syndrome Comprehensive Clinic 2003 – present

Genetics Attending, Angelman Syndrome Comprehensive Clinic 2012 - present

Teach attending third-year pediatric clerkship for one month each year, 2002 – 2003

Attending on the General Pediatric in-patient service for one month each year, 1994 – 2001.

Student Preceptor/Advisor

Post-doctoral:

Medical Genetics Residency Program Director, August 2001 – present
Medical Genetics Residents
Kent McKelvey, MD July 2001 – June 2003
Alice Basinger, MD, PhD July 2003 – 2005
Zheng Fan, MD July 2004 – June 2006
Murugu Manickam, MD July 2006-June 2008
Jennifer Humberson, MD January 2007-December 2008
Heather Baudet, MD, PhD – July 2009 – August 2011
Bernadette Wildemore, MD July 2012-June 2013
Natario Couser, MD – July 2014 - present

Doctoral:

American College of Medical Genetics Summer Scholar – Elizabeth Runge Blythe, 2011.

Doctoral Dissertation Committee, UNC School of Medicine
Demetra Stamm, MD/PhD Student. Projects: Neural tube defects and Native American Myopathy, 2005-2006.

Master's Student:

Masters Thesis Committee, UNC Dental School
Darren Bejan Ravassipour, DDS, Orthodontic Resident, 2002 – 2003. Project: Craniofacial Manifestations Associated with Amelogenesis Imperfecta.

Other:

Pediatric Career Advisor, third and fourth year medical students, University of North Carolina, Chapel Hill, 1999-2009

Preceptor for four students in the Medical Education Development Program, summers 1997 - 1999

Mentor for two undergraduate students through the Womentorship Program from 1995-1997

Science-By-Mail: national program for junior high school students to have scientist penpals and help with at-home learning and science projects; 1995-1996.

Advisor for several junior high school students through the Science-By-Mail program 1995-1996

Genetic counseling student from the Medical College of Virginia, summer 1994

Grants:

Active

1-U19-HD077632-01 (Powell and Berg) 9/5/2013-8/31/2018 3.6 calendar
NICHD and NHGRI $5,885,220
NC NEXUS, North Carolina Newborn Exome Sequencing for Universal Screening
In this pilot project, researchers will identify, confront and overcome the challenges that must be met in order to implement genomic sequencing technology to a diverse newborn population. The researchers will sequence the exomes of healthy infants and infants with known conditions such as phenylketonuria, cystic fibrosis or other disorders involving metabolism. Their goal is to help identify the best ways to return results to doctors and parents. The investigators will the explore the ethical, legal and social issues involved in helping doctors and parents make informed decisions, and develop best practices for returning results to parents after testing. The researchers will also develop a tool to help parents understand what the results mean and examine extra challenges that doctors may face as this new technology is used. This study will place a special emphasis on including multicultural families.
Role: Lead PI

2-P50-HG004488-06 (Henderson) 5/31/2013-05/31/2018 0.52 calendar
NIH/National Center for Human Genome Research $878,079
Center for Genomics and Society
The UNC-CH Center for Genomics and Society focuses on newly emerging ethical, legal and social implications (ELSI) of genomics research as the field matures and shifts its focus from small efforts to those on a much larger scale.
Role: Investigator

2-T73-MC00030-20 (Hooper) 7/1/90-6/30/16 0.52 calendar
Maternal and Child Health Bureau $931,449
North Carolina –LEND/AE
The goal of this project is to provide exemplary interdisciplinary leadership training in screening, assessment, diagnosis, evidence-based treatments, and general health promotion; provide exemplary clinical and community-based interdisciplinary services; and to provide continuing education that fosters family-centered, coordinated care and improves the system-of-care for the ASD population at the state, regional, and national levels.
Role: Investigator

1-U01-HG006487-01(Evans) 12/5/11-11/30/15 .6 calendar
National Center for Human Genome Research $1,107,847
NC GENES: NC Clinical Genomic Evaluation by NextGen Exome Sequencing
In this project we outline a highly inter-disciplinary approach to identifying, confronting and overcoming the major challenges which must be met in order to implement deep sequencing technology in clinical medicine.
Role: Investigator

5-P30-HD003110-41-44 (Piven) 8/01/1997 - 6/30/2014

National Institute of Child Health and Human Development
UNC Developmental Disabilities Research Center
Role: Investigator

Contracts:

ACTIVE
030450 (Powell) 6/1/14-5/31/15 4.8 calendar
NC DHHS-DPH $674,354
UNC-Genetics Services Contract
The primary purpose of this contract is to provide high level genetic services for patients and families with highly complex needs which neither Medicaid nor other third party payers will cover. The major medical center will provide diagnostic, clinical, management and counseling for genetic conditions for select number of patients within targeted service region.
Role: Principal Investigator

Completed

5-P50-HG004488-05 (Henderson) 9/27/07-7/31/12 0.52 calendar
NIH/National Center for Human Genome Research $812,472
Center for Genomics and Society
The UNC-CH Center for Genomics and Society focuses on newly emerging ethical, legal and social implications (ELSI) of genomics research as the field matures and shifts its focus from small efforts to those on a much larger scale.
Role: Investigator

5-P30-HD003110-44 (Piven) 8/1/97-6/30/13 1.2 calendar
NICHD $2,060,504
UNC Developmental Disabilities Research Center
This application seeks support for an Administrative Core and four research cores – the Data Management and Statistical Analysis Core, the Subject Registry Core, the Behavioral Measurement Core; and, the Developmental Neuroimaging Core. These four research cores provide cutting-edge, high-quality and cost-effective support for this integrated, multidisciplinary program of MRDD research. The above funds the following research project:

Family Adaptation to Fragile X Syndrome 07/01/2003 – 06/30/2013
Fragile X Newborn Screening Project
Bailey (PI)
Frank Porter Graham Child Development Institute
The University of North Carolina at Chapel Hill
This project investigates the ethical issues involved in newborn screening for "untreatable" disorders using fragile X syndrome as the model.
Role: Investigator

UNC Dance Marathon Grant: 2011-2012 $15,340
Metabolic Formula and Special Equipment for Genetics Patients
Role: PI, 0% salary support

1P20HG03387-01 (Bailey) 7/1/2004-6/30/2006

NIH National Human Genetics Research Institute $438,000
Centers for Excellence in Ethical, Legal and Social Implications Reasearch, P50 planning grant
ELSI Scale-Up: Large Sample Gene Discovery and Disclosure
This proposal rests on the assumption that rapid expansion of large sample gene discovery and disclosure projects raise major ethical, legal, social, and policy challenges, to such an extent that it constitutes a significant and urgent public health need. By utilizing three unique projects at UNC-CH involving large-sample gene discovery and disclosure, we are proposing a two-year Exploratory (P20) grant to conduct the planning necessary to create a Center of Excellence on ELSI Issues in Large Sample Gene Discovery and Disclosure. Our goal is to develop an infrastructure to maximize collaborative research, create partnerships with relevant constituencies, identify critical issues that must be addressed, and collect sufficient pilot data to propose a wellintegrated center in which state-of-the-art ELSI research can be conducted to inform public policy. 5% effort

A05-0873-001 (Powell) 10/01/2003-09/30/2005
Association of American Medical Colleges and Centers for Disease Control $250,000
Genetic Services for Congenital Hearing Loss
Role: PI 30% effort

97-CDL-184 (Jessica Lord)
Neuropsychological Functioning in Children with Turner Syndrome: Neurocognitive and Neuroaffective Processing
Role: co-investigator no salary support

5R01HG01168-02 (PI Erik Parens, The Hastings Center) 1996-1998
National Human Genome Research Institute
Project on Prenatal Testing for Genetic Disability
Role: consultant No salary support

UNC-CH University Research Council Grant 5/1994-5/1996
$3000
The Prevalence of Prader-Willi Syndrome in an Autistic Population, clinical research project, principal investigator. No salary support

Professional Service

To discipline

National

American Board of Medical Genetics and Genomics Nominating Committee member 2014; chair 2015

American Board of Medical Genetics and Genomics Site Visitor Committee 2014 - present

Association of Professors of Human and Medical Genetics Council Member and Secretary-Treasurer 2014 – present

PhenX Toolkit Rare Conditions Working Group 2014-2015 NIH-funded project conducted by RTI International

NSIGHT Common Data Elements Working Group 2014-present

NBSTRN Clinical Integration Working Group member. NIH-funded project coordinated by ACMG 2014-present.

NSIGHT Projects Committee Chair 2015, member 2013-present (Newborn Sequencing In Genomic medicine and public HealTh) Committee of four project sites, NICHD, NHGRI.

Accreditation Council for Graduate Medical Education: reviewer of the proposed ACGME International Advanced Specialty Requirements in Medical Genetics 2014

American Medical Association Molecular Pathology CPT Coding Workgroup for Next Generation Sequencing Nonsyndromic Hearing Loss Subworkgroup 2013-2014

American College of Medical Genetics and Genomics Professional Practice and Guidelines Committee member, 2013 – present.

American Board of Medical Genetics, Board of Directors, 2006 – 2013
Executive Board (Immediate Past-President) 2013
Executive Board (President) 2012
Executive Board (President-Elect) 2011
Executive Board (Treasurer) 2008-2010

Accreditation Council for Graduate Medical Education, Residency Review Committee for Medical Genetics, 2006 – 2013

Accreditation Council for Graduate Medical Education, Medical Genetics Milestone Project Committee Member, 2011-2012

NIH Study Section: ZRG1 ETTN-H (51) "Improving Interventions for Communication Disorders" NIDCD, 2009.

American College of Medical Genetics, Education Committee member, 1998 – 2006

American Academy of Pediatrics, Section on Genetics and Birth Defects, Executive Committee, 2006 - 2013

American Academy of Pediatrics, Section on Genetics and Birth Defects, Nominating Committee, 2002 – 2004

American Board of Genetic Counseling, site visitor, 2001

Medical Advisory Board, CHERUBS, The Association for Congenital Diaphragmatic Hernia, 1998 - present.

The Hastings Center, Project on Prenatal Testing for Genetic Disability, funded by the ELSI division of the National Human Genome Research Institute, grant 5R01HG01168-02. 1996 – 1998

Moderator, Workshop Session 11, Dysmorphology III: Syndromes, David W. Smith 18th Annual Workshop on Malformations and Morphogenesis, Litchfield Beach, South Carolina, August 16, 1997

Ad hoc manuscript reviewer: Teratology, Journal of Medical Genetics, Behavioral Medicine, American Journal of Medical Genetics, Cleft Palate-Craniofacial Journal, Journal of Neurodevelopmental Disorders, Genetics in Medicine.

Chairman, Scientific Session, International SOFT Conference, The University of North Carolina at Chapel Hill, July 11, 1996

Advisory Board, National Neurofibromatosis Foundation, Metropolitan Washington Chapter, 1979 – 1983

State

North Carolina Physician Advisory Group Genetic Screening Task Force member, 2014.

North Carolina State Newborn Screening Advisory Committee, member 2008 – present.

External Advisory Committee member, University of North Carolina at Greensboro Genetic Counseling Program, 2003 – present

Advisory Board member, North Carolina Collaborative Project for Surveillance, Prevention and Treatment of Birth Defects. Project funded by the National Center on Birth Defects and Developmental Disabilities and the Centers for Disease Control and Prevention 2002 – 2005

Advisory Panel for Association of State and Territorial Health Officers Genomics Toolkit Project, North Carolina site visit member February 21, 2002

North Carolina Medical Genetics Association member 1993 – present
President, June 1998 - September 1999
Secretary-Treasurer, June 1997 - June 1998

North Carolina Medical Genetics Association Bi-Annual Meeting, Planning Committee Chair, 2001

Medical Advisory Board, NC SOFT (Support Organization for Trisomy18, 13, and Related Disorders), 1996

Advisory Board, SOFT (Support Organization for Trisomy 18, 13, and Related Disorders), Tenth Annual International Conference, Chapel Hill and Durham, NC 1996

Chairman, 1996 International SOFT Conference, Scientific Session, Chapel Hill, NC

North Carolina Birth Defects Center member, 1995 – 2003

North Carolina Medical Genetics Association Speaker Committee 1994 - 1995

North Carolina Medical Genetics Association Dysmorphology Committee 1993 – present, chair 1995 - 1997

Within UNC-Chapel Hill

University

Health Sciences Advisory Committee for Promotions 2010 – 2011

Carolina Center for Genome Sciences, faculty member, 2001 - present

University Faculty Grievance Committee member, UNC-Chapel Hill, 3 year term, July 1998 – 2001
Chair, Subcommittee, 2001

Womentoring Program, UNC: mentor for undergraduate students; 1995-1997

School of Medicine

Conflict of Interest Monitoring Committee 2015

Member of Personnel Policies Sub-Committee for the LCME site visit 2011-2012

UNC School of Medicine Committee to Review Appointments and Promotions to Associate Professor with Tenure
Chair 2010 – 2011
Member 2007 – 2011

UNC School of Medicine Block 9 Second Year Curriculum Committee Member 2004 – 2008

Center for Genomics and Society, core faculty and investigator 2004 – present

The University of North Carolina at Chapel Hill School of Medicine, Medical School Admissions Committee 2000 – 2004

Medical Education Development Program, preceptor for aspiring physicians in minority groups: June 1997, June 1999, June-July 2000.

Craniofacial Center Orthodontist Search Committee member, UNC School of Dentistry, 1997 – 1998

UNC School of Medicine Medical Genetics Course (MEDI 226) committee member 1993 – 2002, Course co-director 2002 – 2007

Department of Pediatrics
NCCP Research Grant Review Committee member 2014.

Peer Review Committee Member 2012-2014

Faculty Compensation Plan Workgroup 2012-2013

Promotions Committee 2003 – 2011 and 2012 – present

Advisory Committee to the Chair, member 2011 – 2012

Search Committee for faculty position, Division of General Pediatrics and Adolescent Medicine

Genetics and Metabolism Genetic Counselor Search Committee Chair, 2004

Floyd W. Denny Pediatric Alumni Society, CME Symposium Planning Committee Chair, "Medical Genetics: Practical Topics for the Care of Children", April 20, 2002

Department of Pediatrics, Community Pediatrics Division Chief Search Committee 2002

Department of Pediatrics Mission Statement Task Force 2001

Department of Pediatrics Pediatric Subspecialty Clinic Task Force, 2000

Medical Student Pediatric Career Goal Advisor, 1999 – 2009

Moderator, Boat Evening of Scholarship, platform session, UNC-Chapel Hill, March 31, 1998

Department of Pediatrics, UNC Pediatric Resident Selection Committee 1999 - 2002

Genetics and Metabolism Genetic Counselor Search Committee Chair, 1999

Genetics and Metabolism Clinical Geneticist Search Committee Co-chair, 1998-1999

Department of Genetics

Medical Genetics Residency Program Director, 2001 – present

Department of Genetics Educational Mission Committee 2000 – 2004

Chair, Bryson Faculty Search Committee 2003 - 2005

School of Dentistry

Craniofacial Center Director Search Committee, UNC School of Dentistry, 2000

Craniofacial Center Team Member, 1993 – 2008

Hospital

Clinical Competency Committee, Medical Genetics and 2014-present

Clinical Competency Committee, Molecular Genetic Pathology 2015 - present

Graduate Medical Education Committee UNC Hospitals member 2001 – present
Member, Internal Review Committee - Hematopathology
Chair, Internal Review Committee, Developmental Pediatrics Residency Program, 2009
Chair, Internal Review Committee, Vascular Interventional Radiology, Radiation Oncology, and Neuroradiology Residency Programs, 2004
Chair, Internal Review Committee, Pediatric Nephrology Residency Program, 2002
Chair, Internal Review Committee, Pathology Residency Program, 2002
Internal Review Committee, Urology Residency Program, 2001

Pediatric Task Force Committee Member and Chair of Contracts Subcommittee – 2009
Responsible for review and analysis related to pediatric clinical operations and staffing

Center for Maternal and Infant Health, UNC Hospitals, member 1999 – present
Advisory Committee Member 2005 - 2009

Physician Order Entry Committee, UNC Hospitals 2001

Professional Societies:

American College of Medical Genetics 1994 – present

American Academy of Pediatrics 1993 – present

Association of Professors of Human and Medical Genetics
UNC Chapel Hill Representative 2004 – present
Secretary-Treasurer 2014 - present

Medical Genetics Residency Program Directors Special Interest Group 2009 - present

North Carolina Pediatric Society 1994 - present

American Medical Association 2001 – present

Association of Professional Women in Medicine, 1997 - present

American Cleft Palate-Craniofacial Association, 1996 – 2010

American Association for the Advancement of Science 1995 - 1997

Floyd W. Denny Pediatric Alumni Society 2002 – 2005

North Carolina Medical Genetics Association 1993 - present
Secretary-Treasurer, June 1997 - June 1998
President, June 1998 - September 1999

American Society of Human Genetics 1978 – present

CURRICULUM VITAE

Christine M. Rini, Ph.D.

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UNC Gillings School of Global Public Health
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Campus box 7440, 319C Rosenau Hall
Chapel Hill, NC 27599-7440

Telephone: 919-843-6580 (office)
917-439-3023 (cell)

E-mail: christine.rini@unc.edu

EDUCATION

2001-2004 **Postdoctoral Fellow**, Department of Oncological Sciences, Division of Cancer Prevention and Control, Icahn School of Medicine at Mount Sinai (formerly Mount Sinai School of Medicine), New York, NY

2001 **Doctor of Philosophy, Social Psychology**, University of California, Los Angeles *Minors*: Health psychology, measurement and psychometrics

1995 **Master of Arts, Social Psychology**, University of California, Los Angeles, CA

1993 **Bachelor of Arts, Psychology** California State University, Los Angeles, CA. Graduated *summa cum laude.*

PROFESSIONAL EXPERIENCE

Academic

6/2012-Present **Research Associate Professor**, Department of Health Behavior, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, NC

2010 – 5/2012 **Research Assistant Professor**, Department of Health Behavior, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, NC

2010 - Present **Member, UNC Lineberger Comprehensive Cancer Center**, Chapel Hill, NC

2010 - Present **Adjunct Assistant Professor of Oncological Sciences**, Program for Cancer Prevention and Control. Mount Sinai School of Medicine, New York, NY

2005 - 2010 **Assistant Professor of Oncological Sciences**, Program for Cancer Prevention and Control. Mount Sinai School of Medicine, New York, NY

2004- 2005 **Assistant Professor,** Department of Psychology, Hofstra University, Hempstead, NY

1998-2001, social 1995-1996 **Graduate Research Assistant,** UCLA, Department of Psychology, Stress, support, and pregnancy lab of Christine Dunkel Schetter, Ph.D.

2000 **Research and Statistical Consultant**, Cedars-Sinai Medical Center, Department of Obstetrics and Gynecology, Los Angeles, CA. Calvin Hobel, M.D.

Other academic training/experience

June, 2006 Data analysis workshop on hierarchical linear modeling, University of Connecticut

July, 2006 Fellow, NIH Institute for Randomized Clinical Trials for Behavioral Interventions

May, 2007 APA Advanced Training Institute on structural equation analysis for longitudinal data

June, 2014 ICPSR Summer Program workshop on Latent Growth Curve Models

July, 2015 Dyadic Data Analysis Workshop, Deborah Kashy, Michigan State University

Non-academic

1985-1993 **Marketing and Public Relations**, Candle Corporation, Santa Monica, CA. Created computer-based product demonstrations and training programs for a mainframe computer software company. Developed computer graphics for company presentations and computer-based product demonstrations. Wrote press releases and marketing materials. Worked with company technical experts to ghost-write, co-write, or edit technical articles for placement in industry publications.

HONORS

2015 Inducted into Theta Chapter of Delta Omega national honorary society for individual dedication to quality in the field of public health

2007 Fellow**,** American Psychological Association Advanced Training Institute on Structural Equation Modeling with Longitudinal Data

2006 Fellow**,** National Institutes of Health Summer Institute on Design and Conduct of Randomized Clinical Trials Involving Behavioral Interventions

1998 Bertram R. Raven Award, Outstanding Contribution to the Study of Social Issues, University of California, Los Angeles

1997 Senate Research Grant, University of California, Los Angeles

1996 American Psychological Association Travel Award

1995 Irene Kassorla Research Award, University of California, Los Angeles

1994-1996 National Institutes of Mental Health Health Psychology Predoctoral Training Fellowship

1993 University Fellowship, University of California, Los Angeles

MEMBERSHIPS

Member, Society of Behavioral Medicine
Member, International Society for Research on Internet Interventions (ISRII)
Member, Division 38, Health Psychology, American Psychological Association

BIBLIOGRAPHY

Book Chapters and Unrefereed Publications

Rini, C., Waters, E. A., & Myers, R. E. (2014). Hot topics in health decision making: What, exactly, is a "good" health decision? Perspectives from SBM's Health Decision Making Special Interest Group. *Outlook,* Society of Behavioral Medicine, Fall, 2014.

Austin, J., & **Rini, C.** (2013). Stem Cell and Bone Marrow Transplant. In A. R. Block and D. B. Sarwer (Eds), *Presurgical Psychological Screening:Understanding Patients, Improving Outcomes* (pp. 103-125). Washington, DC: American Psychological Association.

Rini, C., & Dunkel Schetter, C. (2010). The effectiveness of social support transactions in intimate relationships. In J. Davila and K. Sullivan (Eds), *Support Processes in Intimate Relationships* (pp. 26-67). New York, NY: Oxford.

Dunkel Schetter, C., & **Rini, C.** (2004). Preterm delivery: Psychosocial factors. In N. B. Anderson (Ed.), *Encyclopedia of Health and Behavior*. Thousand Oaks, CA: Sage.

Dunkel-Schetter, C., Sagrestano, L. M., Feldman, P., & **Killingsworth [Rini], C**. (1996). Social support and pregnancy: A comprehensive review integrating ethnicity and culture (pp. 375-412). In G. R. Pierce, B. R. Sarason, & I. G. Sarason (Eds.), *The Handbook of Social Support and the Family*. New York: Plenum.

Refereed Papers/Articles

53. Lewis, M. A., Paquin, R., Roche, M., Furberg, R, **Rini, C.,** Berg, J., Powell, C., Bailey, D. (In press). Supporting parental informed decision-making: Development of the NC NEXUS decision aid. *Pediatrics* (special issue).

52. *Khan, C., *Moore, E. G., *Leos, C, **Rini, C.** (In press). Hopes for diagnostic genomic sequencing: The role of illness uncertainty and social status. *European Journal of Human Genetics.*

51. Bennell, K. L., **Rini, C.,** Keefe, F. J., French, S., Bryant, C., Abbott, J. H., Nelligan, r., Staples, M. P., Dobson, F., Dalwood, A., Hodges, P. W., Vincenzino, B., Hinman, R. S. (In press). Internet-based pain coping skills training and education combined with physiotherapy for people with persistent hip pain (HOPE trial): A randomised controlled trial protocol. *Physical Therapy*.

50. **Rini, C.,** *Emmerling, D., Austin, J., Wu, L., Valdimarsdottir, H., Redd, W. H., Woodruff, R., Warbet, R. (In press). The effectiveness of caregiver social support is associated with cancer survivors' experience of their treatment: A linguistic analysis of survivor narratives. *Palliative & Supportive Care.*

49. Nyrop, K. A., Callahan, L. F., **Rini, C.,** Altpeter, M., Hackney, B., Schechter, A., Wilson, A., Muss, H. B. (2015). Adaptation of an evidence-based arthritis program for breast cancer survivors on aromatase inhibitor therapy who are experiencing joint pain. *Preventing Chronic Disease, 12,* 140535. doi: 10.5888/pcd12.140535. PMC4467257.

48. *Elstad, E. A., Sutkowi-Hemstreet, A., Sheridan, S. L., Vu, M., Harris, V. F., Reyna, V. F., **Rini, C.,** Earp, J.A., & Brewer, N. T. (In press). Clinicians' perceptions of the benefits and harms of prostate and colorectal cancer screening. *Medical Decision Making*.

47. *Khan, C. M., **Rini, C.,** Bernhardt, B., Roberts, J. S., Christensen, K. D., Evans, J. P., Brothers, K. B., Roche, M. I., Berg, J. S., & Henderson, G. E. (In press). How Can

Psychological Science Inform Research About Genetic Counseling for Clinical Genomic Sequencing? *Journal of Genetic Counseling.*

46. **Rini, C**., Porter, L. S., Somers, T. J., McKee, D. C., DeVellis, R. F., Smith, M., Winkel, G., Ahern, D. K., Goldman, R., Stiller, J. L., Mariani, C., Patterson, C., Jordan, J. M., Caldwell, D. S., & Keefe, F. J. (2015). Automated, Internet-based Pain Coping Skills Training to Manage Osteoarthritis Pain: A Randomized Controlled Trial. *Pain*, 156(5), 837-848. doi: 10.1097/j.pain.0000000000000121. PMC4402249.

45. Pepper, J. K., Emery, S. L., Ribisl, K. M., **Rini, C. M.,** & Brewer, N. T. (In press). How risky are e-cigarettes? Smokers' beliefs about the health risks of multiple tobacco products. *Journal of Behavioral Medicine.*

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*Collaboration with current or past mentee; **Senior author

Invited Talks

Rini, C., Skinner, D., Raspberry, K., Khan, C., Henderson, G., Roche, M., Berg, J., & Evans, J. *Returning Secondary Genomic Findings to Patients in NCGENES: Intention versus Reality.* National Meeting of the Clinical Sequencing Exploratory Research (CSER) Consortium, Bethesda, MD, April, 2015.

Rini, C. *Clinical Exome Sequencing in a Diverse Patient Population: Correlates and Implications of Baseline Knowledge, Literacy, and Numeracy*. Presentation in invited symposium (*Whole Genome/Exome Sequencing: Patient Expectations, Literacy, and Preferences for Genomic Information;* Moderator: McGuire, A. L.: Speakers: Rini, C., Gracy, S., Bernhardt, B., & McGuire, A. L.), American Society of Human Genetics annual conference, San Diego, CA, October, 2014.

Rini, C. *Reciprocal Benefits of Helping – Complementary Benefits of Creating and Reading Cancer Survivorship Narratives*. Cancer Outcomes Research Program, UNC-Chapel Hill, May, 2014.

Rini, C. *Cancer Survivorship Narratives: Can Patients Benefit Both from Creating them and from Accessing Them*. Comprehensive Cancer Support Program, UNC-Chapel Hill, May, 2014.

Rini, C., *Cancer Survivorship Narratives: Their Content and Potential Value As a Resource for Patients.* Social Medicine Forum, Department of Social Medicine, School of Medicine, UNC-Chapel Hill, NC, November, 2013.

Rini, C., *Evaluating a New Genomic Knowledge Scale.* Semi-Annual Meeting of the North Carolina Medical Genetics Association, UNC-Chapel Hill, NC, October, 2013.

Rini, C., *PainCOACH: A web-based pain coping skills training program that mimics in-person training by experts.* Inaugural Symposium on Using New Technologies to Enhance Healthy Behaviors, UNC-Chapel Hill, NC, October 2013.

Rini, C., *Internet-based Pain Coping skills Intervention for Osteoarthritis.* 8th Annual NIH Pain Consortium Symposium on Advances in Pain Research, Bethesda, MD, May, 2013.

Rini, C., *Social Support and Health: Potenital Effects on Adherence.* Department of Pharmaceutical Outcomes and Policy's Fall Seminar series, November, 2012.

Rini, C., *Improving social support interventions: Benefits of understanding the features and consequences of effective enacted support*. Mary Junck Research Colloquium Series, School of Journalism and Mass Communication, UNC-Chapel Hill, March, 2012.

Rini, C. *Interpersonal decision influence on surgical decision making in high risk patients*. 6th Annual East-West Colorectal Days, organized by the Association of Coloproctology of Hungary, *Hajdúszoboszló, Hungary*, October, 2010.

Rini, C. *'T ain't what you do, it's the way that you do it: The effectiveness of social support attempts in intimate relationships*. Department of Social Psychology, University of North Carolina at Chapel Hill, September, 2010.

Rini, C. *Surgical decision making among IBD patients referred for colectomy*. Ileostomy Association of New York, April, 2009.

Rini, C. *Social support effectiveness: Toward a new conceptualization of enacted social support*. Department of Public Health, Temple University, May, 2008.

Rini, C. *Social support from the spouse and from family and friends among mothers of children treated with bone marrow transplant*. Department of Psychology, Health Psychology Program, University of California at Los Angeles, November, 2007.

Rini, C. *Effective Social Support: What predicts it and why does it matter?* Department of Psychology, State University of New York, Stony Brook. November, 2004.

Refereed unpublished oral presentations and/or abstracts

Rini, C., *Layton, R., *Newell, D., *Cathrton, D., *Margolis, M., *Hurd, S., DeVellis, R., Callahan, L., & Altpeter, M. *Dyadic efficacy: Preliminary findings from a couples-based physical activity study.* Podium presentation at the International Association of Relationships Research mini-conference on Relationships, Health, and Wellness, New Brunswick, NY, June, 2015.

Rini, C., Porter, L., Somers, T., McKee, D., DeVellis, R., Keefe, F. *Significant other support and hindrance for intervention tasks: Implications for interventions to improve health and well-being.* Podium presentation at the International Association of Relationships Research mini-conference on Relationships, Health, and Wellness, New Brunswick, NY, June, 2015.

*Stover, A. M., **Rini, C.**, Mayer, D. K., Earp, J., Linnan, L., Wilder Smith, A., Alfano, C. M., Barbash, R., Baumgartner, K. B., George, S., Reeve, B. B. *Identifying breast cancer survivors with a pattern of low physical activity and high sedentary behavior from pre-diagnosis through 10 years post-diagnosis.* Podium presentation at the annual meeting of National Research Service Award (NRSA) trainees, AcademyHealth, Minneapolis, MN, June, 2015.

Rini, C, Roberts, S., Werner-Lin, A., Rodriguez, L. *Giving patients incidental information from genomic sequencing: Insights from the CSER Consortium*. Symposium presented at the Society of Behavioral Medicine Annual Meeting and Scientific Sessions, San Antonio, TX, April, 2015. Sponsored by the Health Decision Making Special Interest Group of the Society of Behavioral Medicine. Role: Chair and Speaker (*Patient Decision Making about Non-Medically Actionable Incidental Genomic Findings in NCGENES*).

Rini, C., Gibson, B. S., Lewis, M., Balgrosky, J., & Hesse, B. *Using Technology to Address Challenges in Health Decision Making: Case Studies and Recommendations.* Symposium presented at the Society of Behavioral Medicine Annual Meeting and Scientific Sessions, San Antonio, TX, April, 2015. Co-sponsored by the Technology and

Health Decision Making Special Interest Groups of the Society of Behavioral Medicine. Role: Chair.

*Khan, C. M., **Rini, C.,** Berg, J. S., Evans, J. P., & Henderson, G. E. *Roles of Genomic Sequencing Results and Intolerance of Uncertainty on Information-Seeking.* Poster presented at the Society of Behavioral Medicine Annual Meeting and Scientific Sessions, San Antonio, TX, April, 2015.

*Leos, C. **Rini, C.** *Illness Perceptions Predict Healthcare Utilization Differently Among Adult Patients and Parents of Pediatric Patients: Responses to Clinical Use of Diagnostic Genonmic Sequencing.* Poster presented at the Society of Behavioral Medicine Annual Meeting and Scientific Sessions, San Antonio, TX, April, 2015.

*Symes, Y., *Jenkins, K., Green, M., & **Rini, C.** *Effects of Story Type and Transportability on Responses to Cancer Narratives in a Health Population.* Poster presented at the Society of Behavioral Medicine Annual Meeting and Scientific Sessions, San Antonio, TX, April, 2015.

*Pepper, J. K., Emery, S. L., Ribisl, K. M., **Rini, C.,** & Brewer, N. T. *How risky are e-cigarettes? Smokers' beliefs about the health risks of multiple tobacco products.* Podium presentation at the Society of Behavioral Medicine Annual Meeting and Scientific Sessions, San Antonio, TX, April, 2015.

*Campo, R. A., Wu, L. M., Austin, J., Valdimarsdottir, H., & **Rini, C**. M. *Stem Cell Transplant Cancer Survivors' Associations of Personal Resilience Resources with Longitudinal Changes in Distress and Purpose in Life.* Podium presentation at the 2015 World Congress of Psycho-Oncology, Washington, DC; July, 2015.

*Pepper, J. K., Emery, S. L., Ribisl, K. M., **Rini, C. M.,** & Brewer, N. T. *Smokers' perceptions of the health risks of e-cigarettes and other tobacco products: Implications for tobacco control.* Poster and "rapid fire" oral presentation at the Society for Research on Nicotine and Tobacco Annual Meeting, Philadelphia, PA, February, 2015.

Rini, C., Porter, L., Somers, T. J., McKee, D. C., DeVellis, R., Stiller, J., Patterson, C., Winkel, G., Jordan, J. M., Smith, M., Caldwell, D. S., & Keefe, F. J. *Internet-based Pain Coping Skills Training Intervention for People with Osteoarthritis: A Randomized Controlled Trial*. Podium presentation at the 7th Scientific Meeting of the International Society for Research on Internet Interventions (ISRII), Valencia, Spain; October, 2014.

*Symes, Y., **Rini, C.,** Green, M., & Jenkins, K. *Tell me a story: Effects of story type and individual difference variables on responses to cancer narratives in a healthy population.* Podium presentation at the 16th World Congress of Psycho-Oncology and Psychosocial Academy, International Psycho-Oncology Society; Lisbon, Portugal; October, 2014.

*Khan, C., **Rini, C.** *My Illness or Our Illness? Shared Responsibility for Illness Management and Emotional Impact of Receiving Genomic Sequencing Information.* Poster presented at the Society of Behavioral Medicine Annual Meeting and Scientific Sessions, Philadelphia, PA, April, 2014

Rini, C., *Emmerling, D., Austin, J., *Wu, L., Validmarsdottir, H., Redd, W. H., *Woodruff, R. *Challenges to survivors' experience of stem cell transplant and their links to the effectiveness of caregivers' social support*. Talk in symposium, *Challenges to Successful Psychosocial Adaptation to Hematologic Malignancies*. Podium presentation at the Society of Behavioral Medicine Annual Meeting and Scientific Sessions, Philadelphia, PA, April, 2014.

Rini, C., Austin, J., *Wu, L, Winkel, G., Validmarsdottir, H., Stanton, A., Redd, W. H. *Randomized Controlled Trial of an **Expressive Helping** Intervention to Improve Survivorship Problems after Stem Cell Transplant*. Talk in symposium, *New directions in expressive writing research* (Chair: Rini). Podium presentation at the Society of Behavioral Medicine Annual Meeting and Scientific Sessions, Philadelphia, PA, April, 2014.

*Morgan, J. C., **Rini, C.,** Khan, Cl, Henderson. G. *The Relationship Between Illness Uncertainty and Participants' Hopes and Expectations for Whole Exome Sequencing*. Poster presented at the Society of Behavioral Medicine Annual Meeting and Scientific Sessions, Philadelphia, PA, April, 2014

*Morgan, J. C., **Rini, C.,** Khan, Cl, Henderson. G. *The Relationship Between Illness Uncertainty and Participants' Hopes and Expectations for Whole Exome Sequencing: Implications for Cancer*. Podium presentation at the UNC Lineberger Comprehensive Cancer Center Annual Scientific Retreat, Chapel Hill, NC, May, 2014

*Symes, Y., Barrington, C., Austin, J., Wu, L., & **Rini, C**. *Survivors' Advice to Patients Undergoing Stem Cell Transplant: Themes from a Content Analysis of Survivor Narratives*. Poster presented at the annual American Psychosocial Oncology Society conference, Tampa, FL, February, 2014.

*Brown, M., Roche, M., & **Rini, C.** *Medical students' assessment of their training in the clinical application of genomics.* Poster presented at the annual American College of Medical Genetics meeting, Nashville, TN, March, 2014.

*Song, L., **Rini, C.,** Palmer, M., Kinneer, P., Greene, G., Nielsen, M., Mark, B. *Improving couples' QOL through a web-based, couple-oriented prostate cancer education intervention.* Presentation at the Translational Science meeting of the Association for Clinical and Translational Science and the American Federation for Medical Research, Washington, DC, April, 2014.

Rini, C., Porter, L., DeVellis, R., Stiller, J., Somers, T., McKee, D., & Keefe, F. *PainCOACH: Designing, developing, and evaluating an automated web-based pain coping skills training program that mimics therapeutic aspects of in-person training.* Podium presentation at the Annual Conference of the International Society for Research on Internet Interventions, Chicago, IL, May, 2013.

*Khan, C. M., **Rini, C.,** Roche, M.I., & Henderson, G. E. *What Can Psychological Science Offer to Clinical Applications of Next Generation Sequencing?* Poster presented at the American College of Medical Genetics annual meeting, Phoenix, AZ, March, 2013.

*Pearce, E., Roche, M., Brown, M., **Rini, C.,** *Khan, C., Berg, J., Evans, J., & Henderson, G. *Measuring Knowledge and Understanding of Genetics and Genomic Sequencing: The Genomic Knowledge Scale.* Poster presented at the American College of Medical Genetics annual meeting, Phoenix, AZ, March, 2013.

*Wu, L., Austin, J., Kuprian, N., Tannenbaum, M., Valdimarsdottir, H., C. Rowley, S., Isola, L., Redd, W., & **Rini, C**., *Subjective reports of cognitive changes among hematopoietic stem cell transplant survivors.* Poster presented at the Society of Behavioral Medicine annual meeting, San Francisco, CA, March, 2013.

Rini, C., Porter, L., DeVellis, R., Stiller, J., Somers, T., McKee, D., & Keefe, F. *Developing PainCOACH: An automated web-based pain coping skills training program for people with osteoarthritis pain.* Poster presented at the Society of Behavioral Medicine annual meeting, San Francisco, CA, March, 2013.

*****Emmerling, D., **Rini, C.,** Green, M. S., Akiba, C., Woodruff, R., & Simons, J. *Age differences and cognitive processing in the expressive writing paradigm among hematopoietic stem cell transplant survivors.* Poster presented at the American Public Health Association annual meeting, San Francisco, CA, October 2012.

Rini, C., Austin, J., *Wu, L., Valdimarsdottir, H., C. Rowley, S., Isola, L., & Redd, W. *Different functional types of effective caregiver support are differentially associated with people's cancer treatment-related concerns.* Podium presentation at the 26th Annual Conference of the European Health Psychology Society, Prague, Czech Republic, August 2012.

*Woodruff, R. & **Rini, C.** *Return to Work after Hematopoietic Stem Cell Transplantation.* Poster presented at the 2012 Biennial Cancer Survivorship Conference, June, 2012.

Rini, C. *Getting more support is not always best: Benefits of measuring critical features of effective enacted support.* Talk in symposium entitled "*When a Good Thing Isn't a Good Thing: Negative Aspects of Positive Interactions and Social Support"* (K. L. Fingerman, Chair) presented at the 2012 Association for Psychological Science Conference, Chicago, IL, May, 2012.

*Emmerling, D., & **Rini, C.** *Different functional types of effective caregiver social support have different benefits among hematopoietic stem cell transplant survivors.* Poster presented at the Gillings School of Global Public Health Student Research Poster Event, April, 2012, and at the American Public Health Society Annual Conference, San Francisco, CA, October, 2012.

Rini, C., Austin, J., *Wu, L., Valdimarsdottir, H., C. Rowley, S., Isola, L., & Redd, W. *Negative network orientation is associated with worse health-related quality of life after cancer, but not because of deficits in social support.* Poster presentation at Society of Behavioral Medicine Conference, New Orleans, LA, April, 2012.

Rini, C., Henderson, G., Skinner, D., Roche, M., Brewer, N. T., Berg, J. S., & Evans, J. P. *Assessing the Ethical and Psychosocial Implications of Using Whole Exome Sequencing in Clinical Medicine*. Talk presented at the American College of Medical Genetics and Genomics Annual Meeting, Charlotte, NC, March, 2012.

*Woodruff, R. & **Rini, C.** *Return to Work among Women Hematopoietic Stem Cell Transplantation Survivors.* Poster presented at Women's Health 2012: The 20th Annual Congress, Washington, DC, March, 2012.

*Wu, L., Austin, J., Valdimarsdottir, H., Isola, L., Rowley, S., Redd, W. H., & **Rini, C.** *Neurobehavioral symptoms predict psychological distress and quality of life following hematopoietic stem cell transplant*. Poster presented at the International conference on the side effects of cancer (Chemo Brain Symposium: Mechanisms & Assessments), Lexington, KY, October, 2011.

Rini, C. (Symposium Chair). *Advancing social support science and application with new findings on mechanisms related to health.* Symposium presented at the 2011 European Health Psychology Conference, October 2011, Crete, Greece. (Discussant: Luszczynska, A.Speakers: Rini, C., Dunkel Schetter, C., Holt-Lunstad, J., Schwarzer, R.)

Rini, C., Redd, W. H., Dunkel Schetter, C., DuHamel, K. N. *Improving social support interventions: Benefits of understanding the features and consequences of effective enacted support.* Talk in symposium presented at the 2011 European Health Psychology Conference, October 2011, Crete, Greece.

Rini, C., Austin, J., *Wu, L., Valdimarsdottir, H., Dunkel Schetter, C. Rowley, S., Isola, L., & Redd, W. *Social support buffers negative life event stress among cancer survivors, but only if it is effective support from a partner*. Talk at 2011 Multinational Association of Supportive Care in Cancer (MASCC) conference, June 2011, in Athens, Greece.

*Wu, L., Austin, J., Valdimarsdottir, H., Isola, L., Rowley, S., Redd, W. H., & **Rini, C.** *Moving beyond "chemobrain": Understudied neurobehavioral changes following hematopoietic stem cell transplant*. Poster presented at the Multinational Association of Supportive Care in Cancer (MASCC) conference, June 2011, Athens, Greece.

Rini, C., *Wu, L., Austin, J., Valdimarsdottir, H., Dunkel Schetter, C. Rowley, S., Isola, L., & Redd, W. *Enacted support buffers stress among hematopoietic stem cell transplant survivors— but only if it is from a partner and only if it is effective support*. Rapid Communication Poster presented at the Annual Meeting and Scientific Sessions of the Society of Behavioral Medicine, April 2011, Washington, D.C.

*Mosher, C., Lepore, S., *Wu, L., Austin, J., Valdimarsdottir, H., Basmajian K., Rowley, S., Isola, L., & **Rini, C. *Loneliness mediates the impact of social factors in distress following hematopoietic stem cell transplant*. Poster presented at the Annual Meeting and Scientific Sessions of the Society of Behavioral Medicine, April 2011, Washington, D.C.

*Tanenbaum, M., *Jackson, G., *Wai, C., & **Rini, C. *Measuring alexithymia in inflammatory bowel disease: The case for somatic uncertainty as an independent fourth factor.* Poster presented at the Annual Meeting and Scientific Sessions of the Society of Behavioral Medicine, April 2011, Washington, D.C.

*Hamilton, J. G., *Wu, L., Austin, J., Valdimarsdottir, H., Basmajian, K., Vu, A., Rowley, S., Isola, L., Redd, W., & **Rini, C.** *Misery loves company?: Timing of the financial crisis moderates the association between financial stress and quality of life among stem cell transplant survivors.* Poster presented at the Annual Meeting and Scientific Sessions of the Society of Behavioral Medicine, April 2011, Washington, D.C.

*Hamilton, J. G., *Wu, L., Austin, J., Valdimarsdottir, H., Basmajian, K., Vu, A., Rowley, S., Redd, W., Isola, L., & **Rini, C.** *Financial stress is associated with poorer emotional and physical quality of life Among survivors of hematopoietic stem cell transplantation.* Presentation at the 2010 Cancer Survivorship Research Conference in Washington, DC.

Rini, C., Austin, J., *Wu, L.,*Hamilton, J. G., Valdimarsdottir, H., Basmajian, K., Vu, A., Redd, W., Rowley, S., & Isola, L. *Peer support provides experiential information associated with patient coping, adjustment, and quality of life in hematopoietic stem cell transplantation.* Poster presented at the 2010 Cancer Survivorship Research Conference in Washington, DC.

*Wu, L., Austin, J., Basmajian, K., Vu, A., Rowley, S., & **Rini, C.** *Self-efficacy beliefs mediate the relationship between perceived cognitive complaints and distress and quality of life.* 2010 annual meeting of the International Cognition and Cancer Task Force (ICCTF), New York, NY.

Rini, C., Jandorf, L., *Goldsmith, R., Manne, S., Harpaz, N., & Itzkowitz, S. H. *Decision influence from significant others and surgical decision making in high risk patients.* Poster presented at the 2010 annual meetng of the Society for Personality and Social Psychology, Las Vegas, Nevada.

*Jackson, G., *Tannenbaum, M., *Wai, C., & **Rini, C.** *Balance in dyadic support perceptions and relationship satisfaction among patients with chronic illness and their*

partners. Poster to be presented at the 2010 annual meeting of the Society for Personality and Social Psychology, Las Vegas, Nevada.

Rini, C. (Chair), Dunkel Schetter, C., Petrie, K., & Redd, W. H. *International intervention trials: Theory, methodology and findings in diverse populations.* Symposium presented at the 2009 European Health Psychology conference, Pisa, Italy.

Rini, C., Austin, J., *Wu, L., Chee-Chait, J., Basmajian, K., & Valdimarsdottir, H. *Helping others helps Oneself: A novel intervention for survivors of hematopoietic stem cell transplantation*. Presentation as part of the symposium *International Intervention Trials: Theory, Methodology and Findings in Diverse Populations,* presented at the 2009 European Health Psychology conference in Pisa, Italy.

Rini, C., Jandorf, L., Dunkel Schetter, C., Harpaz, N., & Itzkowitz, S. H. *Effective partner support and partner influence on a major medical decision.* Rapid communication poster presented at the 2009 Society of Behavioral Medicine conference, Montreal, Quebec, Canada.

Rini, C., Jandorf, L., & Itzkowitz, S. H. *Surgical decision making among high risk inflammatory bowel disease patients referred for prophylactic surgery to remove their colon.* Presented as part of the symposium on Cultural Variations in Screening Programs for Colorectal Cancer at the International Congress of Behavioral Medicine, August, 2008, Tokyo, Japan.

Rini, C., Manne, S., DuHamel, K. N., Austin, J., Ostroff, J., Boulad, F., Parsons, S., Martini, R., Williams, S., Mee, L., Sexson, S., & Redd, W. H. *Social support and functioning among mothers of critically ill children.* Poster presented at the 2008 American Psychological Association conference, Boston, MA.

Rini, C., DuHamel, K., Dunkel Schetter, C., *Markarian, Y., Labay, L., Burkhalter, J., & Redd, W. H. *Effectiveness of partner support predicts distress among survivors of hematopoietic stem cell transplant.* Paper presented at the July 2008 International Association for Relationships Research conference, Providence, RI.

Rini, C., Jandorf, L., & Itzkowitz, S. H. *Predicting quick surgical decisions in IBD patients at high risk for cancer.* Rapid Communication poster presentation at the 2008 Society of Behavioral Medicine conference, San Diego, CA.

Rini, C., Austin, J., *Lawsin, C., *Markarian, Y., Burkhalter, J., Labay, L., Redd, W. H., & DuHamel, K. *Survivors' stories and decision making: What do cancer patients learn from the experiences of others?* Presented as part of the symposium "Psychosocial Approaches to Understanding and Improving Cancer Decision Making" at the International Psycho-Oncology Society Meeting, September, 2007, London. (Discussant: D. Bowen; Speakers: C. Rini, R. Goldsmith, M. Schwartz).

*Goldsmith, R., Jandorf, L., Duplessi, Y., Itzkowitz, S., & **Rini, C.** *Aspects of Decision-Making among Patients at High Risk for Colorectal Cancer.* Presentation in symposium on Psychosocial Approaches to Understanding and Improving Cancer Decision Making at the International Psycho-Oncology Society Meeting, September, 2007, London. (Discussant: D. Bowen; Speakers: C. Rini, R. Goldsmith, M. Schwartz).

Rini, C., Austin, J., Chee, J., DuHamel, K., Markarian, Y., Labay, L., Burkhalter, J., & Redd, W. H. *Benefits of social support provision for cancer survivors.* Poster presentation at the American Psychological Association annual conference, August, 2007, San Francisco, CA.

Redd, W. H. (Chair) **Rini, C.** (Discussant), Jandorf, L., Weinstein, B. & Tapanya, S. *Old dogs doing new tricks: Translating behavioral interventions to new populations.* Symposium presented at the International Congress of Behavioral Medicine, December, 2006, Bangkok, Thailand.

Rini, C. *Survivors as an informational resource for cancer patients: What are their effects on patients?* Podium presentation at the American Psychosocial Oncology Society 4th Annual Conference, March, 2007, Austin, TX.

Rini, C., Jandorf, L., *Goldsmith, R., & Itzkowitz, S. H. *Dyadic decision-making among chronically ill patients referred for major surgery: Predictors of spouse/partner influence.* Poster presentation at the 2007 Society of Personality and Social Psychology conference, pre-conference on judgment and decision making, Memphis, TN.

*Goldsmith, R., **Rini, C.,** Jandorf, L., Duplessi, Y., Itzkowitz, S. H. Surgical Decision-Making Among Patients at High Risk for Colorectal Cancer. Poster presentation at the Society of Behavioral Medicine annual conference, March, 2007, Washington, D.C.

Rini, C., Manne, S., DuHamel, K. N., Austin, J., Ostroff, J., Boulad, F., Parsons, S., Martini, R., Williams, S., Mee, L., Sexson, S., & Redd, W. H. *Can negative effects of marital strain due to a child's life-threatening treatment be buffered by social support from the spouse and others?* Poster at the Society of Behavioral Medicine Conference, March, 2006, San Francisco, CA.

Rini, C., Jandorf, L., Bakal, H., Brown, K., & Itzkowitz, S. H. *Distress among inflammatory bowel disease patients at high risk for cancer: Objective risk, psychological threat, and perceived social support.* Poster at the Society of Behavioral Medicine conference, April, 2005, Boston, MA.

Rini, C., Jandorf, L., Bakal, H., Brown, K., & Itzkowitz, S. H. *Medical factors and social support as predictors of psychological health among colorectal cancer survivors: Unique effects of the cancer experience compared to chronic inflammatory bowel disease.* Poster at the biennial conference on Cancer Survivorship: Pathways to Health After Treatment, June, 2004, Washington, DC.

Rini, C., Manne, S., DuHamel, K., Austin, J., Ostroff, J., Boulad, F., Parsons, S., Martini, R., Williams, S., Mee, L., Sexson, S., & Redd, W. H. *A longitudinal study of finding benefit in adversity.* Poster presented at the American Psychological Society, May, 2003, Atlanta, GA.

Dunkel Schetter, C., & **Rini, C.** *Perceptions of the effectiveness of social support from partners in pregnant women: Advances in studying social support receipt.* Talk co-presented at the personal relationships pre-conference of the annual meeting of the Society for Personality and Social Psychology, February, 2003, Los Angeles, CA.

Rini, C., Dunkel Schetter, C., Glynn, L., Hobel, C., & Sandman, C. A. *Couples' reports of the effectiveness of enacted support during pregnancy: Concordance and association with individual characteristics*. Poster presented at the Couples Coping with Stress International Conference, sponsored by the Science Directorate of the American Psychological Association and Boston College, October, 2002, Chestnut Hill, MA.

Rini, C., Dunkel Schetter, C., Glynn, L., Hobel, C., & Sandman, C. A. *Measurement and prediction of the effectiveness of enacted social support.* Poster presented at the annual meeting of the American Psychological Association, August, 2002, Chicago, IL.

Rini, C., Dunkel Schetter, C., Glynn, L., Hobel, C., & Sandman, C. A. *Social support effectiveness: A new conceptualization of enacted social support and its relation to*

psychological health during pregnancy. Poster presented at the annual meeting of the Society of Behavioral Medicine, April, 2002, Washington, D.C.

Killingsworth [Rini], C., Dunkel-Schetter, C., Wadhwa, P. D., & Sandman, C. A. Personality, stress, context, and pregnancy: Predicting adverse infant outcomes. In C. Dunkel-Schetter (Chair), *Biopsychosocial approaches to studying stress in pregnancy and effects on birth outcomes.* Symposium at the meeting of the Society of Behavioral Medicine, April, 1997, San Francisco, CA.

Killingsworth [Rini], C., Dunkel-Schetter, C., Wadwha, P. D., & Sandman, C. A. *Personality, Stress, and Pregnancy.* Paper presented at the meeting of the American Psychological Association's Women's Health Conference, September, 1996, Washington, D.C.

Killingsworth [Rini], C., Dunkel-Schetter, C., Wadhwa, P. D., & Sandman, C. A. *Individual differences and life events as predictors of postpartum negative affect*. Poster presented at the annual meeting of the Western Psychological Association, April, 1996, San Jose, CA.

*Current or past student/mentee; **Senior author

Book Reviews

Rini, C. (2007). Book review of *AfterShock: What to do when the doctor gives you—or someone you love—a devastating diagnosis*, J. Gruman. *Psycho-oncology, 16,* 965–966.

Manuscripts Submitted for Peer Review

Song, L., Northouse, L. L., **Rini, C.,** Mood, D. W. *Appraisals, Dyadic Communication, and Quality of Life among Couples Coping with Prostate Cancer: An APIMeM Approach*.

*Symes, Y., Campo, R. A., Austin, J., Wu, L. M., **__Rini, C.__** *Network Orientation and Health-Related Quality of Life in Cancer Survivors: Evaluating Social Resources as Mediators.*(Revise and resubmit underway)

*Leos, C., *Khan, C. M., **__Rini, C.__** *Understanding self-management behaviors in symptomatic adults with uncertain etiology using an illness perceptions framework.* (Revise and resubmit underway)

Nyrop, K. A., Callahan, L. F. **Rini, C.,** Altpeter, M., Hackney, B., DePue, A., Wilson, A., Schechter, A., & Muss, H. B. *Oncology provider communications with breast cancer patients about musculoskeletal side effects of aromatase inhibitors and their potential management through physical activity.* (Revise and resubmit underway)

*Bloom, K., *Bernstein, J., *Bridges, C., *Adler, J., **Rini, C.,** Ripley-Moffitt, C. *Examining Patient Perspectives on Weight Management Support in the Primary Care Setting.*

*Symes, Y., Barrington, C., Austin, J., Wu, L., & **__Rini, C__**. *Survivors' Advice to Patients Undergoing Stem Cell Transplant: Analysis of Survivor Peer Support Narratives*.

Williamson, T. J., Stanton, A. L., Austin, J. E., Valdimarsdottir, H. B., Wu, L. M., Krull, J. L., **__Rini, C__**.** *Helping yourself by offering help: Mediators of expressive helping in survivors of hematopoietic stem cell transplant.* Health Psychology.

*Current or past student/mentee; **Senior author

Manuscripts in Preparation for Submission for Peer Review

*Campo, R. A., Wu, L. M., Austin, J., Valdimarsdottir, H., **Rini, C. *Personal Resilience Resources in Survivors' Adjustment through Meaning Making and Depressive Symptoms.*

*Margolis, M., Austin, J., Wu, L., Winkel, G., Valdimarsdottir, H., Isola, L., Rowley, S., Redd, W. H., **Rini, C.,** *Social support after hematopoietic stem cell transplantation buffers effects of life event stressors, but only if it is effective support from a partner.*

Langer, M., **Rini, C.,** … Roche, M. *Development and Validation of the Genomic Knowledge Scale*.

Roche, M., **Rini, C.,** … *Educating patients about genomic sequencing in clinical research: Consortium approaches and issues to be addressed*.

*Stover, A. M., Reeve, B. B., **Rini, C.,** Mayer, D. K., Earp, J., Linnan, L., Wilder Smith, A., et al. *One size does not fit all: Breast cancer survivors report different physical activity and sedentary behavior patterns from pre-diagnosis through 10 years post-diagnosis: Implications for cancer care.* In Preparation (Dissertation).

*Stover, A. M., Reeve, B. B., **Rini, C.,** Mayer, D. K., Earp, J., Linnan, L., Wilder Smith, A., et al. *New insights into psychosocial constructs that predict physical activity patterns across 10 years of breast cancer survivorship.* In Preparation (Dissertation).

*Stover, A. M., Reeve, B. B., **Rini, C.,** Mayer, D. K., Earp, J., Linnan, L., Wilder Smith, A., et al. *Psychosocial characteristics differentially predict long-term physical activity and sedentary behavior patterns in breast cancer survivors.* In Preparation (Dissertation).

Bennell, K. L., Delany, C., Nelligan, R. K., Rini, C., Keefe, F. J., Bryant, C., Hinman, R. S. *Internet-delivered pain coping skills training for knee osteoarthritis: a qualitative study of patient experience and perception.*

*Current or past student/mentee; **Senior author

TEACHING

Courses

Fall, 2014, 2015 — Guest lecture: Development and Evaluation of Health Promotion and Disease Prevention Interventions (HBEH 811). Discussion of PainCOACH intervention development and evaluation to approximately 9 doctoral students in Health Behavior and Nutrition.

Fall, 2012-2015 non-2012-2015 — Guest lecture: Professional Issues (HBEH 812). Discussion of academic and academic career paths for graduate students pursuing a doctorate in public health.

Fall, 2011 — Guest lecture: Foundations Of Health Behavior And Health Education I (HBEH 815). Discussion of learning theories. UNC Gillings School of Global Public Health, Department of Health Behavior and Health Education (11 students).

Spring, 2011 Spring, 2012 — Guest lecture: Research Grant Proposal Development (HBEH 860). Discussion of grant writing and reviewing. UNC Gillings School of Global Public Health, Department of Health Behavior and Health Education (approximately 11 students each semester).

Fall, Guest lecture: Social and Behavioral Foundations of Health Education (HBEH 730), 2010-2015 *Expressive Helping: An Intervention Applying Riessman's Helper Therapy Principle to Cancer Survivors.* UNC Gillings School of Global Public Health, Department of Health Behavior (55-60 students each semester).

Winter, 2006 *Social Influences on Health and Behavior* lecture for team-taught Behavioral
Fall, 2007 Medicine Seminar, Mount Sinai School of Medicine (approximately 50 students each semester)

Spring, 2005 *Research Seminar in Social Psychology*, upper division undergraduate seminar, Hofstra University, Department of Psychology (21 students)

Fundamentals of Psychology (Psychology of Health and Adjustment), undergraduate lecture course; Hofstra University, Department of Psychology (34 students)

Fall, 2004 *Social Psychology*, upper division undergraduate lecture course, Hofstra University, Department of Psychology (51 students)

Fundamentals of Psychology (Psychology of Health and Adjustment), undergraduate lecture course; Hofstra University, Department of Psychology (31 students)

June, 2002, Instructor, *Social Support and Health* lecture for Behavioral Medicine Seminar,
March, 2003 Mount Sinai School of Medicine (approximately 50 students each semester)

Mentoring and Advising

2014-2015 MPH Capstone Faculty Advisor, Department of Health Behavior, UNC-Chapel Hill

2012-2013 MPH Capstone Faculty Advisor, Department of Health Behavior, UNC-Chapel Hill

2004-Present Undergraduate: 4
Master's Degree: 22
Doctoral: 20
Postdoctoral fellows: 9
Junior faculty: 6

GRANTS

ACTIVE

1U01HG006487-01 (Contact: Evans) 12/1/11 – 11/30/15
NIH/NHGRI
NCGENES: North Carolina Clinical Genomic Evaluation by NextGen Exome Sequencing

In this project we will confront the major challenges that stand between genomic medicine and its broad implementation as a diagnostic tool in a diverse population of patients.
Role: Investigator

2P50HG004488-06 (Henderson) 6/12/13 – 6/11/18
NIH/NHGRI
Center for Genomics and Society

This application focuses on dynamic and reciprocal relationships that exist between the ELSI of genome research and clinical translation, and the society-wide implications of ongoing advances in genomic technology and bioinformatics.
Role: Investigator

1R21CA169492-01A1 (Callahan) 7/1/13 – 6/30/15
NIH/NCI
Walk with Ease (WWE) Adapted for Breast Cancer Patients with Joint Pain

This exploratory study investigates the impact of an evidence-based, self-directed physical activity program (Walk With Ease; WWE) on aromatase inhibitor -associated joint pain and stiffness, and the requirements for tailoring/adapting WWE to the needs and interests of female breast cancer patients with joint pain/stiffness. (No cost extension)
Role: Investigator

1P60AR062760-01 (Jordan) 7/19/13 – 7/18/18
NIH/NIAMS
Multidisciplinary Clinical Research Center: Mitigating the public health impact of osteoarthritis

This project takes a public health approach to reducing adverse effects of osteoarthritis (OA). One of its two projects ("Clarifying critical processes linking partner support to insufficiently active osteoarthritis patients' initiation and maintenance of increased lifestyle physical activity"; PI: Rini) investigates how partner support influences the extent to which insufficiently active people with OA initiate and maintain increased lifestyle physical activity after a couple-focused intervention. Emphasis is on understanding partner support as a social resource that can either facilitate or hinder behavior change and using findings to develop a new intervention approach.
Role: Center Investigator/Project PI

U19 (Powell) 7/1/13 – 6/30/18
NHGRI
NC NEXUS, North Carolina Newborn Exome Sequencing for Universal Screening

We will complete a set of highly multi-disciplinary activities to investigate the utility of genomic sequencing in a diverse pediatric population to augment and extend current newborn screening.
Role: Center Investigator/Project PI

Program Grant 631717 (Bennell)
National Health and Medical Research Council (NHMRC; Australia)
Internet mediated physiotherapy and pain coping skills training for people with persistent knee pain: IMPACT trial

This study will evaluate whether an Internet-based physiotherapy-guided home exercise program, combined with an online pain coping skills training program (PainCOACH) is more

effective in improving pain and function in people with knee osteoarthritis than on-line educational material
Role: Investigator

PENDING

NIH/NCI R01 (Valle) 4/1/16-3/31/20
Promoting Physical Activity in Young Adult Cancer Survivors Using mHealth and Adaptive Tailored Feedback Strategies

This project would conduct a two-arm, 6 month, randomized controlled trial with 200 yound adult cancer survivors to test the efficacy of a theory-based, individually tailored, Internet- and mobile-based physical activity intervention aimed at increasing moderate-to-vigorous intensity physical activity among young adult cancer survivors.
Role: Investigator

NIH/NCI R21 (Rini) 7/1/16-6/30/18
Feasibility of Expressive Helping for patients undergoing stem cell transplant

This project would evaluate a new application of our Expressive Helping intervention, originally developed to reduce physical and psychological symptoms among cancer survivors with persistent symptoms 9-months to 3-years after hematopoietic stem cell transplant. A 2-arm pilot/feasibility randomized controlled trial comparing Expressive Helping with an active comparison group would enable evaluation of the feasibility, acceptability, and potential efficacy of using the intervention during and immediately following transplant to reduce suffering earlier and to prevent development of persistent symptoms after transplant. Scored 22%; revision/resubmission in October, 2015.
Role: Principal Investigator

R01 (Samuel-Hodge) 7/1/14 - 6/30/19
NIH/NIMHD
African American Family Partners in Lifestyle Support (PALS-II)

We will conduct a randomized controlled trial to evaluate a novel family-based behavioral lifestyle intervention wth weight loss as the primary outcome. The intervention targets African Americans with diabetes, who participate in 24 weekly group sessions with an adult family member.
Role: Investigator

Program Grant (Bennell)
National Health and Medical Research Council (NHMRC; Australia)
Effects of pain coping skills training and exercise for people with hip osteoarthritis (HOPE trial)

The purpose of this study is to evaluate whether physiotherapy-guided exercise, combined with education about osteoarthritis and an online pain coping skills training program (PainCOACH), is more effective in improving pain and function in people with hip osteoarthritis than exercise and education alone
Role: Investigator

COMPLETED

UCRF Developmental Research Award (Rini) 7/1/14-6/30/15
Adapting an Internet-based pain coping skills training program to help cancer patients manage pain

We will gather critical data needed to adapt and implement an automated, Internet-based pain coping skills intervention to address the needs of cancer patients with bone pain. Proposed mixed methods activities include a pilot study of cancer patients who use the program at home and provide feedback as well as focus groups with clinicians who treat these patients, gathering feedback on how to integrate use of the adapted program with patients' clinical care.
Role: Principal Investigator

UCRF Developmental Award (Song) 7/1/13-6/30/14
Lineberger Comprehensive Cancer Center
Development of a couple-focused eHealth Intervention for Prostate Cancer Symptom Management
This project will develop a web-based, couple-focused symptom management program for prostate cancer patients and their partners.
(Role: Investigator)

1R01AR57346-1A2 (Rini) 9/20/10 – 6/30/14
NIH/NIAMS
Internet-based pain coping skills intervention

The major goals of this multi-site project are to translate a proven in-person pain coping skills intervention for delivery via the Internet and to evaluate it in a randomized controlled feasibility trial.

K07 CA104701-05 (Rini) 9/01/05 – 08/31/10
NIH/NCI
Predicting Surgical Decisions of High-Risk Inflammatory Bowel Disease Patients (Role: PI)

This project investigated factors predicting the surgical decisions of IBD patients referred for colectomy, with particular emphasis on the role of patients' health beliefs and the influence of their partners or other family members.

RSGPB-07-285-01-CPPB (Rini) 7/01/07 – 6/30/12
American Cancer Society
Reciprocal benefits of helping others: A peer support intervention for bone marrow/stem cell survivors

The major goal of this project is to evaluate the efficacy of a psychosocial intervention for distressed survivors of hematopoietic stem cell transplant using a four-arm randomized controlled trial. (In no-cost extension until 10/31/12).

5-P50-HG004488-05 (Henderson) 8/1/12 – 6/11/13 .
NIH/NHGRI
Center for Genomics and Society

A major aim of the CGS was to conduct integrated research on ELSI issues raised by large-scale genomic studies. The Center was refunded in 2013.

Role: Investigator

UCRF Innovation Award (Reeve) 7/2011-2012
Lineberger Comprehensive Cancer Center
Development and pilot test of the UNC patient-reported symptom monitoring (PRSM) system in the North Carolina Cancer Hospital to enhance quality of care

This project developed and evaluated an electronic system to collect, store, and report patient-reported data on symptom burden, functioning, and quality of life in real time, with the goal of enabling healthcare providers to provide better quality of care
Role: Investigator

R03 (Stadler) 4/1/12 – 3/31/14
NIH/NCI
Multi-Method Study Of Cancer Patients' Medication Adherence After Allogeneic HSCT

This feasibility study will evaluate a theory-based approach to investigating adherence during the first six months after allogeneic transplantation. The study will assess medication adherence using multiple indicators including electronic medication monitoring, self-reported adherence, and blood plasma levels for immunosuppressant medications.
Role: Investigator

PROFESSIONAL SERVICE

To Department, School, and Institution

2015	Member, Student Survey Committee (to gather feedback for departmental planning, the UNC Graduate Program Review, and the Gillings School of Global Public Health's Council on Education for Public Health (CEPH) accreditation). Department of Health Behavior, University of North Carolina at Chapel Hill, Gillings School of Global Public Health.
2014-Present	Member, UNC Lineberger Comprehensive Cancer Center Survivorship Advisory Board, University of North Carolina at Chapel Hill.
2014-Present	Member, Doctoral Advisory Committee, Department of Health Behavior, University of North Carolina at Chapel Hill, Gillings School of Global Public Health.
2013-2015	Reviewer, Developmental Research award applications. UNC Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill.
2013	Member, Committee to update guidelines for promotion of tenure track and fixed term faculty. Department of Health Behavior, University of North Carolina at Chapel Hill, Gillings School of Global Public Health.
2011-2013	Member, UNC Health Care Patient Education Workgroup (planning committee for "Patient Connect" program to link patient-reported data and

electronic medical records at UNC, with the goal of improving quality of care and the patient experience). University of North Carolina at Chapel Hill.

2011-2012 Member, search committee for an open-rank, tenure track behavioral intervention scientist in the field of cancer prevention and control sponsored by the UNC Lineberger Comprehensive Cancer Center in collaboration the UNC Gillings School of Global Public Health and other departments and schools at UNC.

2011 Member, grantee panel, NIH grantsmanship workshop co-sponsored by the Center for Faculty Excellence and NC TraCS, University of North Carolina at Chapel Hill.

2008 Organizer, Speaker Series in the Mount Sinai School of Medicine Program for Cancer Prevention and Control, Icahn School of Medicine at Mount Sinai.

2007-2010 Organizer, Journal Club in the Mount Sinai School of Medicine Program for Cancer Prevention and Control, Icahn School of Medicine at Mount Sinai.

To Discipline

Grant review

2014-present Member, NIH/NIAMS Study Section: AMSC Clinical Trials Review Committee.

2009-2013 Member, American Cancer Society Peer Review Committee for Cancer Control and Prevention: Psychosocial and Behavioral Aspects of Cancer Research (CPPB).

2009 Ad hoc reviewer, National Science Foundation grant submissions.

2008 Ad hoc member, American Cancer Society CPPB Peer Review Committee.

Paper and abstract review

2014-2015 Reviewer, Society of Behavioral Medicine, Health Decision Making Special Interest Group conference abstracts.

2012-2013 Reviewer, Society of Medical Decision Making conference abstracts.

2009-2013 Reviewer, Society of Behavioral Medicine conference abstracts.

2008 Reviewer, American Psychological Association conference abstracts.

1996-present Ad hoc reviewer for various peer reviewed journals including:

Annals of Behavioral Medicine	Anxiety, Stress & Coping
Health Psychology	Journal of Experimental Social Psychology
Journal of Health and Social Behavior	Journal of Personality and Social Psychology
Journal of Women's Health	Journal of Personal and Social Relationships
Pain	Personal Relationships
Psycho-Oncology	Psychosomatic Medicine
Translational Behavioral Medicine	Health Expectations
Journal of Psychosomatic Research	Basic and Applied Social Psychology
Journal of Medical Internet Research	PLOSone

Genetics in Medicine Journal of Behavioral Medicine

Committees and Other Professional Service

2015	Prepared promotion letter for colleague up for promotion to Associate Professor at Rush University, Department of Behavioral Sciences, Rush Medical College.
2014-2015	Chair (2015) and Co-Chair (2014), Health Decision Making Special Interest Group of the Society of Behavioral Medicine.
2014-present	Member, Editorial Board, Annals of Behavioral Medicine.
2014	Society of Behavioral Medicine Health Decision Making Special Interest Group *Outlook* newsletter liaison.
2014	Co-Chair, Outcomes and Measures Working Group of the Clinical Sequencing Exploratory Research (CSER) and Return of Results (RoR) consortia, funded by NHGRI to investigate clinical application of next-generation genomic sequencing technologies.
2013-present	Member, Steering Committee, North Carolina Newborn Exome Sequencing for Universal Screening (NC NEXUS) project.
2012-2013	Co-chair, Award Committee, Health Decision Making Special Interest Group of the Society of Behavioral Medicine, to select recipient of annual Award for Outstanding Training Abstract in Health Decision Making. Recognizes a significant contribution to field of Health Decision Making.
2012	Track Co-Chair, Social Support and Health Track, Annual Conference of the European Health Psychology Society, Prague, Czech Republic.
2011-2013	Member, Measures and Outcomes Working Group of the Clinical Sequencing Exploratory Research (CSER) and Return of Results (RoR) Consortium, National Human Genome Research Institute (NHGRI).
2011-2012	Member, Faculty position search committee, joint hire for Lineberger Comprehensive Cancer Center and Department of Health Behavior.
2010-2015	Member, Cross-Talk Committee, Health Decision Making Special Interest Group of the Society of Behavioral Medicine., tasked with promoting communication between HDM SIG and Society of Medical Decision Making.
2010	Member, NIH National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) Roundtable Discussion on Psychosocial and Behavioral Therapies for Musculoskeletal and Rheumatic Disease Outcomes.
2009-2012	Member, American Cancer Society National Reach to Recovery Volunteer Advisory Workgroup.
2009-2010	Behavioral and Social Scientist Volunteer (BSSV), Socioeconomic Status Related Cancer Disparities Program (SESRCD) (a collaboration of the American Psychological Association and the Centers for Disease Control and Prevention).
2008- 2011	Member, APA Division 38 (Health Psychology) membership committee.

CURRICULUM VITAE

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EDUCATION:

Certificate in Public Health, University of North Carolina-Chapel Hill, 2002
Masters in Medical Genetics, University of Wisconsin, Madison, Wisconsin, 1986
Bachelors of Science, University of Illinois, Champaign, Illinois, psychology major, 1979

BOARD CERTIFICATION:

American Board of Genetic Counseling, re-certification, Genetic Counseling, (2006-present)
American College of Medical Genetics, certification, Genetic Counseling (1990-present)

FACULTY APPOINTMENTS: University of North Carolina-Chapel Hill

2012-present	Clinical Associate Professor, Department of Genetics
2002-present	Associate Professor, Department of Pediatrics, Genetics and Metabolism
1998-2002	Assistant Professor, Department of Pediatrics, Genetics and Metabolism
1990-1998	Clinical Instructor, Department of Pediatrics, Genetics and Metabolism
1986-1990	Lecturer in the Brain Research and Development Center

PROFESSIONAL EXPERIENCE:

Current Clinical Research Positions:

2013-present	Investigator; Project Manager: NC NEXUS, North Carolina Newborn Exome Sequencing as Universal Screening, NHGRI; Powell, C., 2013-18.

2013-present	Investigator, Patient Education Specialist; Center in ELSI Excellence Research, Center for Genomics and Society; The GeneScreen Project, NHGRI; Henderson, G., 2013-2018.
2011-present	Investigator; Project Manager, Patient Education Specialist, Lead Genetic Counselor, NCGENES: North Carolina Clinical Genomic Evaluation by NextGen Exome Sequencing, NHGRI; Evans, J., 2011-15.

Past Clinical Positions:

2008-2011	Director of Pediatric Genetic Counseling Services, Department of Pediatrics, Division of Genetics and Metabolism.
2006-2011	Lead Certified Genetic Counselor, Division of Genetics and Metabolism, Genetics and Metabolism Clinic.

Other Past Positions (Excluding Teaching Positions):

2007-2013	Investigator and Certified Genetic Counselor, Fragile X Newborn Screening Study, NHGRI; Bailey, D., 07/2003–06/2013.
2007-2013	Investigator and Patient Education Specialist, UNC Center for Genomics and Society (CGS), Center for Excellence in ELSI Research (CEER), NHGRI; Henderson, G., 10/07-09/2013.
2005-2006	Lead Science Writer for Genomics Multimedia Project, UNC Institute for Science Learning, NHGRI; Bollenbacher, W., 9/2003-5/2007.
2003-2006	Project Manager and Certified Genetic Counselor, Genetic Services for Congenital Hearing Loss, Division of Genetics and Metabolism, Department of Pediatrics, CDC/AAMC; Powell, C. 10/2003-09/2005.
2003-2005	Clinical Genetics Advisor and Curriculum Chair, North Carolina Center for Genomics & Public Health, CDC: PI: Millikan, R., 2002-2005.
2000-2003	Co-Principal Investigator and Certified Genetic Counselor, Cultural and Family Interpretations of Genetic Knowledge, Frank Porter Graham Child Development Center, NHGRI: PI: Skinner, D., 8/2000–7/2003.

1994-2003	Certified Genetic Counselor, Division of Genetics and Metabolism, UNC Cytogenetic Laboratory.
1994-2004	Certified Genetic Counselor, Pediatric Genetics and Metabolism Clinic, Division of Genetics and Metabolism
1990-1994	Certified Genetic Counselor, Prenatal Genetic Counseling Clinic, Division of Genetics and Metabolism
1989-1990	Genetic Counselor, Prenatal Genetic Counseling Clinic, Department of Obstetrics and Gynecology
1986-1989	Genetic Counselor, Department of Medicine, Div. of Medical Genetics

Awards:

2003-2005	National Society of Genetic Counselors $5,000.00, to co-author "An Ethics Casebook for Genetic Counselors: Ethical Discourse for the Practice of Genetic Counseling."
2003-2004	Bilbro Ethics Fellowship, Parr Center for Ethics, Institute for the Arts and Humanities, 1 semester, 50% salary support to author ethics casebook.

BIBLIOGRAPHY:

Book Publications:

1. Trees, A., Koenig, J. and **Roche, M.I.** Family Narratives. In: *Talking about Genetics: A Family Affair* (C. Gaff, and C. Bylund, eds.) 2010, Oxford Press, NY, NY, pp. 68-86.

2. **Roche M.I.** *An Ethics Casebook for Genetic Counselors: Ethical Discourse for the Practice of Genetic Counseling: second edition* (L. Karns, **M.I. Roche**, and B. Yashar, eds.) 2006, In: Springer Publishing, NY, NY, pp.12-37.

    a. Chapter 1: Introduction to Ethical Theory and Bioethical Principles.
    b. Chapter 4: Autonomy, Informed Consent, and Decisional Capacity.
    c. Chapter 7: A Sampling of Philosophical Theories of Moral Behavior.

3. **Roche M.I.** Genetic Counseling Considerations in Molecular Diagnosis. In: *Molecular Diagnostics for the Clinical Laboratorian, second edition (WB*

Coleman and G. Tsongalis, eds), 2005, Humana Press, Totowa, NJ, pp. 525-544.

4. Kuller J and **Roche M.I.** Autosomal Disorders: Cystic Fibrosis, Tay-Sachs and Huntington Disease. In: *Clinical Decisions in Reproductive Genetics* (J Kuller, N. Chescheir, and R. Cefalo, eds) Mosby Year Book, St Louis, MO, pp. 53-62.

Journal Publications:

1. **Roche, MI** and Berg, JS. Incidental Findings with Genomic Testing: Implications for Genetic Counseling Practice, 2015, *Cur. Genet. Med. Reports*.

2. Bernhard, B., **Roche, MI.,** Scollon, S., Perry, D., Tomlinson, A. and Skinner, D. Experiences with Obtaining Informed Consent for Genomic Sequencing. *Am. J. Med. Genet. Part A*, 2015, DOI 10.1002/ajmg.a.37256.

3. Bailey, D., Wheeler, A., Berry-Kravis, E., Hagerman, R., Tassone, F., Powell, C., **Roche, MI**, Gane, L., and Sideris. Maternal Consequences of the Detection of Fragile X Carriers in Newborn Screening. *Pediatrics,* 2015, doi:10.1542/peds.2015-0414.

4. Couser, N., Masood, M., Strande, N., Foreman, A., Crooks, K., Weck, K., Lu, M., Wilhemsen, K., **Roche, MI**., Evans, J., Berg, J., and Powell, C. The phenotype of the multiple congenital anomalies-hypotonia-seizures syndrome. *Am. J. Med. Genet. Part A*. 2015 Apr 29. doi: 10.1002/ajmg.a.37129.

5. Tomlinson, A., Skinner, D., Perry, D., Scollon, S., **Roche, MI**., and Bernhardt, B. Not Tied Up Neatly with a Bow": Professionals' Challenging Cases in Informed Consent for Genomic Sequencing. *J. Genet. Counsel.* 2015. doi: 10.1007/s10897-015-9842-8.

6. Khan, C., Rini, C., Bernhardt BA, Roberts JS, Christensen KD, Evans JP, Brothers KB, **Roche MI**, Berg JS, and Henderson, GE. How Can Psychological Science Inform Research about Genetic Counseling for Clinical Genomic Sequencing? *J. Genet Counsel.* 2014. doi: 10.1007/s10897-014-9804-6.

7. **Roche, MI** and Prince, A. Genetic Information, Non-Discrimination, and Privacy Protections in Genetic Counseling Practice, *J. Genet. Counsel.* 2014, doi: 10.1007/s10897-014-9743-2

8. **Roche, MI** and Palmer, CSG. Next Generation Genetic Counseling, Introduction to the Special Issue, *J. Genet. Counsel.* 2014, doi:10.1007/s10897-014-9729-0.

9. Clayton, E., McCullough, L., Biesecker, L., Joffe, S., Ross, L., Wolf, S. and the Clinical Sequencing Exploratory Research (CSER) Consortium Pediatrics Working Group. Addressing the Ethical Challenges in Genetic Testing and Sequencing of Children. *J. of Bioeth.* 2014, Volume 14(3), 3-9.

10. Fan, Z., Greenwood, R., Felix, A., Shiloh-Malawsky, Y., Tennison, M., **Roche, M.I.,** Crooks, K., Weck, K., Wilhelmsen, K., Berg, J., and Evans, J. GCH1 heterozygous mutation identified by whole-exome sequencing as a treatable condition in a patient presenting with progressive spastic paraplegia. *J. of Neur.* 2014, doi:10.1007/s00415-014-7265-3.

11. Bailey, D., Lewis, M. Powell, C., and **Roche, M.I.** Family Relations in the Genomic Era: Communicating about Intergenerational Transmission of Risk for Disability. *Family Relations, 2014, DOI: 10.1111/fare.12054. 85-100.*

12. **Roche, M.I**. Moving toward NextGenetic Counseling. *Genet. in Med.* 2012. doi: 10.1038/gim.2012.84.

13. Bailey, D., Bann, C., Bishop, E., Guarda, S., Barnum, L. and **Roche, M.I**. Can a Decision Aid Enable Informed Decisions in Neonatal Nursery Recruitment for a Fragile X Newborn Screening Study? *Genet. in Med*. 2012, doi: 10.1038/gim.2012.135.

14. Bailey, D., Lewis, M., Harris, S., Grant, T., Bann, C., Bishop, E., **Roche, M.I.**, Guarda, S., Barnum, L., Powell, C. and Therrell, B. J. Design and Evaluation of a Decision Aid for Inviting Parents to Participate in a Fragile X Newborn Screening Pilot Study. *J. of Genet Counsel*. 2012, doi:10.1007/s10897-012-9511-0.

15. Skinner, D., Choudhury, S., Sideris, J., Guarda, S., Buansi, A., **Roche, M.I**., Powell, C. and Bailey, D. Parents Decisions to Screen Newborns for Fragile X in a Pilot Research Project, *Pediatrics.* 2011; 127(6): e1455-1463.

16. Skinner, D., Choudhury, S., Sideris, J., Guarda, S., Buansi, A., **Roche, M.I**., Powell, C., Bailey, D. Parents' decisions to screen newborns for *FMR1* gene expansions in a pilot research project, *Ob. and Gyn. Survey*. 2011; 66(10): 616-617.

17. **Roche M.I**. and Skinner D. How Parents Search, Interpret, and Evaluate Genetic Information Obtained from the Internet. *J. of Genet Counsel*. 2009; 18(2):119-129.

18. Bailey DB Jr., Skinner D**, Roche M.I**, Powell CM. Emerging Dilemmas in Newborn Screening. *Virtual Mentor.* 2009,11:709-713.

19. **Roche, M.I**. Genetic Counselling for Dr. Watson. *Nature*. 2008; 453(7193): 261.

20. Irwin, D., Millikan, R., Stevens, R., **Roche, M.I.**, Rakhra-Burris, T., Davis, M., Mahanne, E., Duckworth, S. and Whitesides, H. Genomics and Public Health Practice: A Survey of Nurses in Local Health Departments in N. C., *J. Public Health Management and Pract*. 2004; 10(6): 539-544.

Guest Editor, Journal of Genetic Counseling, 2014

Roche, M.I. and Palmer, C. G. Journal of Genetic Counseling, Special Issue, "Next Generation Genetic Counseling," August, 2014.

Published Abstracts:

1. **Roche, M.I.,** Skinner, D., Powell, C. and Bailey, D. Families' Genetic Counseling Experiences Following a Positive *FMR1* Newborn Screen. *Eur. J. Hum. Genet.*, 2013, 21, (2): P18:34.

2. Hardy, M., **Roche, M.I.,** Cannon, R.E. and Callanan, N**.** "Use of an Interactive Web-Based Learning Module to Increase Undergraduates Interest in the Genetic Counseling Profession", *J. Genet. Counsel*. 2011; 20 (6): 700

3. **Roche, M.I.** and D. Lofland. Analysis of Transcriptomes: A genomics multimedia module. *Am. J. Hum. Genet*. 2006; 79:1156A.

4. **Roche, M.I.,** Skinner, D. and Bailey D. The emotional consequences of informing relatives at risk for Fragile X Syndrome. *J. Genet. Counsel.* 2003:12(6): 503-504.

5. **Roche, M.I.,** Skinner, D., Kuczynski, K. and Shaffer, R. Parents' use of the Internet as a source of genetic information. *J. Genet. Counsel*. 2002:11(6**):** 506-507.

6. **Roche,** M.I., Aylsworth, A., Lachicotte, W., Bailey, D. and Skinner, D. Parental reactions to genetic testing results. *J. Genet. Counsel*. 2001:10(6): 481-482.

7. Skinner, D., **Roche, M.I.** and Bailey, D. Culture and family interpretations of genetic knowledge. *J. Law, Med., and Ethics*. 2001; Special Supplement to Volume 29:2:52.

8. Quigley, D., Kaiser-Rogers, K., **Roche, M.I.** and Powell, CM. Two Cases with Xp Deletions: Characterization Using Sub-telomeric FISH Probes. *Am. J. Hum. Genet.* 2001; 69:833A.

9. **Roche, M.I.,** Rohlfs, E., Booker, J., Zariwala, M., Silverman, L., Shores, C. and Powell, CM. Referrals to Genetics from ENT Following *GJB2* Testing. *Am. J. Hum. Genet.* 2000; 67:1319A.

10. Rohlfs, E., Zariwala, M., Booker, J., **Roche**, **M.I.,** Powell, C., Silverman, L. and Shores, C. Mutation Analysis of *GJB2* in an Unselected Deaf Population. *Am. J. Hum. Genet*. 2000; 67:2276A.

11. Kaiser-Rogers, K., Rao, K., **Roche**, **M.I**. and Powell, C.M. Complex counseling issues associated with a 12;15 translocation. *Am. J. Hum. Genet*. 2000; 67:852A.

12. Kaiser-Rogers, K., Rao, K., **Roche**, **M.I**., Lese, C. and Powell, C.M. A rare terminal deletion involving the distal short arm of chromosome 12. *Am. J. Hum. Genet.* 1999; 65:911A.

13. Kaiser-Rogers, K., **Roche, M.I**., Davenport, M., Aylsworth, A, and Rao, K. Multiple cell lines observed at CVS and amnio in a normal female with 46,XX/47,XX,+der(Y) mosaicism. *Am. J. Hum. Gen.* 1992; 51:82A.

14. Lamb, A., **Roche**, **M.I.,** Kirkman, H., Rao, K., and Aylsworth, A. A Patient with a minute terminal deletion of chromosome 2 long arm. *Am. J. Hum. Genet*. 1990; 47:32A.

Invited Presentations

1. Roche, MI. Clinical Sequencing and Secondary Findings: Genetic Counseling Implications. Joan Marks Graduate Program in Human Genetics, Sarah Lawrence College, Bronxville, NY, April, 2015.

2. Roche, MI. Fragile X Newborn Screening Project, **invited plenary lecture**, National Society of Genetic Counselors, Educational Meeting, Anaheim, CA, October, 2013.

3. Roche, MI. Next Generation Genetic Counseling. Genetics, Ethics, and the Law Conference, University of Virginia Law School, Charlottesville, VA, May 2013; 2009

4. Roche, MI. Families' Genetic Counseling Experiences Following a Positive *FMR1* Newborn Screen, NC Medical Genetics Association meeting, Charlotte, NC, April 2013.

5. Roche, MI. Moving Towards Next Generation Genetic Counseling. Quality Improvement and Public Health Special Interest Group Forum, American College of Medical Genetics meeting, Phoenix, AZ, March 2013.

6. Roche, MI., Powell, C., Skinner, D. and Bailey, D. Families' Genetic Counseling Experiences Following a Positive *FMR1* Newborn Screen, American College of Medical Genetics meeting, Phoenix, AZ., March 2013.

7. Roche, MI. Next Generation Sequencing and Genetic Counseling, Long Island University, Genetic Counseling Training Program, Long Island, NY, October 2013, 2012.

8. Roche, M.I. The Genetic Counseling Experiences of Families with Screen Positive Infant Identified by the Fragile X Newborn Screening Project, International Fragile X Conference, Miami, Fl., July 2012.

9. Roche, M.I. The Genetic Counseling Experiences of Families Whose Newborns Screen Positive for *FMR1* Expansions, International Fragile X Conference, Detroit, MI., July 2010.

10. Roche, M.I., Skinner, D. and Powell, CM. Parents' Decisions to Accept or Decline Newborn *FMR1* Screening, American College of Medical Genetics meeting, Albuquerque, N.M., March 2010.

11. Roche, MI. Parents Use of the Internet: Implications for the MPS Society Website, International Mucopolysaccaridosis Society meeting, Orlando, FL. December 2009.

12. Roche, M.I., Skinner, D. and Powell, CM. Parents' Internet Searching for Genetic Information, American College of Medical Genetics meeting, Phoenix, AZ. March 2008.

13. Roche, M.I., Skinner, D. and Powell, CM. Parents' Internet Searching for Genetic Information, National Society of Genetic Counselors Meeting, KC, MO., October 2007.

14. Roche, M.I. Genetic Causes of Hearing Loss, North Carolina Pediatric Audiology Conference, Greensboro, N.C., April 2005.

15. Roche, M.I. Genomics and Public Health, North Carolina Association of Public Health Nurse Administrators, Winston-Salem, N.C., December 2004.

16. Roche, M.I. Collecting a Family History in a Public Health Setting, North Carolina Medical Genetics Meeting, Chapel Hill, N.C., October 2004.

17. Roche, M.I. and Skinner, D. The Emotional Consequences of Informing At-Risk Relatives, National Society of Genetic Counselors, Charlotte, N.C., October 2003.

18. Roche, M.I. Genetic Causes of Hearing Loss, North Carolina Pediatric Audiology Conference, Chapel Hill, N.C., October 2003.

19. Roche, M.I. The Clinical Impact of Taking a Family History, ELSI Conference for Healthcare Professionals, Chapel Hill, N.C., March 2003.

20. Roche, M.I. and Skinner, D. Parents' Use of the Internet for Genetic Information, National Society of Genetic Counselors, Phoenix, AZ., October 2002.

21. Roche, M.I. and Skinner, D. Parental Reactions to Genetic Testing Results, National Society of Genetic Counselors, Washington, D.C., October 2001.

22. Roche, M.I. Family Interpretations of Genetic Knowledge, North Carolina Medical Genetics Meeting, Asheville, N.C., September 2001.

Invited Presentations by Colleagues

Rini, C., Skinner, D., Raspberry, K., Khan, C., Henderson, G., **Roche, MI**., Berg, J., and Evans, J. Returning Secondary Genomic Findings to Patients in NCGENES: Intention vs Reality, Clinical Sequencing Exploratory Research meeting, Bethesda, MD, April 2015.

Rini, C., Skinner, D., Raspberry, K., Khan, C., Henderson, G., **Roche, MI**., Berg, J., and Evans, J. Patient Decision Making about Non-Medically Actionable, Incidental Genomic Findings in NCGENES. Society for Behavioral Medicine meeting, San Antonio, TX. April 2015.

Henderson, G., RIni, C. **Roche, MI**, Skinner, D. Berg, J., and Evans, J. The GeneScreen Project presented in Genomics for the Healthy: Opportunities and Challenges in Applying Genomics to the Sphere of Public Health - R.

Rodney Howell Symposium, American College of Medical Genetics Meeting, Salt Lake City, UT, March, 2015.

Powell, C., **Roche, M.I**., Skinner, D., Wheeler, A. and Bailey, D. Fragile X Newborn Screening Pilot Study, International Society of Neonatal Screening meeting, Asheville, NC. July 2013.

Bailey, D., Skinner, D., **Roche, M.I**., Wheeler, A., and Powell, C. Fragile X Newborn Screening Pilot Study, American Public Health Laboratory meeting, Atlanta, GA. May 2013.

Rini, C., Henderson, G., Skinner, D., **Roche, MI.**, Berg, J., Evans, J. Assessing the Ethical and Psychosocial Implications of the Use of Whole Exome Sequencing in Clinical Medicine, American College of Medical Genetics meeting, Charlotte, N.C., March 2012.

Hardy, M., **Roche, M.I**., Cannon, R.E. and Callanan, N. An Interactive Web-Based Learning Module to Increase Undergraduates Interest in the Genetic Counseling Profession, National Society of Genetic Counselors Annual Education Conference, San Diego, CA., October 2011.

Powell, CM. and **Roche, M.I**. Assessment of Parental Attitudes about Genetics and Congenital Hearing Loss, ELSI Congress, Chapel Hill, N.C., April 2011.

Powell, CM. and **Roche, M.I.** Assessing Parental Attitudes about Genetic Services for Early-onset Hearing Loss, American College of Medical Genetics meeting, Phoenix, AZ., March 2008.

Irwin, D., **Roche, MI.** and Millikan, R. Colon Cancer Genomics: A Public Health Perspective, Center for Disease Control Meeting, Atlanta, GA., July 2005.

Skinner D, Bailey D, **Roche M.I**. Meanings of Diagnoses, 35th Annual Gatlinburg Conference on Research Theory in Intellectual and Developmental Disabilities, San Diego, CA., August 2002.

Aylsworth, A., Mornet, E., Cardenas, L., Taillandier, A., **Roche, M.I.**, and Wright T. Benign Prenatal Hypophosphatasia: Further Evidence for a Dominant-Negative Mechanism and Possible Maternal Effect. Smith Workshop, Lake Arrowhead, CA, September 2000

Poster Presentations (National Meetings)

1. Roche, M.I., Raspberry, K., Skinner, D., Guarda, S., Forman, A., Lee, K., O'Daniel, J, Powell, B., Skrynzia, C., Berg, J., Evans, J., and Henderson, G. Secondary Findings from Genomic Sequencing: What NCGENES

Participants Say They Want, What They Request, and How They Respond. American College of Medical Genetics, Salt Lake City, UT, March 2015.

2. Brown, M., **Roche, M.I.** and Rini, C. Medical Students' Assessment of Training in Clinical Genomics. American College of Medical Genetics, Nashville, TN, March 2014.

3. Pearce, E., **Roche, M.I**., Brown, M., Rini, C., Khan, C., Berg, J., Evans, J. and Henderson, G. Measuring Knowledge and Understanding of Genetics and Genomic Sequencing: The Genomic Knowledge Scale, American College of Medical Genetics, Phoenix, AZ., March 2013.

4. Berg, J., Carey, T., Crooks, K., Foreman, K., Jensen, B., Juengst, E., Lee, K., Nelson, D., Powell, C., **Roche, M.I**., Skrzynia, C., Weck, K., Wilhelmsen, K., and Evans, J. Development and application of a semi-quantitative metric for measuring clinical actionability of gene-phenotype pairs, American College of Medical Genetics, Phoenix, AZ., March 2013.

5. Khan, C., Rini, C., **Roche, M.I**. Berg, J., Evans, J. and Henderson, G. What Can Psychological Science Offer to Clinical Applications of Next Generation Sequencing? American College of Medical Genetics meeting, Phoenix, AZ., March 2013.

6. Foreman, A., Berg, J.S., **Roche, M.I.,** Weck, K., Wilhelmsen, K., Evans, J. A Year of NCGENES: North Carolina Clinical Genomic Evaluation by NextGen Exome Sequencing, American College of Medical Genetics meeting, Phoenix, AZ. March 2013.

7. Bailey, D., Skinner, D., Powell, C.M., **Roche, M.I**., Wheeler, A. Challenges and Opportunities Associated with Carrier Detection in Newborn Screening for Fragile X Syndrome, American College of Medical Genetics meeting, Phoenix, AZ., March 2013.

8. Roche, M.I. and Skinner, D. Blogs as a Virtual Educational Tools in Genomics for ELSI Trainees, American College of Medical Genetics meeting, Charlotte, N.C., March 2012.

9. Berg, J., Wilhelmsen, K., Weck, K., **Roche, M.I**., Henderson, G, and Evans, J. NCGENES: North Carolina Clinical Genomic Evaluation by NextGen Exome Sequencing, American College of Medical Genetics meeting, Charlotte, N.C., March 2012.

10. Roche, M.I., Skinner, D. and Powell, CM. Families' Experiences in Newborn Screening for Fragile X Syndrome, American College of Medical Genetics meeting, Vancouver, B.C., March 2011.

11. Wheeler, F., Powell, CM., **Roche, M.I**., Booker, J., Weck, K., Kaiser-Rogers, K. Variant Turner Karyotype in a Patient with Hemi-hyperplasia and Developmental Delay, American College of Medical Genetics meeting, Vancouver, B.C., March 2011.

12. Roche, MI. and Powell, CM. North Carolina Parents' Experiences in Obtaining Genetic Services for Congenital Hearing Loss, National Society of Genetic Counselors meeting, Atlanta, GA., October 2008.

13. Roche, MI., Keith, H., Mahanna, E. and Davis, M. Family History Can Identify Women at Increased Risk for Breast Cancer: Development and Evaluation of a Training Module, International Society of Nurses in Genetics meeting, Toronto, Canada, June 2004.

14. Mahanna, E., Irwin, D., Millikan, R., **Roche, M.I.**, Rakhra-Burris, T., and Davis, M. Breast Cancer Training Module: Identifying Women at Increased Risk, National Coalition for Health Professional Education in Genetics, Washington, D.C., February 2004.

15. Roche MI, Skinner D, Lachicotte W, Bailey D, and Aylsworth A. Culture and Family Interpretations of Genetic Disorders, 42nd Annual Short Course in Experimental and Medical Genetics, Bar Harbor, ME, July 2001.

16. Skinner, D. Bailey, D and **Roche MI**. Culture and Family Interpretations of Genetic Disorders, A Decade of ELSI Research Conference, Washington, D.C, February 2001.

Workshop Presentations (National Meetings):

1. **Roche, M.I.** and Adam, Shelin. Evidence-based Genetic Counseling for Clinical Genome Sequencing, American Society of Human Genetics meeting, moderator, Boston, MA., October 2013.

2. **Roche, M. I**. Item Writer Training for the ABGC Certification Examination, National Society of Genetic Counselors meeting, Atlanta, GA., October 2009.

3. Yashar, B., Karns, L. and **Roche, M.I.** Applying the NSGC Code of Ethics, National Society of Genetic Counselors meeting, Charlotte, N.C., October 2003.

4. Yashar, B., Karns, L. and **Roche, M.I**. Genetic Counselors and IRB's, National Society of Genetic Counselors, Washington, D.C., October 2001.

Book Reviews:

1. **Roche, M.I.** New Clinical Genetics by A. Read and D. Donnai. *J. Genet. Counsel*. 2009, 18:103-4.

2. **Roche M.I.** Reshaping Life: Key Issues in Genetic Engineering by G.V. Nossal and Ross Coppel. Cambridge Univ. Press, Cambridge, UK. 2002. *J Genet. Counsel.* 2004, 13(5):460-1.

Patient Educational Pamphlets:

1. **Roche M.I.,** Brown, M., Rini, C., Henderson, G., Evans, J., and Berg, J. "About the NCGENES Project," 2013.

2. **Roche M.I.,** Brown, M., Rini, C., Henderson, G., Evans, J., and Berg, J. "What Is Whole Exome Sequencing?" 2013.

3. **Roche M.I.,** Brown, M., Rini, C., Henderson, G., Evans, J., and Berg, J. "What Can You Learn from Whole Exome Sequencing?" 2013.

4. **Roche M.I.,** Brown, M., Rini, C., Henderson, G., Evans, J., and Berg, J. "Decisions about Learning Incidental Information from Whole Exome Sequencing," 2013.

5. **Roche M.I.,** Rohlfs, E., Powell, C., and Shores, C. 2000. "Genetic Testing for Childhood Hearing Loss," 2000.

On-Line Training Modules:

1. **Roche, M.I.** *Advanced* Item Writing Training for the ABGC Certification Exam, 2010.

2. **Roche, MI**. Item Writing Training for the ABCG Certification Exam, 2010.

3. **Roche, M.I**., McAllister, C., Cooper, J., Jackson, T., Anderson, J., Lofland, D., and Schmidt, M. Analysis of Transcriptomes " an inquiry-based genomics course for undergraduates. Each of the seven subsections contains learning objectives and interactive questions for self-evaluation: http://multimedia.jomc.unc.edu/img/aot/, 2006**.**

4. **Roche, M.I**., Mahanna, E., and Millikan, R. Breast Cancer: Identifying Women at Increased Risk: A Training Module for Public Health Workers, a case-based interactive module for identifying families at risk for hereditary cancer using family history, 2004.

Newsletter Articles:

1. **Roche M.I.** and Mahanna E. Focus on Genomics and Public Health, educate public health nurses about the role of genomics in their practice, 2005.

2. Members of the Ethics Sub-Committee, National Society of Genetic Counselors Code of Ethics. *Perspectives in Genetic Counseling*, 2003, 25(3): 4.

3. Members of the Ethics Sub-Committee. Analyzing Ethics Cases in Genetic Counseling. *Perspectives in Genetic Counseling, 2002,* 24(2): 13.

4. **Roche M.I.** Genetic Counseling: Myths and Misconceptions, National Society of Genetic Counselors, website, 2004.

Genomics Blog:
Center for Genomics and Society Blog
http://genomicsandsociety.wordpress.com/
Creator/administrator/principal author

Blog Entries:

Roche, M.I .The Limits to Fiction: Sequencing the Fetal Genome, April 2012
Roche, M.I. Elizabeth Taylor Was a Mutant, April 2012
Roche, M.I. Telling Fact from Fiction, NPR's April Fools Story, April 2012
Roche, M.I. The ACMG Meeting in Charlotte: Geneticists, Start your Engines, March 2012
Roche, M.I. Trying on Ethical Frameworks to Issues in Genetics, March 2012
Roche, M.I. Tell Us What You Think: Ethical Questions in Genetics, March 2012
Roche, M.I. Student Us of their Own DNA in Classroom Activities, March 2012
Roche, M.I. And What Would You Like for Christmas? 23 and Me? December 2011.
Roche, M.I. Pace of Human Sequencing Far Outpaces Everything Downstream, Including Genetic Counseling, December 2011.
Roche, M.I. Whole Exome Sequencing: the NIH Experience, November 2011.
Roche, M.I. Health Literacy and Communicating Genomic Information, November 2011.
Roche, M.I. Genomic Health Literacy, June 2011.

TEACHING and COURSE DIRECTORSHIP

Course Director

1. Medical Genetics Course
Universidad Autonomia Guadalajara, School of Medicine, Guadalajara, México,
Course Director

Second Year Medical Students (180 students per year)
Biannually: February and August 2011-2013

Lecture Topics: 10 Lectures, 50 minutes each

Clinical Genetic Testing, Taking a Genetic Family History, Newborn Screening, Fragile X Syndrome Case Study, Genetics of Hearing Loss, Next Generation Sequencing, Reproductive Genetics, Genetics of Hereditary Cancer, Clinical Genetics Case Studies

2. Clinical and Counseling Aspects of Human Genetics
UNC, Biology/Genetics 125
Course Director
Undergraduates and Graduate Students (10-15 students)
Spring Semester, 2003 and 2005
14 Lectures (50 minutes), 14 small group session (110 minutes)

Created, designed, and taught a new, semester-long, case-based course that applied clinical genetic principles to show social and ethical implications of genetics.

Lecture Topics

Clinical Genetics, Taking a Family History; Pedigree Construction and Analysis, Mendelian Inheritance and Population Genetics, Clinical and Molecular Cytogenetics, Molecular Genetics and Nontraditional Inheritance, Newborn Screening for Genetic Diseases, Hereditary Predisposition to Cancer, Ethical Issues in Human Genetics and Genetic Testing, Ethical Issues in Human Subjects Research

3. Medical Genetics Course, UNC School of Medicine, MEDI 226
Course Co-Director
Second Year Medical Students (~160 students)
Annually, 18 years, January 1986-2004

Hired to improve the organization, focus, and teaching of the course that had been poorly evaluated by students and failed to show the clinical relevance to medicine.

- Mentored 20 faculty members to improve their lecture and small group teaching.
- Created 7 case-based problem sets (>100 questions) for hands-on experience in solving clinical problems; consistently rated excellent by students (4.5 on a 5 point scale) and remained the highest evaluated part of the course for 18 years.
- Created yearly final exams evaluated “excellent” for problem-based learning.
- Taught small groups of 20-30 students; consistently rated as “excellent”.

Educational Modules

1. Lead Writer: Genomics Multimedia http://multimedia.jomc.unc.edu/img
UNC Institute for Science Learning; 2004-2006

Lead content writer for a multimedia module on DNA microarray techniques. Authored, "Analysis of Transcriptomes", to teach genomics with interactive self-assessments. Led and managed writers, graphic designers, programmers and instructional designers

2. Lead Writer: Public Health Module and Education Advisor
North Carolina Center for Public Health Genomics; 2003-5

Developed a case-based module, Breast Cancer: Strategies for Identifying Women at Increased Risk: a Training Module for Public Health Workers.

Modeled how family history could be elicited with standardized pedigree symbols; created an algorithm to help determine the relevance of a family history; provided age/ethnic specific recommendations for referrals; explained principles of inheritance and genetic testing.

Other Teaching (50 minute sessions unless otherwise specified)

1. Medical Genetics Course, MEDI 226
Second Year Medical Students (25-30 students)
Annually, February 1986-2003 and 2008-2010

Topics (150 minutes each)
Cytogenetics, Single Gene Disorders and Risk Assessment, Molecular Basis of Human Disease, Newborn Screening and Inborn Errors of Metabolism, Genetics of Common Diseases, Clinical Genetic and Genetic Counseling Cases, Ethical Issues in Clinical Genetics.

2. Molecules to Cells, MEDI 140
First Year Medical Students (25-30 students)
October 2003 and 2009

Topics: Case Studies: Duchenne Muscular Dystrophy; Von-Hippel-Lindau; Inborn Errors

Invited Lectures to Residents and Fellows, School of Medicine

1. Molecular Pathology and Cytogenetics Course
Pathology Residents, (~20 students)
Annually, March 2002-2005
Principles of Genetic Counseling

2. Board Certification Review Course
Medical Genetics Fellows and Genetic Counselors

Risk Calculations in Genetic Counseling, May 2008 and 2010
Genetics of Childhood Hearing Loss, February 2002 and 2006

Invited Lectures to Graduate Students and Undergraduates, School of Nursing

1. Genetics and Society Course, NURS 782
Nursing Graduate Students (~20 students) (160 minutes)
Ethical Issues in Genetic Testing, February 2004, 2008, and 2010

2. Pathophysiology Course, NURS 361
First Year Nursing Students, (~150 students) (160 minutes)
Annually: September 1993-2005
Clinical Medical Genetics and Genetic Counseling

Invited Lectures to Other Graduate Students

1. Hearing Disorders Course, SPHS 725
Department of Allied Health Sciences, Speech and Hearing Sciences (~15 students)
ELSI Issues in Genetic Testing for Hearing Loss, November 2009
Genetic Principles and Hearing Loss, February 2004

2. Genetic Epidemiology, EPI 229
Department of Epidemiology, (~20 students)
School of Public Health
Principles of Medical Genetics and Genetic Testing, March 2000

3. Child Development Course, PSYC 500
Developmental Psychology, (~20 students)
School of Arts and Sciences
Annually, April 1991-96
Genetic Causes of Mental Retardation (150 minutes)

Invited Lectures to Undergraduates, Department of Biology

1. Genetics and Molecular Biology, BIO 202 (~150 students)
Debate: Using Student DNA in the Classroom, December 2012

2. Freshman Biology Seminar in Genetic Testing, BIO 57, (20 students)
Ethical Issues in Newborn Screening, November 2005

3, Laboratory in Cell Biology, BIO, 129 (20 students)
Biannually: March 1998, 2000, 2002, and 2004
Clinical Uses of Fluorescent in situ Hybridization
Applications of Genetic Testing to Clinical Genetics

4. Laboratory Experiments in Genetics, BIO, 163 (20 students)
Annually, April 2001-2005
Clinical Aspects of Human Genetics

5. Freshman Seminar in Biotechnology BIO 53 (15 students)
Genetic Testing, November 2000, 2001, 2004

6. Johnston Scholars Seminar: Impact of Genetic Research (20 students)
Counseling and Ethical Issues in Genetics, October 1998 (50 minutes)

7. Freshman, Carolina Summer Reading Leader (35 students)
And the Spirit Catches You and You Fall Down, August 2001 (120 minutes)

Invited Lectures to Faculty and Trainees

1. Faculty and Trainee Seminars in Genomics (~15 trainees/faculty)
UNC Center for Genomics and Society
The NCGENES Project: A Binning Strategy for Incidental Findings and the Role of Penetrance, February 2013 (90 minutes)
The Fragile X Newborn Screening Project: Recruitment, Counseling, and Family Impact, January 2013 (20 minutes)
Ethical Implications of Using Student DNA in a Class, February 2012 (3 sessions).

2. Boot Camps in Genetics, UNC Center for Genomics and Society
Faculty and Trainees (~15 trainees/faculty)
Advances in Genetic Testing, February 2011 (50 minutes)
Advanced Clinical Genetics, November 2010 (50 minutes)
Genetic Principles, September 2010 (50 minutes)

3. Social Genomics Retreat, UNC Carolina Center for Genome Sciences
Faculty in ELSI Research (30 faculty)
Cultural and Family Interpretations of Genetic Knowledge, March 2002 (50 minutes)

Other Invited UNC Seminars

1. Current Topics in Medical and Human Genetics
Faculty, Fellows, Residents and Students in Medical Genetics (20-30 participants)
Semi-annually: 1986- present

- *Genetic Information, Non-Discrimination, and Privacy Protections in Genetics*, April 2014

- *Evidence Based Genetic Counseling for Genome Sequencing,* October 2013
- *AGG Repeats Act as Anchors Stabilizing FMR1 Repeat Expansions*, February 2013
- *Rapid Whole-Genome Sequencing for Genetic Diagnosis in the NICU,* October 2012
- *Case Studies of Ethical Issues in Genome-Wide Arrays*, May 2012
- *Human Genome Sequencing*, January 2012
- *Results from NIH's ClinSeq Project*, October 2011
- *Communication of Genetic Risks in Families*, May 2011
- *Newborn Screening for Fragile X Syndrome Update*, December 2010
- *Genetic Research and Fragile X Newborn Screening,* June 2010
- *Genotype and Phenotypes in Sensorineural Hearing Loss,* October 2009
- *Prognosis: When "Good" News Is Interpreted as "Bad" News,* March 2009
- *The Phenotypes of Fragile X: Resolving the Paradoxes*, November 2008
- *The Evolution of the X and Y Chromosome,* May 2008
- *Wanting Babies Like Themselves*, December 2006
- *Translating Genomics into Educational Modules*, September 2006
- *Genetic and Clinical Features of Hearing Loss*, March 2003
- *Review of 10 Years of ELSI Research Conference*, March 2001
- *Clinical and Genetic Features of Ehrlers Danlos syndrome IV*, June 2000
- *Routine Assessment of Children with Mental Retardation*, February 2000

2. UNC Hospitals Cytogenetic Laboratory Seminar
Faculty and Staff from the Cytogenetic Laboratory (~ 20 participants)

- *Genetic Counseling and Newborn Screening for Fragile X Syndrome*, December 2010
- *Parents' Use of the Internet to Find Genetic Information*, May 2009
- *Ethical and Professional Issues in Genetics*, February 2002
- *Evaluation of Clinical Genetic Services*, June 2001
- *Using Prenatal Genetic Technology*, February 2001
- *Ethical Cases in Medical Genetics*, November 2000
- *GJB2 Testing in Hearing Loss,* July 2000
- *Genetic Testing in Adoption*, June 2000
- *Ethics of Genetic Testing in Children*, January 2000
- *Atypical cri du chat syndrome*, 1999
- *Clinical Cases with Cytogenetic Abnormalities*, November 1998

3. UNC Hospitals Molecular Diagnostic Laboratory Seminar
Faculty and Staff from the Molecular Genetics Laboratory (~ 20 participants)
Using AGG Anchors in FMR1 for Carrier Risk Counseling, December 2011
Genetic Testing for GJB2 Mutations, June 2000

4. UNC Hospitals Department of Medicine Grand Rounds
Faculty and Residents (~ 120 participants)

Renal Artery Stenosis and Neurofibromatosis, with Michael Swift, 1989 (20 minutes)
A Large Family with Idiopathic Thrombocytopenia, with Michael Swift, 1988 (20 minutes)

5. UNC School of Nursing Continuing Education
Public Health Nurses (~ 25 participants)
Women at Risk for Hereditary Breast and Ovarian Cancer, March 2005 (120 minutes)

6. UNC Odum Institute for Social Science
Faculty and Graduate Students in Social Sciences (~ 20 participants)
Genomics for Social Scientists, January 2009 (50 minutes)
Introduction to Genomics, September 2005 (50 minutes)

7. North Carolina Distance Education Advisory Board
Faculty (25 participants)
The Judicious Use of Multimedia in Education, June 2006 (50 minutes)

8. UNC Institute for Science Learning
Faculty and Staff (15 participants)
Genetic Principles for Analysis of Transcriptome module, March, 2006 (50 minutes)

9. UNC Frank Porter Graham Child Development Center
Faculty and Graduate Students (~ 15 participants) (120 minutes each)
Implications of Family History of Fragile X, April 2003
Diagnostic Methods in Pediatric Medical Genetics, December 2002
Parents' Internet Use to Find Genetic Information, March 2002
Principles of Medical Genetics, October 2001
Genetic Disorders, Genotype and Phenotype, April 2001
Medical Genetics and Genetic Counseling, September 2000

10. UNC Parr Center for Ethics, Institute for the Arts and Humanities, Department of Philosophy; Faculty Fellows (20 participants)
Teaching about Ethical Issues in Genetic Testing, October 2003 (120 minutes)

Invited Lectures: Outside UNC-Chapel Hill

Graduate Students in Genetic Counseling

University of North Carolina-Greensboro, Genetic Counseling Training Program
1. Medical Genetics Course, GEN 625
Second Year Students (8 students); **Annually, September 2001-2012**
Genetic Causes of Hearing Loss

2. Risk Calculations in Genetic Counseling, GEN 671
First Year students, (8 students); **Annually, May 2008-2012**
Principles of Probability in Genetic Counseling
Bayes Analysis in Autosomal Dominant Inheritance
Bayes Analysis in Autosomal Recessive and X-linked Inheritance
Communication of Risks and Clinical Applications of Bayes Analysis

3. Principles of Genetic Counseling, GEN 681
First Year Students (8 students)
Case Studies in Pediatric Genetic Counseling, March 2001 and 2008
Breast Cancer Training Module: Public Health Education, May 2004
Techniques Used in Pediatric Genetic Counseling, August 2001
Critique of Student Presentations, February 2001

Invited Lectures to High School Teachers

1. Partnership in Minority Advancement in Science
Summer Program for High School Biology Teachers, Chapel Hill, NC
North Carolina High School Biology Teachers (25 participants)
Ethical Issues in Genetic Testing, August 2010 (120 minutes)

2. Ethics & Leadership in America's Future Conference
North Carolina School of Science and Math, Durham, NC
High School students and their teachers (30 participants)
Ethical Issues in Genetic Testing of Children and Adolescents, March 2001 (120 minutes)

3. Ethics in Human Genetics Workshop
North Carolina High School Biology Teachers (~ 60 participants),
Chapel Hill, Boone, and Greenville, N.C.
Ethical Issues in Genetic Counseling, 1989-90 (180 minutes)

Invited Lectures to High School and Middle School Students

1. North Carolina School of Math and Sciences, Durham, N.C.
Advanced Biology Students (30 students)
Genetic Testing, April 1996
Ethical Issues in Genetic Counseling, October 1993 and1994
The Molecular Basis of Genetic Disorders, March 1991
Duchene Muscular Dystrophy: Genetic Testing and Counseling, March 1990
Genetic Screening, March 1989

2. Granville High School, Granville, N.C.
High School Biology Students (35 students)
Prenatal Diagnosis and Genetic Counseling, October, 1994

3. Githens Middle School, Durham, N.C.
8th Grade Biology Students (25 students)
Ethical Issues in Genetic Counseling, March 1993

4. McDougle Middle School, Carrboro, N.C.
7th Grade Science Students (25 studentS)
Microscopic Look at Cell Types, October 2000

Community Outreach

Covenant Place, Carrboro, N.C.
Senior citizens (25 participants)
Why Knowing Your Family History Is Important, January 2001 (60 minutes)

Jewish Screening Carrier Testing, Durham, N.C., 1998
Genetic Testing, 16 NC Public Health Departments and Private Obstetrical Practices, 1998

CLINICAL TEACHING:

UNC Hospitals Pediatric Genetics and Metabolism Clinic,
Genetic Counseling Student Clinical Rotations, Lead Genetic Counseling Supervisor (2004-2011), Clinical Supervisor (2000-2004)
UNC-Greensboro: 2000-2011, 4 students/year, 7 weeks each
Case preparation, communication of genetic information, and analysis of genetic test results, providing information and family support, advanced counseling techniques

University of Virginia Genetic Counseling Program and University of South Carolina Genetic Counseling Program: 1990-1995, 1-2/year, Prenatal Genetic Counseling Clinic

Clinical teaching, others: 1986-2000 and 2004-2012: fellows, residents, medical students
Administrative liaison for learners rotating in clinic, 2007-2011

University of North Carolina-Greensboro, Genetic Counseling Program Capstone Committee Chair
1. Hardy, M. "Increasing Undergraduates Awareness of Genetic Counseling", 2010-2011,
2. Toler, T. "Ethics and Genetic Research", 2008-2009
3. Gilmore, K. “Resources for Families of Children with Behavior Disturbances” 2002-2003,

Other Clinical Mentorships:
1. Brown, M. UNC medical student, summer clinical research project with NCGENES, developing patient educational materials, 2012-2014.
2. Hogan, K. UNC, Senior lecturer in Biology, Ethical Issues Using Student DNA, 2012
3. Sterling, R. UNC, Ph.D. in Health Policy, Direct to Consumer Genetic Testing, 2009

4. Funches, A. NCSU undergraduate, summer internship in Genetic Counseling, 2008.

GRANT FUNDED POSITIONS

1. 1U19HD077632-01 ($8,000,000)
Powell, Cynthia and Berg, Jonathan (co-PIs**)** 9/01/2013-8/31/2018
NCNEXUS, North Carolina Newborn Exome Sequencing as Universal Screening, NHGRI, NICHD, Departments of Pediatrics and Genetics

Project Goal: outline an interdisciplinary approach to identifying, confronting and overcoming the major challenges to implement genomic sequencing to enhance current newborn screening in a diverse pediatric population.

Role: Investigator: determine attributes affecting informed decision-making and develop best practices regarding return of results; develop novel decision support tools and evaluate use in parental decision making, 25% salary support (2013-present)

2. 2P50HG004488-06 ($5,000,000)
Henderson, Gail (PI) 6/01/2013-05/31/2018
Center for Excellence in ELSI Research (CEER)
NHGRI; Center for Genomics and Society

Project Goal: design and conduct a trial focusing on both highly penetrant rare mutations that place people at risk for preventable conditions.

Role: Investigator; advise on selection of genes suitable for screening, advise the development of educational and consent materials, 10% salary support (2013-present)

3. 1U01HG006487-01 ($6,400,000)
Evans, James (PI): 12/01/2011-11/30/2015
NCGENES: North Carolina Clinical Genomic Evaluation by NextGen Exome Sequencing
NHGRI; Carolina Center for Genome Sciences, Department of Genetics

Project Goal: to evaluate the clinical feasibility and utility of genomic sequencing and assess families understandings and responses to genomic information.

Role: Investigator; project manager; lead genetic counselor; develop patient educational materials; liaison between clinical, bioinformatics and ELSI projects; recruitment and clinical operations, 70% salary support (2012-present)

Previous Funding

1. 3P30HD003110-41S1 ($435,305)
Bailey, Donald (PI): 07/01/2008 – 06/30/2013
Family Adaptation to Newborn Screening for Fragile X Syndrome
NICHD, FPG Child Development Institute
Project Goal: to assess feasibility and parental response to fragile X newborn screening
Role: Investigator; provide genetic counseling/follow-up for screen positive families, 10% salary support (2008-2013)

2. 5P50HG004488-05 ($5,000,000)
Henderson, Gail (PI) 10/01/2007-09/30/2012
Center for Excellence in ELSI Research
NHGRI; Center for Genomics and Society
Project Goal: to support/promote research into ethical, legal and social issues in genetics
Role: Investigator; genetics educator for trainees, 10% salary support (2007-2013)

3. 1R21HD043616-01 ($430,000)
Bailey, Donald (PI): 7/1/2004-4/30/2007
Identifying Newborns for Fragile X: Planning Grant
NHGRI; Planning grant for a Center for Excellence in ELSI Research.
Project Goal: examine how information from large-sample genetic studies is used and disclosed in biomedical research and on newborn screening for fragile X syndrome.
Role: Investigator, genetic counseling advisor, 10% salary support (2004-2007)

4. 4R42HG002983-02 ($514,092)
Bollenbacher, W. 6/26/2003-5/8/2007
Genomics MediaBook for a Technology Society
NHGRI; Department of Biology
Project goal: create a Genomics MediaBook for undergraduate science majors
Role: lead content writer for Genomic MediaBook, 70% salary support (2005-2006)

5. MM-0645-04/04 ($250,000)
Powell, C (PI). 10/01/2003-09/30/2005
Genetic Services for Congenital Hearing Loss
Center for Disease Control/American Association of Medical Colleges

Project goal: Identify barriers to genetic services for children with congenital hearing los
Role: Project manager; designed interview protocol, interviewed families, designed survey, distributed to 105 families, assisted with data collection and analysis; 25% salary support (2004-2006).

6. Millikan, Robert (PI) 2003-2005 ($895,208)
Center for Genomics and Public Health
Center for Disease Control
North Carolina Center for Genomics and Public Health
Project goal: Provide education and awareness of genomics for the public health workforce
Role: genetic education advisor; designed educational module for public health nurses to recognize family histories consistent with hereditary cancer current recommendations for testing/management and how to refer for genetic services; 50% salary support (2003-2005)

7. 1 RO1 HG02164-01 ($650,000)
Skinner, D (PI) 8/1/2000 – 7/30/2003
Culture and Family Interpretations of Genetic Disorders
National Human Genetics Research Institute
Project goal: to assess how ethnically diverse families understand genetic information.
Role: Co-PI, recruited 105 ethnically diverse families, trained ethnographers in genetics, analyzed quantitative and qualitative data; 50% salary support (2000-2003)

CLINICAL SERVICE, GENETICS and METABOLISM CLINIC (1993-2011)

Certified Genetic Counselor: Pediatric Genetics and Metabolism Clinic
Participate in the care of families referred for genetic evaluation and counseling: obtain and interpret medical, genetic, and family histories, provide genetic counseling and education, interpret and explain genetic test results, and serve as patient advocate.

PROFESSIONAL SERVICE

Editorial Appointments (2008-present)

Invited Editorial Board Member, Journal of Genetic Counseling (2008-2013)
Review 3-4 manuscripts/year; counseling/ethical issues
Invited Reviewer, Journal of Genetic Counseling (2008-present)
Review 2-3 manuscripts/year; counseling and ethical issues.
Invited Reviewer, Genetics in Medicine (2009-present)
Review 3-4 papers/year; counseling/ethical issues

Professional Committees:

Centers for Sequencing Exploratory Research (CSER) committees (2011-present)
Genetic Counselors Working Group
Pediatric Working Group

American Board of Genetic Counseling (ABGC) (2003-2011)

Practice Examination Super Committee, ABGC (2011)
Invited member, five-member committee to construct a 100-item practice examination

ABGC Certification Examination Committee, 10 members

Chair (2009-2010): Recruited 35 item writers, mentored 10 item writers (submitted 80 revised items) chaired meetings and conferences, constructed 2 forms of the exam; reported to the board; created/narrated two training modules.

Member (2008-2011): Mentored item writers; revised items for final version and reviewed performance; collaborated on final scoring and determining the final cut score.

Invited item writer, 2003-2011

National Society of Genetic Counselors (NSGC): 1986-present

NSGC Committees:
Genetic Counseling and Clinical Sequencing, 2011-present
Defining the Elements of Genetic Counseling for Payors, 2011
Ethics Sub-committee, invited, 2000-03
Public Health and Genomics, 2003
Genetic Counselors in Research, 2002-2003
Abstract Selection, 2000
Mentoring Students, 2000
Professional Education, 1999

UNC-G Genetic Counseling Program Committees:
External Advisory Committee, 1999-present
Program Re-accreditation, Outcomes Study 2011
Chair, Program Accreditation, Curriculum Study, 2005

Other Memberships:
Chromosome Deletion Outreach Advisory Board, 2003-2010
American College of Medical Genetics, founding associate member, 1993-present
International Society of Nurses in Genetics, member, 2004-2007

Association of Professors of Human/Medical Genetics, member, 1986-2004
American Society of Human Genetics, member, 1986-2000
North Carolina Medical Genetics Association, member, 1986-present

Committees: University of North Carolina-Chapel Hill
Faculty Search Committee, board-eligible genetic counselor for NCGENES, 2013
UNC Bioethics Center, member, 2010-present
Faculty Search Committee, two board-eligible genetic counselors, chair, 2008-2009
Biomedical Institutional Board, member, 2006-2012
Fixed term Faculty Committee, School of Medicine, member, 2006-2009
Diversity Committee, Department of Pediatrics, member, 2006-2008
Faculty Search Committee, Nursing/Genetics, member, 2003
Faculty Search Committee, board-eligible genetic counselor, co-chair, 2000
Faculty Search Committee, board-eligible genetic counselor, member, 1999
Undergraduate Human Genetics Minor, co-chair, 2002-2005
1st/2nd Yr. Med. School Course Directors Committee, member, 1986-2002

Administrative Service: Division of Genetics and Metabolism: (2008-2011)
Oversee the Division's clinic schedule and assign genetic counselors to cases

Faculty Supervision and Mentoring: (2008-2011)
Director of Pediatric Genetic Counseling Services:
Supervised and mentored genetic counselors to promote faculty development.

CONSULTING
Subject matter expert for DNA Direct/Medco Health Solutions, Inc. (2011-2013).
Laboratory and Therapeutic Committee, Medco Health Solutions, Inc. (2010-2013).
Clinical Tools, Inc. Web-Based Curriculum for Med Students, consultant, 2001-4
National Coalition for Professional Education in Genetics, 2001
Center for Disease Control, reviewer, "Genetics in Clinical Practice: A Team Approach", 1999

Continuing Education:
Spanish Language:
Spanish Language Program, IMAC, 10 hours, Guadalajara, Mexico, 2012
Spanish Language Program, Level 3, 16 hours, Carrboro, NC, 2011
Spanish Language Program, Level 2, 16 hours, Carrboro, NC, 2011
Spanish Language Program, IMAC, 10 hours, Guadalajara, Mexico, 2011
Spanish Language Program, Level 1, 16 hours, Carrboro, NC, 2011
Beginning Spanish Language Program, Durham Tech Community College, 15 weeks, 2009
Spanish for Health Professionals, UNC Hospitals, 10 hours, 2009

Other Courses:
Faculty Writing Program, Faculty Development Office, UNC, 2014

Personalized Medicine in the 21st Century, RTI and NC Biotech Ctr, 2010
Multimedia Boot camp, UNC School of Journalism, 1 week, 2006
Writing from the Reader's Perspective, UNC 1 week, 2005
Ethical and Legal Considerations for Genetic Research, NIEHS, RTP, NC, 1 day, 2004
Write Winning Grants: Workshop for Biomedical Research Faculty, UNC, 2 days, 2002
Medical & Experimental Mammalian Genetics, Jackson Lab, Bar Harbor, ME., 2 weeks, 2001
Methods in Clinical Research and Responsible Conduct of Research, UNC. 2 weeks, 2000
Short Course in Qualitative Research in Genetic Counseling, Oakland, CA., 2 days, 1999

CURRICULUM VITAE
Karen E. Weck, M.D.

PERSONAL INFORMATION

Name: **Karen Elizabeth Weck**
Home Address: 4221 Pleasant Green Rd.
Durham, NC 27705
Home Phone: 919-309-9636

EDUCATION AND TRAINING

1993-1995	Research Fellow, Department of Pathology, Washington University School of Medicine, St. Louis, Missouri
1991-1993	Chief Resident in Laboratory Medicine Washington University School of Medicine, St. Louis, Missouri
1988-1991	Resident in Laboratory Medicine Washington University School of Medicine, St. Louis, Missouri
1986-1987	Research Internship, Laboratory of Molecular Microbiology National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland
1984-1988	Duke University School of Medicine, Durham, NC M.D. awarded May 8, 1988
1980-1984	Duke University, Durham, North Carolina B.S. in Zoology, *cum laude,* 1984

Specialty certification

2003	Molecular Genetic Pathology (American Board of Pathology/ American Board of Medical Genetics)
1993	Clinical Pathology (American Board of Pathology)

PROFESSIONAL EXPERIENCE

2010-present	Clinical Professor of Pathology and Laboratory Medicine Clinical Professor of Genetics (joint appointment) Director of Molecular Genetics Associate Director of Molecular Pathology University of North Carolina School of Medicine, Chapel Hill, NC

2007-2013	Associate Director UNC Institute of Pharmacogenomics and Individualized Therapy
2004-2010	Associate Professor of Pathology and Laboratory Medicine Director of Molecular Genetics Associate Director of Molecular Pathology University of North Carolina School of Medicine, Chapel Hill, NC
2008-2010	Associate Professor of Genetics (joint appointment) University of North Carolina School of Medicine
1999-2004	Assistant Professor of Pathology and Assistant Director Molecular Diagnostics, University of Pittsburgh Medical Center, Pittsburgh, PA
1995-1998	Research Instructor in Pathology, Washington University School of Medicine St. Louis, Missouri

HONORS AND AWARDS

Philip M. Blatt Award for Excellence in Resident Training in Clinical Pathology	2008
Young Investigator Award, Academy of Clinical Physicians and Scientists	1996
Clinician Investigator Award (K08), NIAID, NIH	1995
American Cancer Society Physician Research Training Award	1995
Young Investigator Award, Academy of Clinical Physicians and Scientists	1992
Young Investigator Award, Academy of Clinical Physicians and Scientists	1991
National Institute of Neurological Disorders and Stroke Post Doctoral Fellowship	1990
Early Identification Program Acceptance to Duke Medical School	1985

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Refereed Primary Articles:

1. Berg JS, Foreman AK, O'Daniel JM, Booker JK, Boshe L, Carey T, Crooks KR, Jensen BC, Juengst ET, Lee K, Nelson DK, Powell BC, Powell CM, Roche MI, Skrzynia C, Strande NT, Weck KE, Wilhelmsen KC, Evans JP. A semiquantitative metric for evaluating clinical actionability of incidental or secondary findings from genome-scale sequencing. Genet Med. 2015 Aug 13 [Epub ahead of print]. PMID: 26270767
2. Allred SC, Weck KE, Gasim A, Mottl AK. Phenotypic heterogeneity in females with X-linked Alport syndrome. Clin Nephrol. 2015 Aug 7 [Epub ahead of print]. PMID: 26249550

3. Shirts BH, Salama JS, Aronson SJ, Chung WK, Gray SW, Hindorff LA, Jarvik GP, Plon SE, Stoffel EM, Tarczy-Hornoch PZ, Van Allen EM, Weck KE, Chute CG, Freimuth RR, Grundmeier RW, Hartzler AL, Li R, Peissig PL, Peterson JF, Rasmussen LV, Starren JB, Williams MS, Overby CL. CSER and eMERGE: current and potential state of the display of genetic information in the electronic health record. J Am Med Inform Assoc. 2015 Jul 3 [Epub ahead of print]. PMID: 26142422

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5. NL Couser, MM Masood, NT Strande, A K M. Foreman, K Crooks, KE Weck, M Lu, K C Wilhelmsen, M Roche, JP Evans, JS Berg, C M Powell. The Phenotype of Multiple Congenital Anomalies-Hypotonia-Seizures Syndrome 1: Report and Review. Am J Med Genet A. 2015 Apr 29. [Epub ahead of print]. PMID: 25920937

6. Lee K, Berg JS, Milko L, Crooks K, Lu M, Bizon C, Owen P, Wilhelmsen KC, Weck KE, Evans JP, Garg S. High Diagnostic Yield of Whole Exome Sequencing in Participants with Retinal Dystrophies in a Clinical Ophthalmology Setting.Am J Ophthalmol. 2015 Apr 21. [Epub ahead of print] PMID: 25910913

7. Hertz DL, Snavely AC, McLeod HL, Walko CM, Ibrahim JG, Anderson S, Weck KE, Magrinat G, Olajide O, Moore S, Raab R, Carrizosa DR, Corso S, Schwartz G, Peppercorn JM, Evans JP, Jones DR, Desta Z, Flockhart DA, Carey LA, Irvin WJ Jr. In Vivo Assessment of the Metabolic Activity of CYP2D6 Diplotypes and Alleles. Br J Clin Pharmacol. 2015 Apr 24. [Epub ahead of print]. PMID: 25907378

8. Lee JA, Lee CR, Reed BN, Plitt DC, Polasek MJ, Howell LA, Cicci JD, Tasca KE, Weck KE, Rossi JS, Stouffer GA. Implementation and evaluation of a CYP2C19 genotype-guided antiplatelet therapy algorithm in high-risk coronary artery disease patients. Pharmacogenomics. 2015 Mar;16(4):303-13. PMID: 25823779

9. Lyon E, Schrijver I, Weck KE, Ferreira-Gonzalez A, Richards CS, Palomaki GE. Molecular Genetic Testing for Cystic Fibrosis: Laboratory Performance on the College of American Pathologists External Proficiency Surveys, Genet Med 2014 Jul 31. [Epub ahead of print] PMID: 25077647

10. Shapiro AJ, Weck KE, Chao KC, Rosenfeld M, Nygren AOH, Knowles MR, Leigh MW, Zariwala MA. Cri du Chat syndrome and primary ciliary dyskinesia: a common genetic cause on chromosome 5p. J Pediatr. 2014 Oct;165(4):858-61. PMID:25066065

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14. Richards CS, Palomaki GE, Lacbawan FL, et al; CAP/ACMG Biochemical and Molecular Genetics Resource Committee. Three-year experience of a CAP/ACMG methods-based external proficiency testing program for laboratories offering DNA sequencing for rare inherited disorders. Genet Med. 2014 Jan;16(1):25-32. PMID: 23703682
15. Trembath DG, Shaheen NJ, O'Neill S, Weck K, Greene KG. Metastatic melanoma in an esophagus demonstrating Barrett esophagus with high grade dysplasia. BMC Res Notes. 2013 Nov 13;6:457. PMID: 24220097
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17. Tarczy-Hornoch P, Amendola L, Aronson SJ, Garraway L, Gray S, Grundmeier RW, Hindorff LA, Jarvik G, Karavite D, Lebo M, Plon SE, Van Allen E, Weck KE, White PS, Yang Y. A survey of informatics approaches to whole-exome and whole-genome clinical reporting in the electronic health record. Genetics in Medicine 2013; 15:824-832. PMID:24071794
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49. Weck, K.E., Speck, S.H., and Virgin, H.W. Macrophages Are the Major Reservoir of Latent Murine Gammaherpesvirus 68 in Peritoneal Cells. Journal of Virology 73:3273-3283, 1999.

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Review Articles and Editorials:

1. Palomaki GE, Ashwood ER, Weck KE. A flawed challenge but valid recommendation: a response to Takoudes and Hamar. Ultrasound Obstet Gynecol. 2015 Jan;45(1):117. PMID: 25557844

2. N Aziz, Q Zhao, L Bry, DK Driscoll, B Funke, JS Gibson, WW Grody, MR Hegde, GA Hoeltge, D. G.B. Leonard, JD Merker, R Nagarajan, LA Palicki, RS Robetorye, I Schrijver, KE Weck, and KV Voelkerding. College of American Pathologists' Laboratory Standards for Next Generation Sequencing Clinical Tests. Arch Pathol. Lab Med Aug, 2014 [epub ahead of print].

3. I Schrijver, N Aziz, LJ Jennings, S Richards, KV Voelkerding, KE Weck. PERSPECTIVES: Methods-Based Proficiency Testing in Molecular Genetic Pathology. J Mol Diagn. 2014 May;16(3):283-7. PMID:24650895

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Richards KL, Roederer MW, Valgus JM, Riper M, Vernon JA, Zamboni WC, Wagner MJ, Walko CM, Weck KE, Wiltshire T, McLeod HL. Institutional profile. UNC Institute for Pharmacogenomics and Individualized Therapy: interdisciplinary research for individual care. Pharmacogenomics 11(1):13-21, 2010.

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8. Langley M and Weck KE. Role of the clinical laboratory in personalized medicine: challenges and opportunities. Personalized Medicine 5(1):1-4, 2008.
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Book Chapters*:*

1. Jane W. Kimani and Karen E. Weck. Alport Syndrome in Schrijver I. (ed), Diagnostic Molecular Pathology in Practice: A Case-Based Approach, Springer, New York, NY, October 2011, pp.9-15.
2. Kenneth L. Muldrew and Karen E. Weck. Pharmacogenetics in Schrijver I (ed), Diagnostic Molecular Pathology in Practice: A Case-Based Approach, Springer, New York, NY, October 2011, pp. 65-72.
3. Karen Weck. Detection of Resistance to Therapy in Hematolymphoid Neoplasms in Dunphy C, ed. Molecular Pathology of Hematolymphoid Diseases, Springer pub. 2010, pp 165-172.
4. Claudio Mosse and Karen Weck. Molecular Pathology of Burkitt Lymphoma in Dunphy C, ed. Molecular Pathology of Hematolymphoid Diseases, Springer pub. 2010, Chapter 23, pp 277-286.
5. Jennifer Morrissett, Karen Weck, and Cherie Dunphy. Techniques to Detect Defining Chromosomal Translocations/Abnormalities in Dunphy C, ed. Molecular Pathology of Hematolymphoid Diseases, Springer pub. 2010, Chapter 9, pp 129-152.

Published/Proffered Abstracts:

1. N Chahin, et al. “Mutations in MEGF10 in a patient presenting with late onset myopathy and respiratory distress.” American Academy of Neurology 67th Annual Meeting, Washington, DC, April 18 - 25, 2015.
2. Plon, SE, Shirts, B, Salama, J, Aronson, S, Chung, WK, Freimuth, R, Hindorff, LA, Li, Rogling, Overby, C, Starren, J, Tarczy-Hornoch, O, Weck, KE, Williams, MS,

Van Allen, E. “Display of Genetic Information in the Electronic Health Record: From Varied Chaos to Everything in Its Place”. ACMG Annual Meeting, Salt Lake City, Utah, March 24-28, 2015. **(Platform presentation)**

3. DL Hertz, AC Snavely, HL McLeod, CM Walko, JG Ibrahim, S Anderson, KE Weck, P Rubin, O Olajide, S Moore, RRaab, DR Carrizosa, S Corso, G Schwartz, JM Peppercorn, JP Evans, Z Desta, DA Flockhart, LA Carey, WJ Irvin Jr. CYP2D6 intermediate metabolizers includes patient groups with distinct metabolic activity. San Antonio Breast Cancer Symposium (SACBS), San Antonio, TX, December 9-13, 2014.
4. NM Patel, CJ Civalier, ML Gulley, JK Booker, KE Weck. Validation of the Illumina TruSight Tumor Panel for Clinical Testing of Somatic Mutations in Solid Tumors. Association of Molecular Pathology Annual Meeting, Washington, D.C., Nov 2014. J Mol Diagn 2014; 16(6): 747.
5. A Treece, C Civalier, L Dodd, N Patel, M Gulley, K Weck. Cytology Smears as a Source of DNA for Next-generation Sequencing of Solid Tumors. Association of Molecular Pathology Annual Meeting, Washington, D.C., Nov 2014. J Mol Diagn 2014; 16(6): 746.
6. Aziz, N., Zhao, Q., Durtschi, J., Bry, L., Driscoll, Funke, B.H., Gibson, J.S., Grody, W.W., Hedge, M.R., Leonard, D., Merker, J., Nagarajan, R., Palicki, L.A., Robetorye, R.S., Santani, A.B., Schrijver, I. Weck, K.E., Voelkerding, K.V. A Pilot Methods-Based Proficiency Test for Next Generation Sequencing (NGS). Association of Molecular Pathology Annual Meeting, Washington, D.C., Nov 2014. J Mol Diagn 2014; 16(6): 775.
7. Z Hu, Y Li, A Treece K Weck, M Gulley. Verification of the Nanostring Prosigna breast cancer prognostic signature assay in a clinical laboratory. Association of Molecular Pathology Annual Meeting, Washington, D.C., Nov 2014. J Mol Diagn 2014; 16(6): 748.
8. B. C. Powell, A. K. M. Foreman, J. M. O'Daniel, K. Lee, L. Boshe, K. R. Crooks, M. Lu, Z. Fan, J. K. Booker, K. E. Weck, J. P. Evans, J. S. Berg. Look before you leap, and list before you look: the use of a priori curated gene lists to guide exome analysis. American Society of Human Genetics Annual Meeting, San Diego, CA, October 18-22, 2014. (Platform presentation)
9. N. T. Strande, C. Bizon, J. K. Booker, K. R. Crooks, A. K. M. Foreman, G. T. Haskell, M. A. Hayden, K. Lee, M. Lu, L. Milko, J. M. O'Daniel, P. Owen, B. C. Powell, C. Skrzynia, C. R. Tilley, A. Treece, D. Young, K. C. Wilhelmsen, K. E. Weck, J. S. Berg, J. P. Evans. Exploring the diagnostic yield of whole exome sequencing in a broad range of genetic conditions: the first 200 cases in the NCGENES study. American Society of Human Genetics Annual Meeting, San Diego, CA, October 18-22, 2014. (Platform presentation)
10. A. K. M. Foreman, J. K. Booker, L. Boshe, K. R. Crooks, J. P. Evans, B. C. Jensen, K. Lee, D. K. Nelson, J. M. O'Daniel, B. C. Powell, C. M. Powell, M. I. Roche, C. Skrzynia, N. T. Strande, K. E. Weck, K. C. Wilhelmsen, J. S. Berg. Clinical actionability of incidental findings: application of a semiquantitative metric to assess actionability of over 1200 genes. American Society of Human Genetics Annual Meeting, San Diego, CA, October 18-22, 2014.

11. G. T. Haskell, B. C. Jensen, D. S. Marchuk, C. Skrzynia, C. Bizon, K. Wilhelmsen, K. E. Weck, J. P. Evans, J. S. Berg. Whole Exome Sequencing Reveals Rare, Truncating Variants in Nuclear Envelope Genes are Present in a Large Subset of Cardio-Genetic Patients. American Society of Human Genetics Annual Meeting, San Diego, CA, October 18-22, 2014.
12. K. Lee, D. S. Marchuk, M. J. Friez, C. Bizon, D. Young, D. Gillis, P. Owen, K. Wilhelmsen, K. E. Weck, S. Garg, J. P. Evans, J. S. Berg. Analysis of Whole Exome Datasets to Test the Hypothesis of Digenic Inheritance in Stargardt Disease. American Society of Human Genetics Annual Meeting, San Diego, CA, October 18-22, 2014.
13. JA Lee, CR Lee, BN Reed, DC Plitt, JD Cicci, KE Tasca, KE Weck, GA Stouffer. Implementation and evaluation of a *CYP2C19* genotype-guided antiplatelet therapy algorithm in high-risk coronary artery disease patients. ACCP Annual Meeting, Austin, TX, October 15, 2014.
14. Hertz DL, Snavely A, Evans JP, Ibrahim JG, Anderson S, Friedman K, Weck KE, Rubin P, Olajide O, Moore S, Raab R, Carrizosa DR, Corso S, Schwartz G, Peppercorn JM, Graham ML, Canale ST, McLeod HL, Carey LA, Irvin Jr WJ. Does increasing tamoxifen dose to 40 mg/day in patients with diminished CYP2D6 activity increase toxicity? American Society of Clinical Oncology Annual Meeting, May 30-June 3 2014.
15. NM Patel, DA Eberhard, DG Trembath, KA Kaiser-Rogers, KW Rao, ML Gulley, WK Funkhouser, LB Thorne, JS Parker, J Grilley-Olson, NE Sharpless, DN Hayes, KE Weck. Verification and Reporting of Somatic Mutation Results from Massively Parallel Sequencing in a Clinical Setting: The UNCseq Experience. Association of Molecular Pathology Annual Meeting, Phoenix, AZ, Nov 2013. J Mol Diagn 2013; 15(6): 903.
16. Aziz, N., Durtschi, J., Zhao, Q., Bry, L., Driscoll, Gibson, J., Grody, W., Hedge, M., Hoeltge, G., Leonard, D., Merker, J., Palicki, L., Robetorye, R.S., Schrijver, I. Weck, K., Hambuch, T., Harkins, T., Ballinger, D. and Voelkerding, K. Development of a Methods-Based Proficiency Test for Next Generation Sequencing (NGS). American Society of Human Genetics Annual Meeting, Boston, MA, October 2013.
17. KR Sampat, AM Deal, N Patel, K Weck, B O'Neil. Comparing KRAS mutation testing by allele specific primary extension methods versus pyrosequencing in colorectal cancer specimens. American Society of Clinical Oncology Annual Meeting, 2013
18. M Lu, E Runge, A Mottl, KE Weck. Genetic testing for Steroid Resistant Nephrotic Syndrome in an Ethnically Diverse Population. American College of Medical Genetics Annual Meeting, Phoenix, AZ, March 20-23, 2013.
19. KR.Crooks, JL Crooks, C Bizon, J Berg, JP Evans, J Booker, KE Weck. A SNP-based genotyping method for identity confirmation of whole exome sequencing analysis. American College of Medical Genetics Annual Meeting, Phoenix, AZ, March 20-23, 2013.
20. K Lee, K Crooks, L Milko, M Lu, C Biaon, P Owens, KE Weck, JP Evans, JS Berg, S Garg. Investigating whole exome sequencing as a diagnostic test for hereditary

retinal disorders. American College of Medical Genetics Annual Meeting, Phoenix, AZ, March 20-23, 2013

21. Foreman AKM, Berg JS, Roche MI, Weck KE, Wilhelmsen KC, Evans JP. A Year of NCGENES: North Carolina Clinical Genomic Evaluation by NextGen Exome Sequencing. American College of Medical Genetics Annual Meeting, Phoenix, AZ, March 20-23, 2013.
22. O'Neill SS, Chao DC. Gulley ML, Booker JK, Weck KE. PIK3CA mutation detection by three methods: TrimGen Mutector II, Qiagen qBiomarker somatic mutation PCR array, and Sanger sequencing. Association of Molecular Pathology Annual Meeting, Long Beach, CA, Oct 24-27, 2012. J Mol Diagn 2012; 14(6):701.
23. Crooks K, Weck KE, Booker J. Validation of X-inactivation ratio analysis using the AR and SLITRK4 loci. Association of Molecular Pathology Annual Meeting, Long Beach, CA, Oct 24-27, 2012. J Mol Diagn 2012; 14(6):645
24. Aziz N., Bry L., Driscoll D., Gibson J., Grody W., Hedge M., Hoeltge G., Kant J., Leonard D., Merker J., Palicki L., Robetorye R., Schrijver I. Weck K., Voelkerding K. College of American Pathologists' laboratory standards for next generation sequence clinical testing. Association of Molecular Pathology Annual Meeting, Long Beach, CA, Oct 24-27, 2012. J Mol Diagn 2012; 14(6):742.
25. Hu Z, Li Y, Fan C, Weck K, Perou CM, Gulley ML. Validation of PAM50 breast cancer intrinsic subtypes using Nanostring nCounter gene expression assay. Association of Molecular Pathology Annual Meeting, Long Beach, CA, Oct 24-27, 2012. J Mol Diagn 2012; 14(6):647.
26. Lu M, Mottl A, Fine C, and Weck K. Identification of a novel TRPC6 mutation in a family with minimal change disease illustrates the phenotypic heterogeneity associated with heritable forms of nephrotic syndrome. American College of Medical Genetics Annual Meeting, March 28-20, 2012.
27. Crooks K, Berg J, Booker J, Evans J, Foreman K, Weck K. Separating the wheat from the chaff: A gene-based approach for prioritizing analysis of genetic variants in diagnostic whole-exome sequencing in NCGENES. American College of Medical Genetics Annual Meeting, March 28-20, 2012.
28. Foreman AK, Adams M, Lee K, Weck K Evans J, Booker J, Berg K. Implementation of a computational algorithm to assist in the diagnostic interpretation of next-generation sequencing data in a cohort with suspected hereditary breast cancer susceptibility. American College of Medical Genetics Annual Meeting, March 28-20, 2012.
29. Berg J, Wilhelmsen K, Weck K, Roche Y, Henderson G, Evans J. NCGENES: North Carolina Clinical Genomic Evaluation by NextGen Exome Sequencing. American College of Medical Genetics Annual Meeting, March 28-20, 2012.
30. Weck KE, Zehnbauer B, Datto M, Schrijver I. Molecular Genetic Testing for Fragile X Syndrome: Laboratory Performance on the College of American Pathologists Proficiency Surveys (2001-2009). Association of Molecular Pathology Annual Meeting, Dallas, TX, November 17-19, 2011. J Mol Diagn 2011;13(6):713.
31. Chao K and Weck KE. Validation of cytochrome p450 2D6 genotyping by the Luminex xTAG® 2D6 v3 IVD/LX200 detection system. Association of Molecular

Pathology Annual Meeting, Dallas, TX November 17-19, 2011. J Mol Diagn 2011;13(6):772.

32. Irvin WJ, Weck KE, Walko CM, Chiu WK, Rubin P, Olajide OA, et al. Comprehensive CYP2D6 genotyping in a multiracial population shows differences in allele frequencies between races. American Society of Clinical Oncology Annual Meeting, June 3-7, 2011.
33. Dharmavaram J, Stouffer GA , Cammarata MW , Weck KE, Muldrew KL, Walko CM, Gabriel DA, Rossi JS. Outpatient Screening with VerifyNow and CYP2C19 Genotype to Identify Patients at Risk for Clopidogrel Resistance. Journal of the American College of Cardiology April 2011; 57(14) Suppl1: E1207.
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35. Wheeler F, Powell C, Roche M, Booker J, Weck K, and Kaiser-Rogers K. Unsuspected variant Turner syndrome karyotype in a patient with hemihyperplasia and developmental delay. Proceedings of the 18th American College of Medical Genetics Annual Meeting, March 2011.
36. Muldrew KL, Chao K, Civalier C, Blanpain F, Datto MB, Gulley ME, Trembath Y, Weck KE, and Booker JK. Pyrosequencing for Isocitrate Dehyrogenase Mutations in Brain Tumors. Association for Molecular Pathology Annual Meeting, San Jose, CA Nov. 18-20, 2010. J Mol Diagn 2010; 12(6): 904.
37. Fan H, Vadlamudi K. Wang Y, Muldrew K, Chao KC, Naski MC and Weck KE. Detection of Cytochrome P450 2C19 (*CYP2C19)* Genetic Variants Associated with Clopidogrel Response Using a TaqMan Allelic Discrimination Method. Association for Molecular Pathology Annual Meeting, San Jose, CA Nov. 18-20, 2010. J Mol Diagn 2010; 12(6): 859.
38. Berg JS, Zariwala MA, Leigh MW, Evans JP, Knowles MR, and Weck KE. Investigation of Massively Parallel Targeted Exome Sequencing for Clinical Diagnostic Testing and Gene Discovery in Primary Ciliary Dyskinesia. Association for Molecular Pathology Annual Meeting, San Jose, CA Nov. 18-20, 2010. J Mol Diagn 2010; 12(6): 862.
39. Lu M, Mottl A, and Weck K. Molecular genetic testing of podocin *(NPHS2)* mutations in steroid-resistant nephrotic syndrome by high resolution melting curve analysis. American Society of Human Genetics Annual Meeting, Washington DC, November 2-6, 2010.
40. Berg JS, Weck KE, Leigh MW, Knowles MR, Evans JP, Zariwala MA. Investigation of Massively Parallel Targeted Exome Sequencing for Clinical Diagnostic Testing and Gene Discovery in Primary Ciliary Dyskinesia. American Society of Human Genetics Annual Meeting, Washington DC, November 2-6, 2010.
41. J Davies, D Trembath, A Deal, WK Funkhouser, BF Calvo, T Finnegan, K Weck, JE Tepper, BH O'Neil. Relationship between k-Ras and b-Raf status with pAKT, pERK, and outcomes in patients with rectal cancer. American Society of Clinical Oncology 2010 Gastrointestinal Cancers Symposium, Orlando, FL, January 22-24, 2010.
42. M Lu, P Gipson, A Mottl, KE Weck. Mutation scanning by high resolution melting curve analysis (HRMA) to detect mutations associated with focal segmental

glomerulosclerosis (FSGS). Association for Molecular Pathology Annual Meeting, Orlando, FL, Nov 19-22, 2009. J Mol Diagn 2009; 11(6): 617.

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47. D Trembath, K Chao, J Booker, A Fleming, J Spoon, K Weck, M Gulley. Macro-dissection for Tumor Enrichment from Formalin Fixed Paraffin Embedded (FFPE) Tissue for Downstream Molecular Testing. Association for Molecular Pathology Annual Meeting, Orlando, FL, Nov 19-22, 2009. J Mol Diagn 2009; 11(6): 677.
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49. Irvin WJ, Walko CM, Dees EC, et al. Validating CYP2D6 Genotype-guided Tamoxifen Therapy for a Multiracial U.S. Population. San Antonio Breast Cancer Symposium Abstract # 410, Cancer Res 2009.
50. Irvin WJ, Carey LA, Olajide O, et al: Comprehensive CYP2D6 testing in a multiracial U.S. breast cancer population. American Society of Clinical Oncology Annual Meeting, Abstract # 553, 2009.
51. Irvin WJ, Carey LA, Moore S, et al: Patients' attitudes towards a CYP2D6 genotyping study. American Society of Clinical Oncology Breast Cancer Symposium, Abstract # 205, 2009.
52. Irvin WJ, Peppercorn JM, Dees EC, et al: The relationship between CYP2D6 genotype and endocrine symptoms in women taking tamoxifen. American Society of Clinical Oncology Breast Cancer Symposium, Abstract # 238, 2009.
53. Irvin WJ, Carey LA, Olajide L, Dees EC, Peppercorn J, Chiu WK, Walko CM, McLeod HL, Evans JP, and Weck KE . Comprehensive CYP2D6 genotyping in a multiracial USA breast cancer population. American Society of Clinical Oncology Annual Meeting. Orlando, FL, May 29-June 2, 2009. *Merit Awardee*
54. Powell CM, Kimani J, Buchman C, Booker J, Weck K. Etiology in Congenital and Early Onset Hearing Loss. Proceedings of the 16th American College of Medical Genetics Annual Meeting. Tampa, FL, March 25-29, 2009.

55. Zariwala M, Chao K, Langley M, Leigh M, Booker J, Knowles M, Weck K. Clinical Molecular Genetic Testing for Primary Ciliary Dyskinesia. American Society of Human Genetics Annual Meeting, Philadelphia, PA, November 11-15, 2008.
56. Langley M, Booker J, Weck KE. Evaluation of the AutoGenomics INFINITI CYP2D6I Assay. Journal of Molecular Diagn November 2008; 10:574.
57. Ferrell P, McLeod H, Jonas D, Evans J, Valgus J, Deverka P, Weck K. Evaluating the clinical utility of pharmacogenomic markers: a SMARTDRUG review. Journal of Molecular Diagn November 2008; 10:575. *Abstract selected for platform presentation.*
58. Kimani J, Booker J, Powell C, Buchman C, Weck, K. Congenital CMV infection and hearing loss. Journal of Molecular Diagn November 2008; 10:591.
59. Weck K, Langley M, Booker J, McLeod H. Implementing VKORC1/CYP2C9 genotyping for warfarin dosing in a clinical molecular genetics laboratory. Proceedings of the 15th annual American College of Medical Genetics Annual Meeting March 2008, p. 172.
60. Kimani J, Zariwala M, Civalier C, Booker J and Weck K. Detection of the mtDNA 12s rRNA 1555A>G mutation in hearing loss by melting curve analysis on the LightCycler. Proceedings of the 15th annual American College of Medical Genetics Meeting March 2008, p. 167.
61. Langley M, Booker J, McLeod H, Santos C, Lange L, Susswein L, Weck KE. Pharmacogenomic testing in a clinical laboratory: technical and interpretative challenges in implementing VKORC1/CYP2C9 testing for warfarin dosing. Journal of Molecular Diagn November 2007; 9:660.
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63. Chao KC, Zariwala MA, Langley MR, Warren C, Knowles MR, and Weck KE. Mutation Scanning for Mutations associated with Primary Ciliary Dyskinesia by High-Resolution Melting Analysis. Journal of Molecular Diagn November 2007; 9:653.
64. Rasmussen K, Singh Z, Langley MR, Booker J, Kaiser-Rogers K, Rao K, and Weck KE. Detection of Acquired Gleevec Resistance in CML: report of interesting cases. Journal of Molecular Diagn November 2007;9:667.
65. Singh Z, Rasmussen K, and Weck KE. HFE gene mutation testing for hereditary hemochromatosis: evaluation of clinical practice and the appropriate use of testing. Journal of Molecular Diagn November 2007;9:654.
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67. C Civalier, J Booker, J Sailus, M Gulley and K Weck. Detection of T-cell Receptor Gamma rearrangement by PCR and Capillary Electrophoresis. Association for Molecular Pathology Annual Meeting, Orlando, FL, November 2006.
68. J Sailus, J Booker, H Parker, A Fleming, M Gulley, and K Weck. Contamination of DNA Samples Extracted on the MagnaPure LC. Association for Molecular Pathology Annual Meeting, Orlando, FL, November 2006.

69. M Langley, J Booker and K Weck. Validation of Testing for Gleevec Resistance Mutations in an Academic Medical Center. Association for Molecular Pathology Annual Meeting, Orlando, FL, November 2006.

70. M Langley, M Zariwala, J Booker, M Leigh, M Knowles and K Weck. Incorporation of Sequence Analysis as a Diagnostic Tool in Primary Ciliary Dyskinesia. Journal of Molecular Diagn November 2006; 8:630.

71. Betz SL, Booker JK,Weck KE, and Farber RA. Validation of PCR Amplification and Capillary Electrophoresis for Accurate Sizing of Fragile X CGG Repeats. American Society of Human Genetics Annual Meeting, Salt Lake City, Utah, June, 2005.

72. Scanga, L., et al. Diagnosis of Congenital Cytomegalovirus Infection by PCR of Dried Blood Spots on Perinatal Cards. Association for Molecular Pathology Annual Meeting, Los Angeles, CA, November 2004. The Journal of Molecular Diagnostics 6(4): 422, 2004.

73. Melan MA, Weck KE, and Kant JA. Comparison of Three Methods for Determination of HCV Genotype. Association for Molecular Pathology Annual Meeting, Los Angeles, CA, November 2004. The Journal of Molecular Diagnostics 6(4): 426, 2004.

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75. Im, L.T., Kowalski, R.P., Mah, F.S., and Weck, K. The use of PCR for Diagnosis of Atypical VZV Ocular Disease. Proceedings of the Association for Research in Vision and Ophthalmology Annual Meeting, 2003.

76. Vats, A., Shapiro, R., Randhawa, P., Pattison, J., Hilton, R., Beattie, J., Gonwa, T., Mathias, R., Brewer, J., Green, M., Weck, K., and Scantelbury, V. BK Virus Associated Nephropathy And Cidofovir: Long Term Experience. Proceedings of the American Transplant Society Congress Annual Meeting, Washington, DC, May 2003.

77. Randhawa,P., Ho, A., Vats, A., Shapiro, R. and Weck, K. Quantitative measurement of BK virus load in urine and plasma of renal transplant patients: correlation with biopsy findings. Proceedings of the American Transplant Society Congress Annual Meeting, Washington, DC, May 2003.

78. Paulus-Thomas, J.E., Weck, K.E., and Kant, J.A. Utility and Challenges using an Online Database to Monitor Quality Improvement in the Clinical Molecular Diagnostics Laboratory. Association for Molecular Pathology Annual Meeting, Dallas, TX, November, 2002. The Journal of Molecular Diagnostics, 4(4): 260, 2002.

79. Weck, K. J. Ahmad, and O. Shakil. HCV Genomic Diversity and Outcome after Liver Transplantation. Association for Molecular Pathology Annual Meeting, Philadelphia, PA, November, 2001. The Journal of Molecular Diagnostics, 3(4): 204, 2001.

80. Suzow, J., A. Vats, B. Crum, and K. Weck. Development of a Quantitative PCR assay for detection of BK and JC viruses. Association for Molecular Pathology Annual Meeting, Philadelphia, PA, November, 2001. The Journal of Molecular Diagnostics, 3(4): 202, 2001.

81. Ahmad, J., K. Weck, and O. Shakil. Correlation of HCV genomic diversity with outcome after liver transplant. The American Association for the Study of Liver Diseases: New Therapeutic Strategies for Hepatitis C, Chicago, IL, June 15-16, 2001.

82. Kowalski RP, Suzow J, Karenchak LM, Romanowski EG, Weck KE, and Gordon YJ. Laboratory diagnosis of Ocular Adenovirus Infection: Is there really one best test? Invest Ophthalmol Vis Sci 2001;42(4,suppl):3106.

83. Vats AN, Saxena M, Gautam A, Ellis D, Randhawa PS, Shapiro R, Scantelbury V, Vivas C, Yodakamalla R, and Weck KE. Development of Quantitative PCR for BK Virus Detection in Urine And Its Role In Management of Allograft Viral Infection Masquerading As Acute Rejection. American Society of Transplantation, Chicago, IL, May, 2000.

84. Hofgaertner WT Kant JA and Weck KE Hepatitis C Virus Quantitation: Optimization of Testing Strategies for Low Level Viremia. Association for Molecular Pathology Annual Meeting, St. Louis, MO, November 4-7, 1999. The Journal of Molecular Diagnostics, 1(1): 50, 1999.

85. Chen Z, Kant JA, and Weck KE. Comparison of INNO-LiPA and NS5B Region Sequencing for Hepatitis C virus Genotype Determination. Association for Molecular Pathology Annual Meeting, St. Louis, MO, November 4-7, 1999. The Journal of Molecular Diagnostics, 1(1): 50, 1999.

86. Weck KE, Kim SS, Virgin HW, and Speck SH.. B Cells Are Important For Regulating γHV68 Latency. 24th International Herpesvirus Workshop, July, 1999.

87. Weck KE, Kim SS, Speck SH and Virgin HW. Macrophages Are A Major Reservior of γHV68 Latency. 24th International Herpesvirus Workshop, July, 1999.

88. Dal Canto, A.R., K.E. Weck, J.D. Gould, A.K. O'Guin, K.A. Roth, J.E. Saffitz, S.H. Speck, and H.W. Virgin. γHV68 Induce Arteritis: A Model for Herpesvirus Induced Vascular Disease. American Society for Virology 17th Annual Meeting, July, 1998.

89. Weck, K.E., H.W Virgin, and S.H. Speck. B Cells Are Important For Clearance of Murine γHV68 Latency in vivo. American Society for Virology 17th Annual Meeting, July, 1998.

90. Gendelman, H.E., Leonard, J., Weck, K.E., Rabson, A.R., Capon, Martin, M.A., and Ostrove, J. Herpesviral Transactivation of the Human Immunodeficiency Virus (HIV) Long Terminal Repeat Sequence. Third International Conference on AIDS, Washington, DC, June, 1987.

91. Weck, K.E., Clouse, Gendelman, H.E., Ostrove, J.M., Folks, T., and Rabson, A.R. Regulation of HIV Long Terminal Repeat (LTR) Activity. Third International Conference on AIDS, Washington, DC, June, 1987.

Invited Seminars and Presentations:

1. "Whole Exome Sequencing: Opening the floodgates," Next Generaton Dx Summit, Washington, DC, August 19-20, 2015

2. "Whole Exome Sequencing: Opening the floodgates," UNC Department of Pathology and Laboratory Medicine CME event, Chapel Hill, NC, May 2, 2015

3. "Pharmacogenomics," Duke School of Medicine Department of Genetics, April 8, 2015

4. “Laboratory Performance Revealed: 10 years of CAP Molecular Genetics Proficiency Testing Surveys,” Association for Molecular Pathology annual meeting, Washington, DC, November 13, 2014.
5. “Diagnosing Genetic Diseases Using Exome Sequencing,” Personalized Diagnostics Virtual Conference, American Association of Clinical Chemistry, October 29, 2014.
6. “NextGen Sequencing: clinical applications and research discovery at UNC,” Duke/UNC Melanoma Retreat, David Thomas Center, Durham NC, September 12, 2014.
7. “Clinical genomic-based research at UNC,” UNC Hematology/Oncology Scientific Retreat, Rizzo Center, Chapel Hill, NC, September 5, 2014.
8. “Clinical Molecular Testing for Secondary Drug Resistance in Cancer,” Next Generaton Dx Summit, Washington, DC, August 22, 2013.
9. “Genetic Testing in the Era of Personalized Medicine,” North Carolina society of Pathologists Annual Meeting, Asheville, NC, April 5, 2013.
10. “Laboratory Performance on Molecular Genetic Proficiency Testing,” College of American Pathologists Workshop, American College of Medical Genetics Annual Meeting, Phoenix, AZ, March 19, 2013.
11. “Clinical Genomic Testing in the Era of Personalized Medicine,” Speck-tacular Gammaherpesvirus Research Symposium, Department of Microbiology and Immunology & Emory Vaccine Center, Emory University, February 16, 2013.
12. “Advances in Molecular Diagnostic Cancer Testing,” Next Generation Diagnostics Summit, Washington, DC, August 20, 2012
13. “Application of Pharmacogenomic Technologies in the Clinical Laboratory,” 2nd Latin American Pharmacogenomic and Personalized Medicine Congress, Rio deJaneiro, Brazil, June 28-29, 2012.
14. “Pharmacogenomic Testing to Direct Clinical Therapy at UNC,” Gentris Corporation, Research Triangle Park, Morrisville, NC, December 15, 2011
15. “Genetic Testing Principles Applied to Case Studies: BRCA1 and BRCA2 Analysis & Cystic Fibrosis Mutation Analysis” Association for Molecular Pathology Outreach Course, Dallas, TX, November 16, 2011.
16. “Pharmacogenomic Testing to Individualize Cancer Therapy,” Next Generation Diagnostics G2 Summit, Washington, DC, August 22, 2011.
17. *“*Anecdotes of Success in Personalized Medicine: Pharmacogenomics,” Association for Pathology Chairs Annual Meeting, Monterey, California, July 13-15, 2011.
18. “Pharmacogenetic testing”, Duke School of Medicine Department of Genetics, June 29, 2011
19. “CYP2D6 Genotyping to Guide Use of Tamoxifen in Breast Cancer,” 21st International Congress of Clinical Chemistry and Laboratory Medicine, Berlin, Germany, May 15-19, 2011.
20. “Pharmacogenomics in the Clinical Laboratory,” G2 Intelligence Conference on Molecular Diagnostics, Boston, MA, April 13-15, 2011

21. “Case Studies in Molecular Genetics: Cystic Fibrosis Mutation Analysis and BRCA1/2 Analysis”, Association for Molecular Pathology Outreach Course, San Jose, CA, November 17, 2010.
22. “CYP2D6 Genotyping to Guide use of Tamoxifen in Breast Cancer”, Symposium on Pharmacogenomics in Clinical Practice, American Association of Clinical Chemistry annual meeting, Anaheim, CA, July 29, 2010.
23. “The Multidisciplinary Approach to Personalized Medicine: Fitting together the pieces of the ‘P-4’ Puzzle (Predictive, Preventive, Personalized, and Participatory)”. Invited panelist, Conference on Personalized Medicine in the 21st Century, RTI International and the North Carolina Biotechnology Center, Durham, NC, June 15, 2010.
24. “Pharmacogenetic testing for individualized therapy” American Association of Clinical Chemistry, Capital Section Annual Spring Meeting, Richmond, VA, May 13th, 2010
25. “Genetic Testing for Personalized Medicine – *Are we there yet*?” North Carolina Medical Genetics Association Annual Meeting, Asheville, NC, April 16, 2010.
26. “Pharmacogenetic testing”, Duke School of Medicine Department of Pathology, June 2009
27. “ABL1 Kinase Mutation Analysis”, Invited speaker and panel participant, Association of Molecular Pathology Annual Meeting Workshop, Dallas, TX, Nov 2, 2008
28. “GeneticTesting Principles Applied to Case Studies: Cystic Fibrosis, BRCA1/2, and DNA Sequencing Analysis”, College of American Pathologists Annual Meeting, San Diego, CA, September 30, 2008.
29. “Genetic Testing for Identity, Medical Diagnosis and Forensic Analysis”, International Judicial Academy Conference in Mendoza, Argentina and Santiago Chile, May, 2008
30. “The Right To Health”, Speaker and Panel Participant, International Judicial Academy conference in Buenos Aires, Argentina, May 2008
31. “Genetic Testing for Primary Ciliary Dyskinesia”, Annual Update in Clinical and Laboratory Medicine, Park City, Utah, March 3-7, 2008
32. “Pharmacogenetic Testing for Warfarin Response”, Annual Update in Clinical and Laboratory Medicine, Park City, Utah, March 3-7, 2008
33. “Mutation detection in the clinical laboratory: the new frontier of genomic medicine”, UNC Department of Genetics Research Colloquium, February 13, 2008
34. “Implementation of a Program to Aid in Warfarin Dosing”, Invited speaker and panel discussant, Association for Molecular Pathology Annual Meeting Workshop, Los Angeles, CA, November 10, 2007
35. “Genetic Testing Principles Applied to Case Studies: Cystic Fibrosis, BRCA1/2, and DNA Sequencing Analysis”, College of American Pathologists Annual Meeting, Chicago, IL, October 2, 2007.
36. “Genetic Testing for Focal Segmental Glomerulosclerosis”, 22nd Annual Meeting of the Glomerular Disease Collaborative Network, Chapel Hill, NC, May 19-20, 2007

37. “Individualized Therapy”, Session Chair, 2nd Annual Chapel Hill Drug Conference: Pharmacogenomics, May 18, 2007
38. “Molecular Classification of Cancer: Practical Applications for the Surgical Pathologist”, Organizer and Moderator, AMP companion meeting, USCAP Annual meeting, San Diego, CA, March 2007
39. “Development and Quality Control of Sequencing Assays,” Workshop Moderator and Presentation of an Unusual Case, Association for Molecular Pathology Annual Meeting, Orlando, FL. November 2006
40. “New Applications of Mutation Detection in Diagnostic Medicine,” UNC Department of Pathology and Lab Medicine Annual Research Symposium, Chapel Hill, NC, September, 2006
41. “An Interesting Fragile X Case,” Genetic Puzzlers Workshop, Association for Molecular Pathology Annual Meeting, Scottsdale, AZ, November 2005
42. “Development and validation of real-time PCR assays for viral load monitoring: CMV and BK viruses,” Workshop Moderator, Association for Molecular Pathology Annual Meeting, Los Angeles, CA, November 2004.
43. “Quantitative PCR for Diagnosis and Monitoring of BK Virus Post-transplant Nephropathy,” Academy of Clinical Laboratory Physicians and Scientists Annual Meeting, Denver, CO, June 2004.
44. “Molecular Testing of Antibiotic Resistance,” Workshop Moderator, Association for Molecular Pathology Annual Meeting, Orlando, FL, 2003.
45. “Macrophages are a Major Reservoir of γHV68 Latency,” 24th International Herpesvirus Workshop, 1999
46. “B cells are important for clearance of murine γHV68 latency *in vivo,”* American Society for Virology 17th Annual Meeting, 1998
47. Workshop on Gammaherpesviruses, 22nd International Herpesvirus Workshop, 1997
48. Workshop on Gammaherpesviruses, 21st International Herpesvirus Workshop, 1996
49. Academy of Clinical Laboratory Physicians and Scientists Annual Meeting, 1996
50. XVIII Symposium of the International Association for Comparative Research on Leukemia and Related Diseases, Kyoto, Japan, 1995
51. Academy of Clinical Laboratory Physicians and Scientists Annual Meeting, 1992
52. Academy of Clinical Laboratory Physicians and Scientists Annual Meeting, 1991

Interviews

Medical genetics labs shine in 10-year proficiency test data. CAP Today, January 19, 2015.

Genomics and Personalized Medicine in Pathology at the University of North Carolina, USCAP TV, March 2012.

An Immense New Power to Heal: the Promise of Personalized Medicine, by Lee Gutkind and Pagan Kennedy, In Fact Books, Publishers Group West, Berkeley, CA, March 2012.

Next Generation Sequencing in the Clinical Laboratory. CAP Today, April 2011.

Experts split on need for greater FDA oversight of diagnostic tests. Elsevier Global Medical News, MD consult www.mdconsult.com. March 4, 2009.

PGx tests for warfarin dosing – how soon? CAP Today, January 2009, Feature story

Too fast or too slow on PGx testing? CAP Today, March 2008. C*over story*.

Pharmacogenomics enables more targeted treatment. Cancer Lines, UNC Lineberger Comprehensive Cancer Center Newsletter, Spring, 2007.

Are drug companies stalling the pharmacogenomic revolution? Diagnostic Testing and Technology Report. June 2004, cover story.

Welcoming resistance tests, old and new. CAP Today, May 2004. *Cover story.*

TEACHING RECORD

Research Advisory Committees and Mentorships

2011-2014	PhD Thesis Advisory committee, Vindhya Kunduru, University of North Carolina/North Carolina State University Joint Dept. of Biomedical Engineering
2007-2009	Research Advisor/ Mentor for Brent Ferrell MSIII, UNC School of Medicine Holderness Distinguished Medical Scholar
2001	Faculty mentor for Dr. Jawad Ahmad, Clinical Fellow in Gastroenterology and Hepatology, University of Pittsburgh, to conduct research investigating diversity in hepatitis C and correlation with clinical outcome in orthoptic liver transplantation. *~25 contact hours.*

Lectures/ small group teaching

2014-2015	"Molecular Genetic Testing", 1st year Medical Student Course, Principles of Medicine, University of North Carolina School of Medicine *1 contact hour, ~150 students*
2014	Small Group Learning lab preceptor for 1st year Medical Student Course, Principles of Medicine, University of North Carolina School of Medicine. *2 contact hours, ~20 medical students.*
2010-present	"Pharmacogenetics," Medical Genetics course for clinical genetic fellows and residents, UNC School of Medicine
2009-present	"Pharmacogenomic Testing in the Clinical Laboratory," DPET 832 Introduction to Applied Pharmacogenomics, UNC Eshelman School of Pharmacy Graduate course. *2 contact hours*
2007-present	Lecture "Translating Genetics to Clinical Medicine," Translational Pathology and Laboratory Medicine Graduate Course, University of North Carolina. *2 contact hours*

2005-present Director of Molecular Case Conference (monthly conference) presented by fellows in molecular pathology, University of North Carolina School of Medicine

2005-present Attending supervision and teaching of clinical fellows in Molecular Genetic Pathology and Clinical Molecular Genetics, University of North Carolina

2005-present Molecular Diagnostics/Cytogenetics course for Pathology residents and fellows, University of North Carolina School of Medicine. Lectures and Workshops, *8-10 contact hours.*

2006-2015 Lecture "Pharmacogenetics" for 2nd year Medical Student Course in Reproductive Biology and Genetics, University of North Carolina School of Medicine *1 contact hour, ~150 students*

2006-2013 Small Group Learning lab preceptor for 2nd year Medical Student Course in
Reproductive Biology and Genetics, University of North Carolina School of Medicine. *5 contact hours, ~20 medical students.*

2011 "Pharmacogenomic Testing to Predict Response to Cancer Therapy," Genomics in Society course, UNC School of Nursing, April 8, 2011.

2010-2011 Course Director, "Current Applications of Molecular Pathology: Real time updates and case studies," Association of Molecular Pathology Outreach Course, November 17, 2010 San Jose, CA and November 16, 2011, Dallas, TX

2010-2011 "Pharmacogenomics in Clinical Practice," Fourth year Medical Student Basic Science Elective, UNC School of Medicine

2007-2011 Small Group Learning lab preceptor for 1st year Medical Student Course in Cell and Molecular Biology: Molecules to Cells, University of North Carolina School of Medicine. *6 contact hours, ~20 medical students.*

2010 "Genetic Testing in the Era of Personalized Medicine, Genomics in Society Course, UNC Nursing and Undergraduate, October 19, 2010, *2 contact hours*

2007-2009 "Genetic Testing Principles Applied to Case Studies: Cystic Fibrosis, BRCA1/2, and DNA Sequencing Analysis", Half Day Course, College of American Pathologists Annual Meeting.

2006-2007 Lecture "Dominant Negative Mutations" to Genetics Residents and Fellows, University of North Carolina

2006 Lecture "Molecular Genetics" to graduate students, Methods in Pathology course, University of North Carolina

2000-2004 Course Director for Continuing Topics in Laboratory Medicine Series for Residents in Pathology, University of Pittsburgh Medical Center, Department of Pathology

1998-2004 Attending supervision and teaching of clinical residents and fellows in Pathology rotating on the Molecular Diagnostics Service. University of

Pittsburgh Medical Center, Department of Pathology*. ~200 contact hours per year.*

2004 Lecture on "Emerging Infectious Diseases: West Nile Virus and SARS." Molecular Pathogenesis of Infectious Disease Course, MSI, University of Pittsburgh School of Medicine. *1 contact hour, ~150 first year medical students*

2000-2004 Lectures on "Introduction to Virology," "Antiviral Therapy," and "Blood/transplant borne infections," Molecular Pathogenesis of Infectious Disease Course, 1st year medical students, University of Pittsburgh School of Medicine. 5 *contact hours, ~150 first year medical students per year*

2001-2004 Problem Based Learning lab preceptor for Molecular Pathogenesis of Infectious Disease Course, 1st year medical students, University of Pittsburgh School of Medicine. *12 contact hours, ~10 first year medical students per year.* ***Received highest evaluation of all course preceptors in 2001.***

2001-2003 Lecture on "Hepatitis Viruses: Acute and Chronic Hepatitis" and proctor for student journal club, Molecular Pathobiology Course (Graduate Level), University of Pittsburgh School of Medicine. *2 contact hours, 12 Graduate students*

2001-2003 Faculty Mentor for MSI Journal Club, University of Pittsburgh School of Medicine. *4 contact hours, ~20 first year medical students per year.*

2000-2003 Lectures on "Hepatitis C: molecular testing in diagnosis and management" and "Clonal Analysis of Hematolymphoid Disorders," Didactic Lecture Series for Residents in Pathology, University of Pittsburgh School of Medicine. *2 contact hours, ~10 pathology residents per year*

2001 Lectures on "Human Cytomegalovirus molecular biology" and "Animal herpesviruses as models." Advanced Topics in Herpesviruses Course (Graduate Level), University of Pittsburgh School of Medicine. *2 contact hours, 12 graduate students*

2001 Problem Based Learning lab preceptor for Molecular and Human Genetics course, MSI, University of Pittsburgh School of Medicine. Covered Down Syndrome, Prader-Willi, Huntington Disease, Fragile X Disease, and Neurofibromatosis*. 12 contact hours, 9 first year medical students*

1999-2001 Lecture "Hepatitis Viruses," Viral Pathogenesis Course, Dept. of Infectious Diseases and Microbiology, Graduate School of Public Health, University of Pittsburgh. *2 contact hours, ~15 graduate students per year*

1999 Course Director for Review Course in Molecular Diagnostics for Clinical Technologists, University of Pittsburgh School of Medicine, Division of Molecular Diagnostics.

1996	Markey Pathway Lecture Series, "EBV Vaccine strategies," Markey Graduate Students, Washington University School of Medicine, St. Louis, MO
1992-1996	Lecture on "Introduction to Clinical Medicine/ History and Physical Exam," to Occupational Therapy students, Washington University School of Medicine, St. Louis, MO
1991-1993	Course Director for Third Year Medical Student Lecture Series in Laboratory Medicine, Washington University School of Medicine, St. Louis, MO
1988-1993	Lecture on "Blood Components and Transfusion Reactions," Third Year Medical Student Laboratory Medicine Lecture Series, Washington University School of Medicine
1988-1990	Practical Lab Preceptor: "Blood and Urine Cultures," Second Year Medical Students, Washington University School of Medicine, St. Louis, MO

RESEARCH

Grant funding

1U19HD077632-01 (Cynthia Powell, PI)
NHGRI 9/05/13-8/31/2018 $6.1 million
NC NEXUS: North Carolina Newborn Exome Sequencing in Universal Screening
Co-investigator/ 5% effort

U01HG006487 (Karen Weck, PI)
NHGRI 12/05/11 – 11/30/15 $ 1.1 million (direct)
NC GENES: North Carolina Clinical Genomic Evaluation by NextGen Exome Sequencing
Co-Principle Investigator/ 15% Effort

UL1TR001111 (John Buse, Tim Carey, CoPIs)
Clinical Translational Science Award (CTSA) 2008-2118
NC Translational and Clinical Sciences (TraCS) Institute NIH/NCRR $7,352,734
NC TraCS Core Investigator/ 10% Effort

KG100355 (William Irvin, PI)
Susan G. Komen 05/18/10 – 05/17/13 $119,911
Validating CYP2D6 Genotype-guided Tamoxifen Therapy for a Multiracial U.S. Population
Co-Investigator/ 2.5% effort

Evaluating the Role of Genotype in Tamoxifen Therapy for Breast Cancer (PI: Lisa Carey)
UNC Lineberger Comprehensive Cancer Center, University Cancer Research Fund
Co-sponsors: LabCorp, Roche Molecular Diagnostics
Co-investigator/ 3% effort 2008-2010 UNC/UCRF

UNC Investments for the Future Grant (PI: James P Evans)
Closing the Gap- Bringing Genetics to Clinical Medicine
Co-Investigator / 10% effort 2007-2010 UNC $840,000

UNC Program in Translational Science (PI: Weck)
Development of a Translational Renal Genetics Program
Program Director/ 20% effort 2006-2008 UNC $230,000

NCBC 2006-MRG-1117 (PI Weck)
Development of Novel Diagnostic Testing for Primary Ciliary Dyskinesia
Multidisciplinary Research Grant (MRG)/North Carolina Biotechnology Center (NCBC)
Principal Investigator/ 10% effort 2006-2008 NCBC $250,000

Collaboration Education and Test Translation Program (CETT)/
NIH Office of Rare Diseases (PI: Zariwala, Weck)
Development of clinical genetic testing for primary ciliary dyskinesia
Principle co-investigator 2006-2007 NIH ORD $26,000

1U01DK060329
Multicenter Virahep-C Study of Viral Resistance to Antiviral Therapy of Chronic Hepatitis C
Consultant/ 3% effort 2001-2005 NIH

R01 AI51227-01 (PI: Randhawa)
Polyomavirus Infection After Kidney Transplantation
Co-Investigator/ 10% effort 2003-2005 NIH $175,000

1 RO3 DK 60619-01 (PI: Shakil)
Outcome of Hepatitis C Following Liver Transplantation
Consultant/ 3% effort 2003-2005 NIH $200,000

Roche Study
The Utility of MecA Gene PCR Assays for Detection of MRSA
Principle Investigator/ 10% Effort 2003-2005 Roche $25,000

50795K (K08 Award)
Regulation of the EBV Lytic Switch Gene BZLF1
Principle Investigator /100% effort 1995-1998 NIH

Inventions

2001 Method for detecting BK virus and related compositions (Co-inventor), licensed to Viracor laboratory, St. Louis, MO

PROFESSIONAL SERVICE

Membership In National Societies

American College of Medical Genetics (ACMG) Affiliate Specialist 2012-present

Fellow, College of American Pathologists (FCAP)	2005-present
American Society of Human Genetics (ASHG)	2004-present
Academy of Clinical Laboratory Physicians and Scientists (ACLPS)	1993-1998, 2006-present
American Association for the Advancement of Science (AAAS)	2000-2006
American Society of Microbiology (ASM)	1999-2005
Pan American Society for Virology (PASCV)	1998-2005
American Society for Virology (ASV)	1998-2005

Service on National Committees

2015-	Chair, Molecular Pathology Cluster and Council of Scientific Affairs member, College of American Pathologists
2014-present	Clinical and Laboratory Standards Institute (CLSI) Consensus Committee on Molecular Methods, Advisor
2012-present	Chair, Biochemical and Molecular Genetics Resource Committee, College of American Pathologists/ American College of Medical Genetics
2012-present	Chair, Pharmacogenetics Workgroup, College of American Pathologists
2011-present	Molecular and Clinical Genetics Devices Panel of the US FDA Medical Devices Advisory Committee
2014-2015	Nominating Committee, Association of Molecular Pathology Solid Tumors Subdivision (elected office)
2013	College of American Pathologists House of Delegates member (elected office)
2011-2013	Next Generation Sequencing Workgroup, College of American Pathologists
2009-2011	Chair, Training and Education Committee and Council Member, Association for Molecular Pathology (elected office)
2009-2010	Pharmacogenetics Workgroup, College of American Pathologists
2005-2010	Biochemical and Molecular Genetics Resource Committee, College of American Pathologists/ American College of Medical Genetics
2008	ABL Mutation Working Group, Association for Molecular Pathology Clinical Practice Committee
2007	Chair of the Program Committee and Council Member, Association for Molecular Pathology (elected office)
2006	Chair Elect of the Program Committee, Association for Molecular Pathology (elected office)

2004	Chair of Infectious Disease Subdivision and Council Member, Association for Molecular Pathology (elected office)
2003	Chair Elect of Infectious Disease Subdivision, Association for Molecular Pathology (elected office)
2000-2001	Association for Molecular Pathology Training and Education Committee, Infectious Disease Subdivision, (elected office)

Consultancies

2014-2015	Continuing Medical Education Advisory Committee, Laboratory Corporation of America Holdings (LabCorp)
2012-present	Consultant Advisory Panel, BlueCross BlueShield of North Carolina
2013	Advisory Board, Illumina Corporation
2010-2013	Consultant Laboratory Director, Gentris Corporation, Morrisville, NC
2006-2012	Advisory Board Member, Roche Molecular Diagnostics
2008-2010	Consultant to the FDA Molecular and Clinical Genetics Devices Panel of the Medical Devices Advisory Committee
2008-2010	Advisor, World Science Festival, New York City, NY http://www.worldsciencefestival.com
2008-2010	College of American Pathologists Liaison to Model Genetics Test Report Workgroup, RAND Corporation/ CDC-funded
2008-2010	Consultant Laboratory Director, ParagonDx, Morrisville, NC
2005-2006	Consultant, Third Wave Technologies
2004 –present	Ad Hoc consultant to McKenzie & Company, Gerson Lehrman Group, and Easton Associates

Editorial Boards

2013-present	American Journal of Pathology
2010-present	Expert Review of Molecular Diagnostics
2010-present	Genetics in Medicine, Associate Editor of Molecular Genetics and Pharmacogenetics
2009-2010	Genetics in Medicine, Associate Editor of Pharmacogenomics and Personalized Medicine
2006-present	Journal of Molecular Diagnostics

Other Service

2015	Reviewer for Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 6th ed. 2015 Elsevier Press

2013	American Medical Association CPT Molecular Pathology coding workgroups on Aortic Dysfunction/ Dilation and Hearing Loss
2003-present	Ad Hoc Molecular Laboratory Inspector, College of American Pathologists
1999-present	Ad Hoc Reviewer for Gene, Human Mutation, Journal of Virology, Journal of Clinical Virology, Molecular Diagnosis, Transplantation, Bone Marrow Transplantation, Molecular and Cellular Probes, Clinical Chemistry, Clinical & Diagnostic Laboratory Immunology, Clinica Chimica Acta, International Journal of Research in BioSciences, and Laboratory Medicine

University of North Carolina

2004 –present	Director Molecular Genetics, UNC Hospitals McLendon Clinical Laboratories
2010 -present	Department of Pathology and Laboratory Medicine Research Advisory Committee
2013-2015	Proposal review committee for UNC School of Medicine Translational Team Science Award (TTSA) program
2013-present	NC TraCS Institute/CTSA Translational Advancements Resource Committee
2013-present	Biospecimen Processing Facility Internal Advisory Board

Personal Information:

Name: Kirk Child Wilhelmsen, M.D., Ph.D.

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Home address: 321 Bayberry Dr.
Chapel Hill, NC 27517

Phone: 919-966-1373 (office)
919-969-2990 (home)

E-mail: kirk@med.unc.edu

Education:

Neurology Resident	Columbia-Presbyterian Medical Center Department of Neurology; 1987-91
Medical Intern	Columbia-Presbyterian Medical Center Department of Medicine; 1986-87
M.D.	University of Wisconsin, Madison; School of Medicine; 1986
Ph.D.	University of Wisconsin, Madison; Department of Molecular Biology; Advisor - Howard M. Temin, Ph.D.; 1984
B.S.	University of California, San Diego; Chemistry; 1978

Professional Experience:

Director Bioinformatics; Renaissance Computing Institute; 1/11-present

Domain Scientist for Biology and Genomics; Renaissance Computing Institute; 1/07-present

Member Neuroscience Center; 8/05 – present

Member Lineberger Comprehensive Cancer Center; 6/04 – present

Associate Professor of Genetics and Neurology; University of North Carolina at Chapel Hill; 2/04 – present

Member Carolina Center for Genome Science; 2/04 – present

Member Bowles Center for Alcohol Studies; 2/04 – present

Co-Director Bioinformatics Core TraCS Institute (UNC CTSA) 1/07-1/08[LU1][SH2]

Visiting Professor of Genetics; University of North Carolina at Chapel Hill; 10/03-2/04

Associate Professor of Neurology; University of California, San Francisco; 5/99-10/03

Assistant Professor of Neurology; University of California, San Francisco; 2/95-5/99

Assistant Professor of Neurology; Columbia University College of Physicians and Surgeons; 10/90-2/95

Postdoctoral Fellow, DANA Fellow and Keck Scholar; Columbia-Presbyterian Medical Center, Departments of Neurology and Psychiatry, Laboratory of Conrad Gilliam, Ph.D.; 1988-90

Honors and Awards:

2008	Carol Masters Schiller Scholar in Neurology
1999	Potamkin Prize for Neurology
1999	Decade of the Brain Lecture at the AAN
1990-94	Herbert Irving Assistant Professor of Neurology
1991	Klingenstein Fellow Award[LU3][SH4]
1986	Forester Award for Neurology
1984	Price Award for Cancer Research
1975	California State Scholarship

Bibliography:

Book Chapters:

Wilhelmsen,K.C. (2006). Frontotemporal dementia - current concepts. In Dementia and Motor Neuron Disease, M.J.Strong, ed. (Abingdon: Informa Healthcare Ltd), pp. 9-21.

Wilhelmsen,K.C., Clark,L.N., Geschwind,D.H., and Miller,B.L. (2000). Mutational analysis of tau in chromosome 17-linked dementia. In Fatal Attractions: Protein Aggregates in Neurodegenerative Disorders (Research and Perspectives in Alzheimer's Disease), V.M.Y.Lee, J.Q.Trojanowski, L.Buee, and Y.Christen, eds. (Berlin: Springer), pp. 19-28.

Clark,L.N. and **Wilhelmsen,K.C.** (1998). The genetics of Pick Complex and Adult-Onset Dementia. In Pick's Disease & Pick Complex, A.Kertesz and D.G.Munoz, eds. (New York: John Wiley & Sons, Inc.), pp. 269-280.

Wilhelmsen,K.C., Moskowitz,C.B., Weeks,D.E., Neystat,M., Nygaard,T.G., Clark,L., Dancoup,M., Sobrevega,E.E., Rosales,R., Gamez,G.L., Pacioles,O., Perez,M., and Fahn,S. (1998). Molecular genetic analysis of Lubag. In Dystonia 3, S.Fahn, C.A.Marsden, and M.R.DeLong, eds. (Philadelphia: Lippincott-Raven Publishers), pp. 341-348.

Wilhelmsen,K.C. (1998). Genetic interventions in geriatric neuropsychiatry. In American Psychiatric Press Textbook of geriatric neuropsychiatry, C.E.Coffey and J.L.Cummings, eds. (Washington, D.C.: American Psychiatric Press, Inc.), pp. 905-914.

Wilhelmsen,K.C. and Clark,L.N. (1998). Chromosome 17-linked dementias. In Neuropathology of Dementing Disorders, W.R.Markesbery, ed. (London: Edward Arnold Publsihers), pp. 170-180.

Wilhelmsen,K.C. (1997). Disinhibition-dementia-parkinsonism-amyotrophy complex (DDPAC) is a non-Alzheimer's frontotemporal dementia. In Advances in Research on Neurodegeneration, Y.Mizuno, M.B.H.Youdim, D.B.Calne, R.Horowski, W.Poewe, and P.Riederer, eds. (New York: Springer Wein), pp. 269-275.

Brzustowicz,L.M., **Wilhelmsen,K.C.**, and Gilliam,T.C. (1991). Genetic analysis of childhood-onset spinal muscular atrophy. In Amyotrophic Lateral Sclerosis, L.P.Rowland, ed. (New York: Raven Press), pp. 181-187.

Wilhelmsen,K.C., Tarpley,W.G., and Temin,H.M. (1984). Identification of some of the parameters governing transformation by oncogenes in retroviruses. In Cancer Cell. Vol. 2 Oncogenes and Viral Genes., (New York: Cold Spring Harbor Press), pp. 303-308.

Temin,H.M., Chen,I.S.Y., Watanabe,T., and **Wilhelmsen,K.C.** (1983). Evolution of retro-viruses. In Perspectives on Genes and the Molecular Biology of Cancer. A.H.Robbins and G.F.Saunders, eds. (New York: Raven Press), pp. 243-254.

Wilhelmsen,K.C., Chen,I.S., and Temin,H.M. (1983). The organization of c-rel in chicken and turkey DNAS. In Oncogenes and Retroviruses: Evaluation of Basic Findings and Clinical Potential., T.E.O'Connor and F.J.Jr.Raucher, eds. (New York: Alan R. Liss Inc.), pp. 43-56.

Refereed papers:

O'Bryant, S.E., Xiao, G., Barber, R., Cullum, C.M., Weiner, M., Hall, J., Edwards, M., Grammas, P., **Wilhelmsen, K.**, Doody, R., Diaz-Arrastia, R., Texas Alzheimer's, R., and Care, C. (2014). Molecular neuropsychology: creation of test-specific blood biomarker algorithms. Dement Geriatr Cogn Disord 37, 45-57.

Norden-Krichmar, T.M., Gizer, I.R., Libiger, O., **Wilhelmsen, K.C.**, Ehlers, C.L., and Schork, N.J. (2014). Correlation analysis of genetic admixture and social identification with body mass index in a Native American community. American journal of human biology : the official journal of the Human Biology Council 26, 347-360.

Fan, Z., Greenwood, R., Felix, A.C., Shiloh-Malawsky, Y., Tennison, M., Roche, M., Crooks, K., Weck, K., **Wilhelmsen, K.**, Berg, J., and Evans, J. (2014). GCH1 heterozygous mutation identified by whole-exome sequencing as a treatable condition in a patient presenting with progressive spastic paraplegia. J Neurol 261, 622-624.

Bizon, C., Spiegel, M., Chasse, S.A., Gizer, I.R., Li, Y., Malc, E.P., Mieczkowski, P.A., Sailsbery, J.K., Wang, X., Ehlers, C.L., and **Wilhelmsen, K.C.** (2014). Variant calling in low-coverage whole genome sequencing of a Native American population sample. BMC genomics 15, 85.

Szigeti, K., Lal, D., Li, Y., Doody, R.S., **Wilhelmsen, K.**, Yan, L., Liu, S., Ma, C., Texas Alzheimer, R., and Care, C. (2013). Genome-wide scan for copy number

variation association with age at onset of Alzheimer's disease. Journal of Alzheimer's disease : JAD 33, 517-523.

Gizer, I.R., Gilder, D.A., Lau, P., Wang, T., **Wilhelmsen, K.C.**, and Ehlers, C.L. (2013). Contributions of ethnicity to differential item functioning of cannabis abuse and dependence symptoms. J Stud Alcohol Drugs 74, 320-328.

Berg, J.S., Adams, M., Nassar, N., Bizon, C., Lee, K., Schmitt, C.P., **Wilhelmsen, K.C.**, and Evans, J.P. (2013). An informatics approach to analyzing the incidentalome. Genetics in medicine : official journal of the American College of Medical Genetics 15, 36-44.

Li, Y., Shaw, C.A., Sheffer, I., Sule, N., Powell, S.Z., Dawson, B., Zaidi, S.N., Bucasas, K.L., Lupski, J.R., **Wilhelmsen, K.C.**, Doody, R., and Szigeti, K. (2012). Integrated copy number and gene expression analysis detects a CREB1 association with Alzheimer's disease. Translational psychiatry 2, e192.

Gizer, I.R., Ehlers, C.L., Vieten, C., Feiler, H.S., Gilder, D.A., and **Wilhelmsen, K.C.** (2012). Genome-wide linkage scan of antisocial behavior, depression, and impulsive substance use in the UCSF family alcoholism study. Psychiatr Genet 22, 235-244.

Crowley, J.J., Adkins, D.E., Pratt, A.L., Quackenbush, C.R., van den Oord, E.J., Moy, S.S., **Wilhelmsen, K.C.**, Cooper, T.B., Bogue, M.A., McLeod, H.L., and Sullivan, P.F. (2012). Antipsychotic-induced vacuous chewing movements and extrapyramidal side effects are highly heritable in mice. The pharmacogenomics journal 12, 147-155.

Cabanski, C.R., Cavin, K., Bizon, C., Wilkerson, M.D., Parker, J.S., **Wilhelmsen, K.C.**, Perou, C.M., Marron, J.S., and Hayes, D.N. (2012). ReQON: a Bioconductor package for recalibrating quality scores from next-generation sequencing data. BMC Bioinformatics 13, 221.

Barber, R.C., Edwards, M.I., Xiao, G., Huebinger, R.M., Diaz-Arrastia, R., **Wilhelmsen, K.C.**, Hall, J.R., and O'Bryant, S.E. (2012). Serum granulocyte colony-stimulating factor and Alzheimer's disease. Dementia and geriatric cognitive disorders extra 2, 353-360.

Webb, A., Lind, P.A., Kalmijn, J., Feiler, H.S., Smith, T.L., Schuckit, M.A., and **Wilhelmsen, K.** (2011). The investigation into CYP2E1 in relation to the level of response to alcohol through a combination of linkage and association analysis. Alcohol Clin Exp Res 35, 10-18.

Shaw, C.A., Li, Y., Wiszniewska, J., Chasse, S., Zaidi, S.N., Jin, W., Dawson, B., **Wilhelmsen, K.**, Lupski, J.R., Belmont, J.W., Doody, R.S., and Szigeti, K. (2011). Olfactory copy number association with age at onset of Alzheimer disease. Neurology 76, 1302-1309.

O'Bryant, S.E., Xiao, G., Barber, R., Reisch, J., Hall, J., Cullum, C.M., Doody, R., Fairchild, T., Adams, P., **Wilhelmsen, K.**, Diaz-Arrastia, R., Texas Alzheimer's, R., and Care, C. (2011). A blood-based algorithm for the detection of Alzheimer's disease. Dement Geriatr Cogn Disord 32, 55-62.

O'Bryant, S.E., Xiao, G., Barber, R., Huebinger, R., **Wilhelmsen, K.**, Edwards, M., Graff-Radford, N., Doody, R., Diaz-Arrastia, R., Texas Alzheimer's, R., Care, C., and Alzheimer's Disease Neuroimaging, I. (2011). A blood-based screening tool for Alzheimer's disease that spans serum and plasma: findings from TARC and ADNI. Plos One 6, e28092.

Gizer, I.R., Ehlers, C.L., Vieten, C., Seaton-Smith, K.L., Feiler, H.S., Lee, J.V., Segall, S.K., Gilder, D.A., and **Wilhelmsen, K.C.** (2011). Linkage scan of alcohol dependence in the UCSF Family Alcoholism Study. Drug Alcohol Depend 113, 125-132.

Gizer, I.R., Ehlers, C.L., Vieten, C., Seaton-Smith, K.L., Feiler, H.S., Lee, J.V., Segall, S.K., Gilder, D.A., and **Wilhelmsen, K.C.** (2011). Linkage scan of nicotine dependence in the University of California, San Francisco (UCSF) Family Alcoholism Study. Psychol Med 41, 799-808.

Gizer, I.R., Edenberg, H.J., Gilder, D.A., **Wilhelmsen, K.C.**, and Ehlers, C.L. (2011). Association of alcohol dehydrogenase genes with alcohol-related phenotypes in a Native American community sample. Alcohol Clin Exp Res 35, 2008-2018.

Ehlers, C.L., Gizer, I.R., Gilder, D.A., and **Wilhelmsen, K.C.** (2011). Linkage analyses of stimulant dependence, craving, and heavy use in American Indians. Am J Med Genet B Neuropsychiatr Genet 156B, 772-780.

Webb, A., Lind, P.A., Kalmijn, J., Feiler, H.S., Smith, T.L., Schuckit, M.A., and **Wilhelmsen, K.** (2010). The Investigation into CYP2E1 in Relation to the Level of Response to Alcohol Through a Combination of Linkage and Association Analysis. Alcohol ClinExpRes 35, 10-18.

Gizer, I.R., Seaton-Smith, K.L., Ehlers, C.L., Vieten, C., and **Wilhelmsen, K.C.** (2010). Heritability of MMPI-2 scales in the UCSF family alcoholism study. J Addict Dis 29, 84-97.

Ehlers CL, G.I., Schuckit MA,**Wilhelmsen KC** (2010). Genome-wide scan for self-rating of the effects of alcohol (SRE) in American Indians. Psychiatr Genet.[LU5][SH6] 221-8

Ehlers, C.L., Phillips, E., Gizer, I.R., Gilder, D.A., and **Wilhelmsen, K.C.** (2010). EEG spectral phenotypes: heritability and association with marijuana and alcohol dependence in an American Indian community study. Drug Alcohol Depend 106, 101-110.

Ehlers, C.L., Gizer, I.R., Vieten, C., and **Wilhelmsen, K.C.** (2010). Linkage analyses of cannabis dependence, craving, and withdrawal in the San Francisco family study. Am J Med Genet B Neuropsychiatr Genet 153B, 802-811.

Ehlers, C.L., Gizer, I.R., Vieten, C., Gilder, D.A., Stouffer, G.M., Lau, P., and **Wilhelmsen, K.C.** (2010). Cannabis dependence in the San Francisco Family Study: age of onset of use, DSM-IV symptoms, withdrawal, and heritability. Addict Behav 35, 102-110.

Ehlers, C.L., Gizer, I.R., Vieten, C., Gilder, A., Gilder, D.A., Stouffer, G.M., Lau, P., and **Wilhelmsen, K.C.** (2010). Age at regular drinking, clinical course, and heritability of alcohol dependence in the San Francisco family study: a gender analysis. The American journal on addictions / American Academy of Psychiatrists in Alcoholism and Addictions 19, 101-110.

Ehlers, C.L., Gizer, I.R., Schuckit, M.A., and **Wilhelmsen, K.C.** (2010). Genome-wide scan for self-rating of the effects of alcohol in American Indians. Psychiatr Genet 20, 221-228.

Ehlers, C.L., Gizer, I.R., Phillips, E., and **Wilhelmsen, K.C.** (2010). EEG alpha phenotypes: linkage analyses and relation to alcohol dependence in an American Indian community study. BMC Med Genet 11, 43.

Lind, P.A., Eriksson, C.J., and **Wilhelmsen, K.C.** (2009). Association between harmful alcohol consumption behavior and dopamine transporter (DAT1) gene polymorphisms in a male Finnish population. Psychiatr Genet 19, 117-125.

Ehlers, C.L., Gilder, D.A., Gizer, I.R., and **Wilhelmsen, K.C.** (2009). Heritability and a genome-wide linkage analysis of a Type II/B cluster construct for cannabis dependence in an American Indian community. Addict Biol 14, 338-348.

Sherva, R., **Wilhelmsen, K.**, Pomerleau, C.S., Chasse, S.A., Rice, J.P., Snedecor, S.M., Bierut, L.J., Neuman, R.J., and Pomerleau, O.F. (2008). Association of a single nucleotide polymorphism in neuronal acetylcholine receptor subunit alpha 5 (CHRNA5) with smoking status and with 'pleasurable buzz' during early experimentation with smoking. Addiction 103, 1544-1552.

Ehlers,C.L., Lind, P.A., **Wilhelmsen, K.C.** (2008) Association between single nucleotide polymorphisms in the Mu Opioid receptor gene (*OPRM1*) and self reported responses to alcohol in Southwest California Indians. BMC Medical Genetics *9*, 35.

Lind,P.A., Eriksson, C.J.P and **Wilhelmsen, K.C.** (2008) The role of aldehyde dehydrogenase-1 (aldh1a1) polymorphism in harmful alcohol consumption in a Finnish population. Human Genomics *3,* 24-35.

Webb, A.E, Miller, B.L. Bonasera, S. Boxer, A., Karydas, A. **Wilhelmsen, K.C.** (2008) The Role of the *Tau* Gene Region Chromosome Inversion in PSP, CBD and related disorders. Archives of Neurology *65*, 1473-1478

Ehlers,C.L., Gilder,D.A., Slutske,W.S., Lind,P.A., and **Wilhelmsen,K.C.** (2008). Externalizing disorders in American Indians: Comorbidity and a genome wide linkage analysis. Am J Med. Genet B Neuropsychiatr. Genet. *147B,* 690-698.

Quinzii,C.M., Vu,T.H., Min,K.C., Tanji,K., Barral,S., Grewal,R.P., Kattah,A., Camano,P., Otaegui,D., Kunimatsu,T., Blake,D.M., **Wilhelmsen,K.C.**, Rowland,L.P., Hays,A.P., Bonilla,E., and Hirano,M. (2008). X-linked dominant scapuloperoneal myopathy is due to a mutation in the gene encoding four-and-a-half-LIM protein 1. Am J Hum Genet *82*, 208-213.

Ehlers,C.L., Slutske,W.S., Lind,P.A., and **Wilhelmsen,K.C**. (2007). Association between single nucleotide polymorphisms in the cannabinoid receptor gene

(CNR1) and impulsivity in southwest California Indians. Twin Res Hum Genet *10*, 805-811.

Ehlers,C.L., Wall,T.L., Corey,L., Lau,P., Gilder,D.A., and **Wilhelmsen,K.C.** (2007). Heritability of illicit drug use and transition to dependence in Southwest California Indians. Psychiatr. Genet *17*, 171-176.

Guo,G., **Wilhelmsen,K.C.**, and Hamilton,N. (2007). Gene-lifecourse interaction for alcohol consumption in adolescence and young adulthood: Five monoamine genes. Am J Med. Genet B Neuropsychiatr. Genet *144*, 417-423.

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Invited Presentations:

2012,2013,2014 Invited Speaker, NIDA Genetics Consortium, Bethesda MD[LU7][SH8]

2013 Invited Speaker and Conference Organizer, National Center for Data Science Conference on the data science of genomics, Chapel Hill, NC.

2012 Invited Speaker, UNC Center for Alcohol Studies, Chapel Hill NC

2012 Invited Speaker, Health Science Seminar Series, Fort Worth, TX.

2012 Invited Speaker, Systems Biology Seminar Series, Chapel Hill, NC.

2010 Invited Speaker, UNC Department of Computer Science Bioinformatics Seminar Series, Chapel Hill, NC.
2010 Invited Speaker, Bioinformatics Seminar Series, Blacksberg, VA.
2010 Invited Speaker, Baylor Alzheimer Center Seminar Series, Houston, TX
2010 Invited Speaker and Session Chair, International Conference on Alzheimer's Disease, Honolulu HI.
2008 Invited Speaker, Mt. Sinai Department of Genetics Seminar Series, NY
2008 Invited Speaker, UNC MD-PhD Colloquium Series, Chapel Hill NC
2008 Invited Speaker, UNC Center for Alcohol Studies, Chapel Hill NC
2008 Invited Speaker, UNC Center for Alcohol Studies, Chapel Hill NC
2007, 2005 Invited Speaker, Lineberger Comprehensive Cancer Center Cancer Genetic Seminar Series
2006 Invited Speaker, AAAS symposia on Tobacco susceptibility, St Louis, Mo
2006 Invited Speaker, 5th International Meeting on Frontotemporal Dementia, San Francisco, CA
2005 Invited Speaker, 11th Annual Duke Nicotine Conference, Durham NC
2005 Invited Speaker, UNC Genetics Symposia, Chapel Hill NC
2005 Invited Speaker, UNC Center for Alcohol Studies, Chapel Hill NC
2005 Invited Speaker, HapMap in Neurogenetics of Alcoholism Conference, Washington DC
2005 Invited Speaker, Molecular Genetics of Tau and the Frontotemporal Dementias, Genetics of Alzheimer's Disease and Related Disorders Conference, Dallas TX
2005 Invited Speaker, UNC Medical Genetics Conference, Chapel Hill NC
2004 Invited Speaker, Society for Research on Nicotine and Tobacco Meeting, Phoenix AZ
2004 Invited Speaker, Duke – UNC Cell Biology/Genetics Retreat, Wilmington. NC.
2004 Invited Speaker, Research Society on Alcoholism Annual Meeting
2004 Invited Speaker, Molecular Pathology Seminar, TX A&M
2004 Invited Speaker, Missouri Alcoholism Research Center's Fourth Guze Symposium St. Louis MO
2003 Invited Speaker, ALS Investigators Workshop, Philadelphia, PA
2003 Invited Speaker, Traumatic Brain Injury Network, New York
2003 Invited Speaker, Alzheimer’s Legislative Advocacy, Sacramento CA
2003 Invited Speaker, University of North Carolina, Chapel Hill, Center for Alcoholism Study
2003 Invited Speaker, 4th International Conference on Frontotemporal Dementia, Lund, Sweden
2002 Invited Speaker, Saunders-Brown Institute of Aging, Lexington, KY
2002 Invited Speaker, UCSF-Genetic epidemiology Seminar Series
2002 Invited Speaker, ACNP workshop on Alcoholism Endophenotypes, San Juan, Puerto Rico
2001 Invited Speaker, Rotman Institute Conference on Dementia, Toronto Canada

2001 Invited Speaker, Duke University Mini-symposia Genetics of Behavior, Durham, NC
2001 Invited Speaker, UCSF Program in Phamacogenomics Retreat, Tomalas Bay, CA
2001 Organizer, First International Meeting on Autism Research
2001 Invited Speaker, Society for Research on Nicotine and Tobacco Meeting
2001 Invited Speaker, ALS Investigators Workshop, Boston, MA
2000 Invited Speaker, American Academy of Neurology, San Diego, CA
2000 Invited Speaker, World AD Congress, Washington, DC
2000 Invited Speaker, International Society for Biomedical Research on Alcoholism, Yokohama, Japan
2000 Invited Speaker, Society for Research on Nicotine and Tobacco Meeting, San Diego CA
1999 Plenary Lecturer, Decade of the Brain, American Academy of Neurology, Toronto, Canada
1999 Invited Speaker, American Academy of Neurology, Toronto, Canada
1999 Invited Speaker, Rotary Club, Modesto California
1999 Invited Speaker, Ninth Congress of the International Psychogeriatric Association, Vancouver, Canada
1999 Invited Speaker, California Society of Addiction Medicine, Marina del Rey, California
1999 Invited Speaker, Association for Research in Nervous and Mental Disease
1999 Invited Lecturer, University of California, Davis, Dept. of Viticulture and Enology
1999 Invited Speaker, Fatal attractions within neurons: Intracytoplasmic protein aggregates in AD and related degenerative diseases. Paris, France
1999 Invited Speaker, Cure Autism Now Symposia, Marina Del Rey CA
1998 Invited Speaker, University of Wisconsin, Madison, Grand Rounds
1998 Invited Speaker, International Parkinson's Disease Expert Form, Kyoto Japan
1998 Invited Lecturer, Epilepsy Lecture Series, University of California, San Francisco
1998 Invited Lecturer, American Academy of Neurology, Minneapolis, MN
1998 Invited Lecturer, Bay Area Alzheimer’s Disease Study Group, University of California, S.F.
1998 Invited Lecturer, Northern CA Neuropsychology Forum, University of California, Berkeley
1998 Invited Speaker, Bay Area Population Genetics Group, University of California, San Francisco
1998 Invited Speaker, Sixth International Conference on Alzheimer’s Disease and Related Disorders, Amsterdam
1998 Invited Lecturer, Wonderfest: The Bay Area Festival of Science, San Francisco
1998 Invited Speaker, American Society for Cell Biology Annual Meeting
1998 Invited Speaker, Department of Pathology University of Lund, Sweden

1997 Invited Lecturer, University of California, Davis, Dept. of Viticulture and Enology
1997 Invited Speaker, University of California, Davis, Alzheimer's Center
1997 Invited Speaker, University of California, San Diego - Alzheimer's Center
1997 Plenary Lecturer at American Neuropsychiatric Association
1997 Invited Speaker, Advances in Social Brain, French Foundation
1996 Invited Speaker, Harbor - University of California Los Angeles, Medical Center
1996 Invited Speaker, John Muir Medical Center
1996 Invited Speaker, Epilepsy Lecture Series, University of California, San Francisco
1996 Invited Speaker, Sequana
1996 Invited Speaker, University of California, San Diego
1996 Invited Speaker, Third International Symposium On Dystonia
1996 Invited Speaker, Toronto Meeting on Frontotemporal Dementia
1996 Invited Speaker, Winter Conference on Brain Research, Snowmass Village, Co.
1996 Invited Speaker, American Neuropsychiatric Association, 8th Annual Meeting
1994 Invited Speaker, Neurology Grand Rounds, Columbia University
1994 Platform Speaker, American Academy of Neurology
1994 Invited Speaker, The Brain Institute, Staten Island, New York
1994 Invited Speaker, Mt. Sinai Department of Genetics
1994 Invited Speaker, Department of Human Genetics, Medical College of New Jersey
1993 Invited Speaker, Neurology Grand Rounds, Columbia University
1992 Invited Speaker, Neurology Grand Rounds, Columbia University
1991 Platform Speaker, American Academy of Neurology

Teaching record:

Lectures

2009-present Carolina Institute for Developmental Disabilities Postdoctoral training 1 lecture/year
2004-2012 Genetic Epi (299) Graduate Students 1 lecture/year[LU9][SH10]
2005-2007 Integrative Function and Cellular Basis Section Leader/Lecture - 4.5 hours
2004-2006 Neurogenetics Neurology Residents 1 lecture/year
2004 Principals of Genetic Analysis I Graduate Students 1 lecture
2001-2004 Pharmacogenomics Graduate Students 1 lecture

Grand Rounds

-UNC

2010, 2007, 2004, 2002 Invited Speaker, UNC Department of Neurology Grand Rounds
2010, 2005 Invited Speaker, UNC Department of Genetics Colloquium

2002 Invited Speaker, University of North Carolina, Chapel Hill, Department of Genetics

-Outside UNC

2011 Invited Speaker, Psychiatry Grand Rounds2010, Los Angeles, CA
2011 Invited Speaker, Neurology Grand Rounds2010, Los Angeles, CA
2010, 2002 Invited Speaker, UCSF CPC, San Francisco, CA[LU11][SH12]
2006 Invited Speaker, UT Southwestern Neurology and Psychiatry Grand Rounds, Dallas TX
2005 Invited Speaker, Einstein Medical School Grand Rounds, Bronx NY
1998, 2002 Invited Speaker, UCSF Grand Rounds, San Francisco, CA

Continuing Education Lectures

-UNC

2005 Update on Movement Disorders, Wilmington, NC
2004 Update on Movement Disorders, Pinehurst, NC

-Outside UNC

2007,2008,1997-2004 American Academy of Neurology Non-Ad Dementia Course (Faculty participant, lecture)
2002 Invited Speaker, UCSF-Fresno Mini-Symposia on Aging, Fresno VA, CA
1998 Invited Speaker, Advances in Alzheimer's Disease and Related Dementia Course, University of California, San Diego
1996 UCSF Twenty-Ninth Annual Recent Advances in Neurology (Faculty participant, lecture)
1995 Columbia University: Basic and Clinical Neurosciences (Faculty participant, lecture)
1995 Columbia University: Comprehensive Review of Current Neuropsychiatry (Faculty participant, paper, lecture)

Lab or Research Teaching/Mentorships

-Attending on Clinical Service

2007-2012 Movement Disorder/Neurogenetics Clinic 1 day/month
2004-2012 Neurology Ward Service, Resident and Med Student Supervision 35hours/week for 2 weeks
2005-2006 Movement Disorder/Neurogenetics Clinic ¾ day/week
2004-2006 Night Attending Call 17 nights/year
2004-2005 Neurogenetics Clinic 24 half day clinics/year

-Graduate Supervision, Committees

2008-2014	Gabi Cameron	Ph.D. thesis advisor
2004-2010	Amy Webb	Ph.D. thesis advisor[LU13][SH14]
2009	Janet Dolittle	Rotation Supervisor
2006-2008	Natilie Surzenko	Thesis committee
2006-2008	Kyle Gaulton Thesis committee	

2005-2008	Hind Muallem	Thesis committee
2004	Amanda Nave	Ph.D. rotation student
2004	Kyle Gaulton	Ph.D. rotation student
2004	Demetra Stamm	M.D.-Ph.D. rotation student

-Other Supervision

2011-2012	Micheal Spiegel	Postdoctoral Fellow
2007-2010	Ian Gizer, Ph.D.	Postdoctoral Fellow
2004-2006	Carla Nester, M.D.	Clinical Research Fellow[LU15][SH16]
2005-2005	Zheng "Jane" fan	Medical Genetics Fellow
2002-2005	Penelope Lind, Ph.D.	Postdoctoral Fellow
2000-2004	Dina Phyere, Ph.D.	Postdoctoral Fellow
1999-2001	Sietske Nora Heyn, Ph.D.	Postdoctoral Fellow
1999-2001	Paul Hengen, Ph.D.	Postdoctoral Fellow
1999-2000	Barbara Kloeckener-Gruissem, Ph.D.	Postdoctoral Fellow
1995-2000	Lorraine Clark, Ph.D.	Postdoctoral Fellow
1999-2000	Anthony Ashworth, Ph.D.	Postdoctoral Fellow
1998-1999	Cassandra Vieten, Ph.D.	Postdoctoral Fellow
1996-1998	Katherine Dains, Ph.D.	Postdoctoral Fellow
1995-1998	Dan Mirel, Ph.D.	Postdoctoral Fellow
1995-1996	Lise Jeppensen (Ph.D. cand.)	Postdoctoral Fellow
1993-1995	Michael Neystat, Ph.D.	Postdoctoral Fellow
1993-1995	Timothy Lynch, Ph.D.	Clinical Research Fellow
1991-1994	Tobjoern Nygaard, M.D.	Clinical Research Fellow

Grants:[LU17]

ACTIVE:

5R01DA030976-04 (Wilhelmsen) 9/30/10-5/31/15 3.86 calendar
Role: PI 30% effort
NIH $2,478,414
Deep Sequencing Studies for Cannabis and Stimulant Dependence

5U01HG006487-02 (Evans, Wilhelmsen, Henderson, Berg) 12/01/11-11/30/15 1.8 calendar
Role: PI project 2 9% effort
NHGRI $1,146,209
NC GENES: North Carolina Clinical Genomic Evaluation by NextGen Exome Sequencing

1U19HD077632-01(Powell,Berg) 9/05/2013-8/31/2018 0.65 calendar
Role: Co-Investigator 5.4% effort
NIH $903,419
NC NEXUS, North Carolina Newborn Exome Sequencing for Universal Screening
RFA-HD-13-010

1U01HG007437-01(Berg,Evans,Philip,Ledbetter,Watson) 09/23/2013-5/31/2017 0.72 calendar
Role: Co-Investigator 6% effort
NIH $1,121,537

A Knowledge Base for Clinically Relevant Genes and Variants

5P01-DK058335-14(Falk) 9/01/2000-7/31/2015 0.96 calendar
Role: Investigator 4.8% effort
NIH $983,089
ANCA Glomerulonephritis: From Molecules to Man

1UL1TR001111-01(Runge) 9/16/2013-4/30/2018 0.6 calendar
Role: Investigator 5% effort
NIH $4,209,603
North Carolina Clinical and Translational Science Award (NC TraCS)

5U24 AA020024-04 (Crews) 9/01/10-8/31/15 1.1 calendar
Role: Investigator 9.2% effort
NIH $270,432
UNC-CH NADIA Administrative Core

5R01-ES017794-03 (Whitsel) 8/01/10-5/31/14 0.3 calendar
NIH $0 NCE
Modification of PM-Mediated Arrhythmogenesis in Populations

Professional Service:

Editorial Board:

2004-2014 Associate Editor Alcoholism: Clinical and Experimental Research

Journal Reviewer:

Annals of Neurology
Archives of Neurology
Archives of Neurology
BMC Medical Genetics
Biological Psychiatry
Epilepsia
Experimental Neurology
Genes to Cells
Genomics
Hepatology
Human Molecular Genetics
JAMA
Lancet
Muscle and Nerve
Movement Disorder Journal
Neurobiology of Disease
Neurogenetics
Neuropsychiatric Genetics
Neurology
New England Journal of Medicine
Psychophysiology

Consultant:

2003-2006 Advisory Board for Stanford University/Mayo Clinic Bioethics of Nicotine Genetics Research

Committees:

-UNC:

2011-2012 Genomics Data Task Force
2010 Next Generation Sequencing Advisory Committee.
2010 Pangano Award Selection Committee.
2007, 2008, 2009, 2010 Neurology/Neuroscience Faculty Search Committee

2008-2009	Chair North Carolina Collaboratory for Bioinformatics State-Wide Engagement Project
2008-2009	UNC Enterprise Data Warehouse Operation Committee
2008	RENCI UNC Provost Commissioned Self Study Committee[LU18][SH19]
2005-2007	Faculty advisory Committee for Research Computing
2005-2007	High Throughput Genotyping Committee
2004-2007	Specimen Procurement Planning Committee [LU20][SH21]
2005-2006	Department of Genetics Retreat Planning Committee
2005-2006	UNC faculty grant nominating committee
2004-2005	UNC Faculty Competitive Grant Applicant Selection Committee
2004	Department of Neurology Informatics Planning Committee
2004	Bioinformatics Faculty Search Committee
2004	Statistical Genetics Faculty Search Committee

-other

1997-2004	UCSF Human Genetics Program Curriculum Committee[LU22][SH23]
1997-2000	UCSF Student Dean's Prize Committee: 1997-2000
1999-2000	UCSF Neurogenetics Chair Search Committee
1997	UCSF Search Committee, Human Genetics, Department of Orthopedic Surgery
1996-1997	UCSF Search Committees for Distinguished Chair (Classen Chair)

Study Section/Review Panels

Reviewer Special Study Section NHGRI, Intragenic Variant Analysis 2014
Reviewer Special Study Section NHGRI, Genomic Medicine Demonstration Projects 2013
Reviewer Special Study Section NINDS, Epilepsy Genomics 2013
Reviewer Special Study Section NIA, Alzheimer Genomics 2013
Permanent member Study Section NIA-N 2010-2013
Amyotrophic Lateral Sclerosis Scientific Review 2001,2002,2005,2007
NIAAA Biomedical Research Review Subcommittee adhoc reviewer 2004,2005,2006&2007
Ad hoc reviewer Genetics of Human Disease Study Section Oct 2006
Ad hoc reviewer Mammalian Genetics Study Section 2001,2002,2005
NIDA CIDR Scientific Review Oct 2005
NINDS Review for Morris Udall Centers Nov 2003
Genome Canada Scientific Review 2002
Cure Autism Now Scientific Review 1997-2001
Neuropathy Association 1997-2001
John Douglas French Foundation 2000
Alzheimer's Disease Association 1998-2000
NIAA Center Grant Site Review Team 1999 and for resubmission
NINDS Special Study Section 1997
NIH Marshfield Genotyping Center 1996

Research Statement

The Wilhelmsen laboratory is engaged in the genetic mapping of susceptibility loci for complex traits using traditional linkage and association approaches and now using massively parallel sequncing. A theme that runs through my work is to use genetic analysis to not only map susceptibility loci but to help define disease syndromes. This approach depends on large populations with rich phenotypic data.

I have mapped many simple Mendelian traits. My most important work has been when I used genetics to define the phenotype that segregates with a locus. My original identification of linkage of the syndrome disinhibition-dementia-parkinsonism-amyotrophy complex to 17q21-22, now referred to as Frontotemporal Dementia and Parkinsonism linked to chromosome 17 (FTDP17), has been an important development in Neurogenetics because contributed to the recognition that Frontotemporal Dementia is one of the most important causes of presenile dementia. This work was based on the study of a family with a complex syndrome that had not been previously defined. The phenotype was defined based on susceptibility to a clinically hetergeneous neurodegenerative process that resembled a mix of Schizophrenia, Alzheimer's and Parkinson's and ALS defined the disease. My collaborators and I have subsequently identified eight named neurodegenerative syndromes that appear to be due to different mutations in the same locus. Based on this work co-organized a NIH sponsored conference on this subject which named the condition FTDP17. Subsequently mutations in the tau gene were shown to be responsible for many cases FTDP17. My current research activity in this area is devoted to identifying genes that contribute to the major causes of neurodegeneration that are not due to simple patterns of inheritance.

My long term interest is in the genetic dissection of complex traits. When I moved from Columbia University to UCSF in 1995 I established a clinical and laboratory program to identify alcoholism susceptibility genes. There are few conditions where there is a genetic susceptibility that shows more complex interactions between genes and the environment than alcoholism. Using genetic epidemiology it has been possible to show a heritable component to alcoholism. These studies do not, however, allow us to infer how many genes are involved, how these genes interact with each other, or how they are affected by the environment. Our strategy is to not only map loci that affect behavior but to let the data define the phenotype that is produced by the alternative alleles of these loci. In each of the four large addiction related cohorts that my lab and collaborators have established we are exploring phenotype genotype relationships that depends on having rich phenotype data.

While still at UCSF my group invested heavily in automation that enables large scale genotype determination, which is the currency of genetic analysis and data processing. They developed a system that could determine the genotypes of 815 evenly spaced markers (3 cM average spacing) for 10 people per day. Data collection was completed for four large family studies related to addiction. The

first lab has identified the first gene that affects how persons perceive alcohol. On going analysis is focused on identifying additional sequence variations that affect susceptibility to addiction behavior.

To extend this work I saw the need to develop high throughput association analysis. Since moving to UNC I have transitioned to chip based and sequencing based approaches. In contrast to the approach, used by most investigators that try to find the sequence variations that cause a specific disease, each of my projects is an exploration of phenotype genotype relationships in projects with rich phenotypic data. We are trying to increase the power to detect an association between a phenotype and genotype by exploring phenotypic space to define the optimal phenotype that segregates with alternative alleles.

My group current efforts are to develop analytic methods to explore phenotype genotype relationships. We expect this approach to be most successful for determining the phenotype of common sequence variations when rich phenotypic data is available. I believe that this approach will lead to the most efficient annotation of the human genome. Our work collecting data for genome-wide association analysis has led us to develop a new computationally intensive strategy, called Convergent Haplotype Association Tagging, to begin determining the affect of rare sequence variation on disease. This approach allows the detection previously undetectable mutations based on long range haplotype sharing in distant relatives.

In the last 4 years my group has sequenced more than three thousand whole genomes. First as Chief Domain Scientist and Now as Director of Bioinformatics at RENCI I have led a team that supports many research and medical genomics projects. During the next few years we expect use these bioinformatics approaches for genetic analysis on data sets that can also be used for other, more general, purposes. The recognition that data aggregation and reuse can be a powerful resource for answering important questions in biology and medicine has led me to increase my involvement with the Renaissance Computing Institute where I have been working to increase the institute's portfolio of biomedical research where computational resources can lead to important insights.

Teaching Philosophy:

I like teaching students in small groups using the Socratic method. My favorite leading question after students read a paper or see a lecture is: If this were your data what is the most important thing to do next? All the students that reach my sphere of influence are accomplished. My students routinely can suggest an experiment that will make an incremental advance, but rarely ask a testable question that will challenge a field. I believe that the ability to ask the critical question can be learned with practice. When teaching students I try to demonstrate the value of their broad experience and push them to use the vast body of knowledge to which they have been exposed.

University of North Carolina at Chapel Hill
Information Sheet: Phase I of NCNEXUS
Adult Participants, "Diagnosed" Cohort
Biomedical Form

IRB Study # 13-2409
Information Sheet: 5/15/2015
Title of Study: North **C**arolina **N**ewborn **Ex**ome **S**equencing as **U**niversal **S**creening **(NC NEXUS)**
Principal Investigators: Cynthia Powell, M.D. and Jonathan S. Berg, MD, PhD
UNC-Chapel Hill Department: Genetics
UNC-Chapel Hill Phone number: 919-966-7043
Email Address: powellcm@med.unc.edu; jsberg@med.unc.edu
Co-Investigators: Donald Bailey, James P. Evans, Karen Weck, Kirk Wilhelmsen
Funding Source: National Human Genome Research Institute (National Institutes of Health)
Study Contact:
Study Contact telephone number:
Study Contact email:

This information sheet is for couples thinking about joining Phase I of NCNEXUS.

What are some general things you should know about research?
Research studies are done to learn information that may help others in the future. You and your child may *not* get any direct benefits from joining and there may be risks. Joining a study is up to you.

It is important that you understand the information on this sheet so that you can make an informed choice about whether or not to join. You have the right to ask, and have answered, any questions you have about this study by contacting the researchers listed at the top of this form.

What is the purpose of the NCNEXUS study?
The purpose of this study is to learn whether a new kind of testing, called "genomic sequencing" can help identify children who have or are likely to develop some kinds of genetic health conditions.

Newborn screening is done to look for health conditions that can be successfully treated when they are found early. Screening can identify some, but not all, genetic health conditions.

Genomic sequencing looks for **genetic** differences, called "variants," that cause conditions like the ones identified by newborn screening and many more. The technology

that allows many genes to be studied at once is called "Next-generation sequencing." It is a new way to test for these conditions.

We are interested in knowing how parents decide whether or not to have sequencing of their child and, if so, how they understand and respond to the different kinds of information they can learn from testing.

NCNEXUS study has two phases; Phase I and Phase II.
In Phase I, we want to find out what information parents need to help them decide whether or not to have genomic sequencing of their child to find conditions like those identified by newborn screening.

At the end of Phase I, parents will be asked if they want their child to have sequencing. Parents who consent will enter Phase II. Parents who decline will complete a questionnaire and then end their participation in the study. Phase II is described on a separate consent form. If you join Phase I, you do not have to join Phase II.

You have agreed that we can contact you by phone to ask whether or not you want to join **Phase I.** To help you decide before we call, we have sent you the following materials:

- A **brochure** that tells about the study, how genomic sequencing is done, the kinds of genetic health conditions might be found, and information to help you decide whether or not to join.

- This **information form**. Please read it before we call you.

When we call to ask if you want to join **Phase I**, you can tell us your answer over the phone**.** If both parents are involved in the child's life, they **both** have to agree to join but each member of the couple will complete the questionnaires on his or her own. If only one parent has custody of the child, he or she can join by him or herself.

What happens if you do not want to join Phase I?
We will ask you some questions about yourself and your reasons for declining. After you answer the questions, your part in the study will end and we will shred your identifying information.

The rest of this information sheet is about what happens if you decide to join Phase I.

How many people will take part in the study?
We expect to have about 400 children and their parents complete the whole study.

How long will your part in Phase I last?
Phase I lasts until both parents either agree to sequencing or decline and complete the questionnaire.

What will happen after you join Phase I?
You will complete an **intake form.**

If you have access to an Internet-enabled computer:
1) We will give you a link to complete a questionnaire.
2) We will then give you a link to an online electronic decision guide. The guide has information about sequencing, describes the types of results you might learn, the risks and benefits of testing and helps you think about if sequencing for your child would be the right decision for you and your family.

At the end of the decision guide, you will be asked to pick one of the following 3 options:

(1) We **do not** want our child to have genomic sequencing for conditions like those found in newborn screening and **do not** want to schedule a study visit;
(2) We **are** interested in genomic sequencing for our child and **want** to schedule a study visit; or,
(3) We **are undecided** about genomic sequencing and **want** to schedule a study visit to learn more.

If you come to the study visit, it does not mean that you have to consent to sequencing.

If you do not have an Internet-enabled computer
We will send you a questionnaire to complete and return it in the pre-paid envelope. If you are interested in scheduling a visit to view the decision guide and learn more, please let us know.

What happens next?
If you decide to schedule a study visit, you will meet with a genetic counselor to discuss why you may or may not want to have sequencing of your child. This visit will last about 1 hour but may last longer if you have more questions.

You will then be asked if you want to consent to having genomic sequencing of your child.

If both parents consent, you will both sign the consent form and a form so we can obtain your child's health records.

Parents who consent to sequencing will enter into Phase II. They will be randomized to 1 of 2 groups. One group will be asked to decide if they want to request additional genetic information about conditions that are not related to those found with newborn screening. The other group will not be asked to make these decisions.

If you decide not to consent, you will answer a questionnaire and then your part in the study will end.

What are the possible benefits to you of participating in Phase I?
There is little chance that you will benefit, but it will help us learn how parents make these decisions.

What are the possible risks or discomforts to you by participating in Phase I of this study?
You may be uncomfortable answering some questions on the forms. You can refuse to answer a question or stop completing the forms but not completing them means you can't continue in the study.

You will *not* be charged for any of the activities in NC NEXUS.
You will be paid with a $20 VISA card for completing each questionnaire.

We will give you any new information that might affect your willingness to continue participation. You can stop participating at any time, without penalty, by contacting the researchers on the first page.

Who is sponsoring this research?
This research is funded by a grant from the National Human Genome Research Institute and the National Institutes of Child Health and Development at the National Institutes of Health. The research team is paid to carry out the study but they do *not* have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about your rights as a research participant?
The Institutional Review Board (IRB) reviews all research on human volunteers in order to protect their rights and welfare. If you have questions or concerns about you and your child's rights as research participants, you may contact the IRB at 919-966-3113 or IRB_subjects@unc.edu. You do not have to use your name.

You will be asked to give your verbal consent to join Phase I over the phone.

University of North Carolina at Chapel Hill
Information Sheet: Phase I of NCNEXUS
Adult Participants, "Well-Child" Cohort
Biomedical Form

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If you come to the study visit, it does not mean that you have to consent to sequencing.

If you do not have an Internet-enabled computer
We will send you a questionnaire to complete and return it in the pre-paid envelope. If you are interested in scheduling a visit to view the decision guide and learn more, please let us know.

What happens next?
If you decide to schedule a study visit, you will meet with a genetic counselor to discuss why you may or may not want to have sequencing of your child after birth. This visit will last about 1 hour but may last longer if you have more questions.

You will then be asked if you want to consent to having genomic sequencing of your child.

If both parents consent, you will both sign the consent form and a form so we can obtain your child's health records after birth.

Parents who consent to sequencing will enter into Phase II. They will be randomized to 1 of 2 groups. One group will be asked to decide if they want to request additional genetic information about conditions that are not related to those found with newborn screening. The other group will not be asked to make these decisions.

If you decide not to consent, you will answer a questionnaire and then your part in the study will end.

What are the possible benefits to you of participating in Phase I?
There is little chance that you will benefit, but it will help us learn how parents make these decisions.

What are the possible risks or discomforts to you by participating in Phase I of this study?
You may be uncomfortable answering some questions on the forms. You can refuse to answer a question or stop completing the forms but not completing them means you can't continue in the study.

You will *not* be charged for any of the activities in NC NEXUS.
You will be paid with a $20 VISA card for completing each questionnaire.

We will give you any new information that might affect your willingness to continue participation. You can stop participating at any time, without penalty, by contacting the researchers on the first page.

Who is sponsoring this research?
This research is funded by a grant from the National Human Genome Research Institute and the National Institutes of Child Health and Development at the National Institutes of Health. The research team is paid to carry out the study but they do *not* have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about your rights as a research participant?
The Institutional Review Board (IRB) reviews all research on human volunteers in order to protect their rights and welfare. If you have questions or concerns about your rights as a research participant, you may contact the IRB at 919-966-3113 or IRB_subjects@unc.edu. You do not have to use your name.

You will be asked to give your verbal consent to join Phase I over the phone.

University of North Carolina at Chapel Hill
Consent to Participate in a Research Study: Phase II of NC NEXUS
Parental Permission for Child Participants: Diagnosed Cohort
Biomedical Form

IRB Study # 13-2409
Consent Form Version Date: 5/15/2015
Title of Study: North **C**arolina **N**ewborn **Ex**ome **S**equencing as **U**niversal **S**creening **(NC NEXUS)**
Principal Investigators: Cynthia Powell, M.D. and Jonathan S. Berg, MD, PhD
UNC-Chapel Hill Department: Genetics
UNC-Chapel Hill Phone number: 919-966-7043
Email Address: powellcm@med.unc.edu; jsberg@med.unc.edu
Co-Investigators: Donald Bailey, James P. Evans, Karen Weck, Kirk Wilhelmsen
Funding Source: National Human Genome Research Institute and National Institutes of Child Health and Development (National Institutes of Health)
Study Contact:
Study Contact telephone number:
Study Contact email: IRB Study #

What are some general things you should know about research?

The goal of research is to learn information that may help other people in the future. You and your child may ***not*** receive any direct benefit from joining this study and there may be risks.

You may refuse for your child to take part in this study. If your child is a patient with an illness, he or she does not have to be in a study to get treatment. Joining the study is voluntary.

It is important for you to understand the information in this consent form so that you can make an informed choice. You will be given a copy. You have the right to ask, and have answered, any questions you may have about this research. Please contact the researchers listed at the top of this form.

What is the purpose of the NC NEXUS study?

The purpose of this study is to learn whether a new kind of testing, called "genomic sequencing" can help identify children who have or are likely to develop some kinds of genetic health conditions.

Newborn screening is done to look for health conditions that can be successfully treated when they are found early. Screening can identify some, but not all, genetic health conditions.

Genomic sequencing looks for **genetic** differences, called "variants," that cause conditions like the ones identified by newborn screening and many more. The technology

that allows many genes to be studied at once is called "Next-generation sequencing." It is a new way to test for these conditions.

We are interested in knowing how parents decide whether or not to have sequencing of their child and, if so, how they understand and respond to the different kinds of information they can learn from testing.

How long will your and your child's part in this study last?
We ask that you and your child to join for a total of up to **4** years. Periodically, we plan to review the information from your child's sequencing. If we find new information that would affect your child's medical care, or your willingness to continue participation in the study, we will contact you.

How many people will take part in this study?
We expect **~ 400** children will have genomic sequencing in this study.

You are currently participating in Phase I of NC NEXUS.
You have been sent this consent form because you have been scheduled for the first study visit.

What will happen at the first study visit?
You will be asked a few questions about your understanding of genetics. A genetic counselor will review this consent form with you and discuss sequencing and the types of results you could learn.

What is genomic sequencing?
Genomic sequencing looks for differences, called "variants", in many genes at once to identify those that cause genetic health conditions.

What types of information could you learn from the genomic sequencing done in NC NEXUS?

- Although all of your child's genes will be sequenced, only a selected group of genes will be analyzed and interpreted.

- Genomic sequencing might find genetic variants that provide information about the health condition that made your child eligible for NC NEXUS.

  We will look for variants in those genes that have been reported as being connected with your child's diagnosis. Variants in these genes will be classified in 1 of 3 categories:

  1. Positive result: a gene variant is identified that explains your child's diagnosis.

  2. Uncertain result: a gene variant is identified that **might** explain your child's diagnosis but we are **uncertain** if it explains it or not. This is called a "variant of uncertain significance" or "VUS."

3. Negative result: no variant has been identified that explains your child's health condition in the group of genes that was studied.

- If your child's sequencing identifies a variant of uncertain clinical significance (VUS), we might be able to clarify the meaning by testing family members. Genomic sequencing will not be done on the family samples but rather they will **only** be tested to study the meaning of the VUS found in your child's sample. This testing is part of the research study and you will not be charged for it.

- Genomic sequencing might also find variants in a group of genes that provide information about **other** genetic health conditions like those identified by newborn screening.

    - In this part of the NC NEXUS study, we will analyze and interpret variants in those genes that provide information about health conditions like those identified by newborn screening. These conditions have symptoms that begin in infancy or childhood and have treatments. We call this genetic screening, "**NGS-NBS**."

    - **Everyone** who consents to sequencing of their child will learn about the results of the **NGS-NBS screen**. Only "positive" results that strongly indicate the presence of a genetic disorder will be reported. Most participants in the study will screen "negative" for these conditions.

- We call this analysis, the "**Next-generation Sequencing Newborn Screen" (NGS-NBS Screen).**

- **In the NGS-NBS Screen, we will analyze and interpret gene variants to provide information about genetic health conditions that are very similar to those found by newborn screening.**

    - Newborn screening identifies children who have, or are likely to develop, health conditions that

        - can be successfully treated when they are found early.

        - have symptoms that begin in infancy or childhood.

    - **All** parents who consent to sequencing of their child will learn results from the **NGS-NBS Screen**.

    - Only those results that **strongly** indicate the presence of a genetic health condition as described above will be reported. These results are considered to be "**positive."**

    - Most children will have "**negative**" results for these conditions.

- It is possible that your child's results will indicate that other family members are at

risk. When genetic testing for that condition is clinically available, it would *not* be paid for by the study.

What will happen if you and your child join Phase II of NC NEXUS?

(1) After the first study visit, you will **complete a questionnaire** on line that asks questions about your decision to consent for your child to be sequenced. You *may* choose *not* to answer a question for any reason. We will send you a $20 Visa card for completing it.

(2) We will **obtain the sample at this visit** or **schedule a visit to obtain the sample.** An experienced nurse will use up to 5 sponges to swab the inside of your baby's cheeks and along the gums.

(3) After your child's results from the **NGS-NBS Screen** have been analyzed, we will **schedule the second study visit** to discuss them with you. You will be **randomized** to one of two study groups and told which group you are in (described below).

What will happen at the second study visit?
We will explain the results of your child's sequencing and provide genetic counseling to help you understand the meaning and implications of their results.

Most children will have a **negative** result.

If your child has a **positive** result:

> We will confirm it in the CLIA-approved, Medical Genetics Laboratory (MGL) at UNC Hospitals. Once confirmed, we will give you a clinical report that can be placed into your child's UNC Hospitals electronic medical record (EMR).
>
> We will ask you to decide whether or not you want the clinically confirmed positive results to become a permanent part of your child's EMR.
>
> If you choose to do so, we will enter the report so that other health care providers taking care of your child can be aware of this result.
>
> If you choose **not** to do that, we will **not** enter the report into your child's EMR.
>
> We will ask you to sign a form to indicate your decision.

(4) Randomization Procedure
Before the second study visit, parents will be randomized into two groups. Both parents will be in the same group. We will tell you which group you are in when we schedule the visit with you and give you more information at that time.

- The "experimental" group will use an electronic decision guide to help them make decisions about whether or not to learn information from three additional categories.

- The "control" group will not make decisions about learning additional information.

(5) After learning these results, you will **complete two more questionnaires** over the next 3 months.
You will be asked questions about your decision to consent for your child to be sequenced. You *may* choose *not* to answer a question for any reason. We will send you a $20 Visa card for completing it.

(6) Some parents will be asked if they will agree to be interviewed by phone and to have the study visits observed. You can decline these optional activities but still remain in the NC NEXUS study. Declining to participate in these optional activities will *not* affect your child's medical care at UNC.

There are a few other things you should know about this study:

- We will ask you to sign a HIPAA authorization so we can access your child's medical records and other health-related information from visits to UNC Hospitals. This information will include his or her health history, family history, and relevant laboratory test results.

- Since our knowledge about genomics is growing quickly, we plan to periodically review the information from your child's sequencing. If we learn new information that would affect your child's medical care, we will contact you for a follow-up visit that will be part of the study.

- NC NEXUS researchers may observe the study visits to help us improve our explanations.

What will happen to your child's sample?
We will code your child's sample with a unique participant identifier (ID) so that his or her personal health information will *not* be available to the study personnel that process and analyze the sample.

One half of the sample will be sent to the UNC Biospecimen Processing Facility to extract and store the DNA. Some of this sample will be sent to Dr. Jonathan S. Berg's laboratory for sequencing.

The other half of the sample will be sent to the UNC Hospitals' Clinical Laboratory to extract and store the DNA. It will be used to confirm the variants found by sequencing and for quality control testing.

We will use the samples for an undetermined period of time but may choose to destroy them when the study is complete.

Who owns the specimens?

Any samples or sequence data obtained for this study become the exclusive property of the UNC-Chapel Hill. The researchers may retain, preserve or dispose of these specimens and may use these specimens for research that may result in commercial applications. There are *no* plans to compensate you or your baby for any future commercial use of these coded specimens.

What are the possible benefits to you?

Research is designed to benefit society by gaining new knowledge. There is little chance you or your child will benefit from being in this research study. Your and your child's participation will contribute to our understanding of how to use this new genomic test in the future and help us learn how people might respond to learning different kinds of information from this testing.

What are the possible risks or discomforts involved with participation in this study?

(1) Physical risk: The physical risks in this study are minimal. An experienced nurse will collect the cheek swab. It should only take a few minutes to obtain but might cause your child some discomfort.

(2) Psychological Risks: Genetic testing can provide information about the risk for health conditions in a family. This knowledge may affect your or your child's emotional well-being. Some people may experience stress, anxiety and/or depression. We will explain your child's results to you and provide genetic counseling to help you understand their meaning and implications for family members.

Learning that your baby has a **negative** result is not expected to affect your emotional well being.

To study how parents respond to genomic sequencing and learning the results, we will ask you questions about your experiences in the study. You can choose *not* to answer any question at any time.

(3) DNA Storage: The foreseeable risks of storing your child's genetic material are low.

(4) Risk to Confidentiality and Privacy: Som**e** parents of children who get positive results may want to keep that information private. This study has many protections to protect the privacy of your and your child's participation in the study and to protect information arising from the study.

> **Use of Participant ID Numbers:** We will code your child's samples and all study materials with a unique participant ID number. The link between the ID number and your child's personal identifying information will be kept in a

secured database with restricted access. Electronic information, including that from your child's medical records, will also be stored on secure drives in password-protected databases with restricted access.

Paper Documents: Paper documents, including this signed consent form, will be stored in a locked filing cabinet in a locked office at UNC.

Report of Positive Results: We will ask for your consent before putting any clinically confirmed positive results into your child's UNC electronic medical record.

Publications about the Research:
When we report findings from this research, we will not identify you nor your child.

We will make every effort to keep research records private but there may be times when federal or state law requires their disclosure, including of personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, the information could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

(5) Risk for Genetic Discrimination
A Federal law called the "Genetic Information Nondiscrimination Act" (GINA) generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against someone based on their genetic information.

GINA does *not* protect people against discrimination based on an already-diagnosed genetic condition or disease. The Americans with Disabilities Act (ADA) applies to them.

The Affordable Care Act (ACA) prohibits health insurance companies from discriminating against patients with genetic diseases by refusing coverage because of 'pre-existing conditions'.

GINA and the ACA do *not* protect people against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance

(6) Other risks to study participation: There may be uncommon or other risks that we don't know about. You should report any concerns to the researchers listed on the first page of this form.

Who is sponsoring and paying for this research?
NC NEXUS is being paid for by a grant from the National Human Genome Research Institute and the National Institutes of Child Health and Development at the National

Institutes of Health (NIH). The researchers are paid to carry out the study but they do *not* have a direct financial interest with the sponsor or in the final study results.

Data Sharing with Qualified Researchers
By signing this consent form, you are allowing us to share the DNA or the sequence data obtained from your child's samples with researchers at UNC or other institutions to study the clinical use of sequencing. Your child's personal identifying information will *not* be included and will *not* be sent.

The NIH is the government agency that funds most of medical research in the US. By collecting the genetic information obtained from many research centers, the NIH and other data banks will store it so other qualified researchers can use it to do more studies. Researchers can be from the government, academic, or a commercial site and studies may be done at many places at the same time.

Risks to Privacy and Confidentiality by Data Sharing
We think that the risks to your privacy and confidentiality by sharing your child's genetic information with other databanks is low; however, we cannot predict how genetic information will be used in the future. These databases have safeguards to protect information while it is stored and used for research. If your child has a genetic condition, this information will be sent with only a code number and personal identifying information will *not* be included and will *not* be sent.

You will not receive any results produced from your child's participation in the national databases unless it is considered medically relevant. If you no longer want your child's data in these databases, you can choose to withdraw your consent at anytime with no penalty. However, data that has already been sent to researchers cannot be retrieved from them.

Will researchers seek approval from you to do future studies involving the specimens?
A committee called the Institutional Review Board (IRB) protects the rights and welfare of research participants in current and future research.

For your child's data to be used in a future research study, the IRB may require that you be re-contacted and asked for your consent. You have the right, at that future time, to refuse to allow your child to participate. This refusal will *not* affect your or your child's medical care or result in loss of benefits to which you are or your child is entitled. In other cases, the IRB may determine that future research on your child's specimen is acceptable without re-contacting you. For example, your child's uniquely coded specimen and sequence data may be useful for other genetic research studies *not* directly related to genomic sequencing in children.

You may opt-out of future genetic research studies unrelated to this consent form by initialing:

_____I do *not* want my child's sample or data to be used in future genetic studies unrelated to those described in this consent form

Can you withdraw from participation in this study?
You can withdraw from this study at any time, without penalty by contacting the researchers listed on the front page of this form. We will then destroy any remaining samples. If you withdraw after you have consented for your child's results to be entered into the UNC electronic medical record, this report cannot be removed and will remain a permanent part of the medical record. Analyses that are complete or in progress when you withdraw will continue to be used in the study.

What will happen if you are or your child is injured by this research?
All research involves a chance that something bad might happen to participants. This may include the risk of personal injury. In spite of all safety measures, your child might develop a reaction or injury from having the sample collected. UNC-Chapel Hill has *not* set aside funds to pay for any such reactions or injuries, or for the related medical care. However, by signing this form, you do *not* give up any of your or your child's legal rights.

Will there be any cost to you for participating in NC NEXUS?
You will *not* be charged for the visits or the sequencing done as part of the study.

Will you receive anything for your participation?
We will not pay you nor your child for allowing the samples to be taken or for coming to the visits. You will receive parking vouchers and a $20 VISA card for completing each questionnaire for a total of $80.

What if you have questions about your rights as a research participant?
The IRB reviews all research on human volunteers in order to protect your rights and welfare. If you have questions or concerns about your rights as a research participant you may contact, the IRC at 919-966-3113 or to IRB_subjects@unc.edu. You do not have to use your name.

Participant Agreement:
I have read the information provided above and have asked all the questions I have at this time. I voluntarily agree to my and my child's participation in **the N**orth **C**arolina **N**ewborn **Ex**ome **S**equencing as **U**niversal **S**creening **(NC NEXUS); Principal Investigators:** Cynthia Powell, MD and Jonathan S. Berg, MD, PhD

_____ _____
Signature of Research Participant's Parent or Guardian Date

_____
Printed Name of Research Participant's Parent or Guardian and Relationship

_____ _____

Signature of Research Participant's Parent or Guardian Date

_____

Printed Name of Research Participant's Parent or Guardian and Relationship

_____ _____

Signature of Research Team Member Obtaining Consent Date

_____

Printed Name of Research Team Membe

University of North Carolina at Chapel Hill
Consent to Participate in a Research Study: NC NEXUS: Phase II
Parental Permission for Child Participants: Well-Child Cohort
Biomedical Form

IRB Study # 13-2409
Consent Form Version Date: 5/15/2015
Title of Study: North **C**arolina **N**ewborn **Ex**ome **S**equencing as **U**niversal **S**creening **(NC NEXUS)**
Principal Investigators: Cynthia Powell, M.D. and Jonathan S. Berg, MD, PhD
UNC-Chapel Hill Department: Genetics
UNC-Chapel Hill Phone number: 919-966-7043
Email Address: powellcm@med.unc.edu; jsberg@med.unc.edu
Co-Investigators: Donald Bailey, James P. Evans, Karen Weck, Kirk Wilhelmsen
Funding Source: National Human Genome Research Institute and National Institutes of Child Health and Development (National Institutes of Health)
Study Contact:
Study Contact telephone number:
Study Contact email:

What are some general things you should know about research?

The goal of research is to learn information that may help other people in the future. You and your child may ***not*** receive any direct benefit from joining this study and there may be risks.

You may refuse for your child to take part in this study. If your child is a patient with an illness, he or she does not have to be in a study to get treatment. Joining the study is voluntary.

It is important for you to understand the information in this consent form so that you can make an informed choice. You will be given a copy. You have the right to ask, and have answered, any questions you may have about this research. Please contact the researchers listed at the top of this form.

What is the purpose of the NC NEXUS study?

The purpose of this study is to learn whether genomic sequencing can help identify children who have, or are likely to develop, some kinds of genetic health conditions.

After a baby is born, newborn screening is done to look for health conditions that can be successfully treated when they are found early. Screening can identify some, but not all, genetic health conditions.

Genomic sequencing looks for **genetic** differences, called "variants," that cause conditions like the ones identified by newborn screening and many more. The technology

that allows many genes to be studied at once is called "Next-generation sequencing" and is a new way to test for these conditions.

We are interested in knowing how parents decide whether or not to have sequencing of their child and, if so, how they understand and respond to the different kinds of information they can learn from testing.

How long will your and your child's part in this study last?
We ask that you and your child to join for a total of up to **4** years. Periodically, we plan to review the information from your child's sequencing. If we find new information that would affect your child's medical care, or your willingness to continue participation in the study, we will contact you.

How many people will take part in this study?
We expect **~ 400** children will have genomic sequencing in this study.

You are currently participating in Phase I of NC NEXUS.
You have been sent this consent form because you have been scheduled for the first study visit.

What will happen at the first study visit?
You will be asked a few questions about your understanding of genetics. A genetic counselor will review this consent form with you and discuss sequencing and the types of results you could learn.

What is genomic sequencing?
Genomic sequencing looks for differences, called "variants", in many genes at once to identify those that cause genetic health conditions.

What types of information could you learn from the genomic sequencing done in NC NEXUS?

- Although all of your child's genes will be sequenced, only a selected group of genes will be analyzed and interpreted.

- We call this analysis, the "**Next-generation Sequencing Newborn Screen" (NGS-NBS Screen).**

- **In the NGS-NBS Screen, we will analyze and interpret gene variants to provide information about genetic health conditions that are very similar to those found by newborn screening.**

  - Newborn screening identifies children who have, or are likely to develop, health conditions that

    - can be successfully treated when they are found early.

    - have symptoms that begin in infancy or childhood.

- **All** parents who consent to sequencing of their child will learn results from the **NGS-NBS Screen**.

- Only those results that **strongly** indicate the presence of a genetic health condition as described above will be reported. These results are considered to be "**positive."**

- Most children will have "**negative**" results for these conditions.

- It is possible that your child's results will indicate that other family members are at risk. When genetic testing for that condition is clinically available, it would *not* be paid for by the study.

Parents who consent to genomic sequencing for their child will join Phase II of NC NEXUS.

Parents who do not consent to sequencing will complete a questionnaire that asks about this decision. We will send them a $20 VISA card and this will end their participation in the study.

What will happen if you and your child participate in Phase II of NC NEXUS?

(1) After the first study visit, you will **complete a questionnaire** on line that asks questions about your decision to consent for your child to be sequenced. You *may* choose *not* to answer a question for any reason. We will send you a $20 Visa card for completing it.

(2) After your baby is born, we will **schedule a visit to obtain the sample.** An experienced nurse will use up to 5 sponges to swab the inside of your baby's cheeks and along the gums.

(3) After your child's results from the **NGS-NBS Screen** have been analyzed, we will **schedule the second study visit** to discuss them with you. You will be **randomized** to one of two study groups and told which group you are in (described below).

What will happen at the second study visit?
We will explain the results of your child's sequencing and provide genetic counseling to help you understand the meaning and implications of their results.

Most children will have a **negative** result.

If your child has a **positive** result:

We will confirm it in the CLIA-approved, Medical Genetics Laboratory (MGL) at UNC Hospitals. Once confirmed, we will give you a clinical report that can be placed into your child's UNC Hospitals electronic medical record (EMR).

We will ask you to decide whether or not you want the clinically confirmed positive results to become a permanent part of your child's EMR.

If you choose to do so, we will enter the report so that other health care providers taking care of your child can be aware of this result.

If you choose **not** to do that, we will **not** enter the report into your child's EMR.

We will ask you to sign a form to indicate your decision.

(4) Randomization Procedure
Before the second study visit, parents will be randomized into two groups. Both parents will be in the same group. We will tell you which group you are in when we schedule the visit with you and give you more information at that time.

- The "experimental" group will use an electronic decision guide to help them make decisions about whether or not to learn information from three additional categories.

- The "control" group will not make decisions about learning additional information.

(5) After learning these results, you will **complete two more questionnaires** over the next 3 months.
You will be asked questions about your decision to consent for your child to be sequenced. You *may* choose *not* to answer a question for any reason. We will send you a $20 Visa card for completing it.

(6) Some parents will be asked if they will agree to be interviewed by phone and to have the study visits observed. You can decline these optional activities but still remain in the NC NEXUS study. Declining to participate in these optional activities will *not* affect your child's medical care at UNC.

There are a few other things you should know about this study:

- We will ask you to sign a HIPAA authorization so we can access your child's medical records and other health-related information from visits to UNC Hospitals. This information will include his or her health history, family history, and relevant laboratory test results.

- Since our knowledge about genomics is growing quickly, we plan to periodically review the information from your child's sequencing. If we learn new information that would affect your child's medical care, we will contact you for a

follow-up visit that will be part of the study.

- NC NEXUS researchers may observe the study visits to help us improve our explanations.

What will happen to your child's sample?
We will code your child's sample with a unique participant identifier (ID) so that his or her personal health information will *not* be available to the study personnel that process and analyze the sample.

One half of the sample will be sent to the UNC Biospecimen Processing Facility to extract and store the DNA. Some of this sample will be sent to Dr. Jonathan S. Berg's laboratory for sequencing.

The other half of the sample will be sent to the UNC Hospitals' Clinical Laboratory to extract and store the DNA. It will be used to confirm the variants found by sequencing and for quality control testing.

We will use the samples for an undetermined period of time but may choose to destroy them when the study is complete.

Who owns the specimens?
Any samples or sequence data obtained for this study become the exclusive property of the UNC-Chapel Hill. The researchers may retain, preserve or dispose of these specimens and may use these specimens for research that may result in commercial applications. There are *no* plans to compensate you or your baby for any future commercial use of these coded specimens.

What are the possible benefits to you?
Research is designed to benefit society by gaining new knowledge. There is little chance you or your child will benefit from being in this research study. Your and your child's participation will contribute to our understanding of how to use this new genomic test in the future and help us learn how people might respond to learning different kinds of information from this testing.

What are the possible risks or discomforts involved with participation in this study?

(1) Physical risk: The physical risks in this study are minimal. An experienced nurse will collect the cheek swab. It should only take a few minutes to obtain but might cause your child some discomfort.

(2) Psychological Risks: Learning that your baby has a **positive** result may affect your emotional well being and some people may experience stress, anxiety and/or depression. We will explain your child's results to you and provide genetic counseling to help you understand their meaning and implications for family members.

Learning that your baby has a **negative** result is not expected to affect your emotional well being.

To study how parents respond to genomic sequencing and learning the results, we will ask you questions about your experiences in the study. You can choose *not* to answer any question at any time.

(3) DNA Storage: The foreseeable risks of storing your child's genetic material are low.

(4) Risk to Confidentiality and Privacy: Some parents of children who get positive results may want to keep that information private. This study has many protections to protect the privacy of your and your child's participation in the study and to protect information arising from the study.

Use of Participant ID Numbers: We will code your child's samples and all study materials with a unique participant ID number. The link between the ID number and your child's personal identifying information will be kept in a secured database with restricted access. Electronic information, including that from your child's medical records, will also be stored on secure drives in password-protected databases with restricted access.

Paper Documents: Paper documents, including this signed consent form, will be stored in a locked filing cabinet in a locked office at UNC.

Report of Positive Results: We will ask for your consent before putting any clinically confirmed positive results into your child's UNC electronic medical record.

Publications about the Research:
When we report findings from this research, we will not identify you nor your child.

We will make every effort to keep research records private but there may be times when federal or state law requires their disclosure, including of personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, the information could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

(5) Risk for Genetic Discrimination
A Federal law called the "Genetic Information Nondiscrimination Act" (GINA) generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against someone based on their genetic information.

GINA does *not* protect people against discrimination based on an already-diagnosed genetic condition or disease. The Americans with Disabilities Act (ADA) applies to them.

The Affordable Care Act (ACA) prohibits health insurance companies from discriminating against patients with genetic diseases by refusing coverage because of 'pre-existing conditions'.

GINA and the ACA do *not* protect people against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance

(6) Other risks to study participation: There may be uncommon or other risks that we don't know about. You should report any concerns to the researchers listed on the first page of this form.

Who is sponsoring and paying for this research?

NC NEXUS is being paid for by a grant from the National Human Genome Research Institute and the National Institutes of Child Health and Development at the National Institutes of Health (NIH). The researchers are paid to carry out the study but they do *not* have a direct financial interest with the sponsor or in the final study results.

Data Sharing with Qualified Researchers

By signing this consent form, you are allowing us to share the DNA or the sequence data obtained from your child's samples with researchers at UNC or other institutions to study the clinical use of sequencing. Your child's personal identifying information will *not* be included and will *not* be sent.

The NIH is the government agency that funds most of medical research in the US. By collecting the genetic information obtained from many research centers, the NIH and other data banks will store it so other qualified researchers can use it to do more studies. Researchers can be from the government, academic, or a commercial site and studies may be done at many places at the same time.

Risks to Privacy and Confidentiality by Data Sharing

We think that the risks to your privacy and confidentiality by sharing your child's genetic information with other databanks is low; however, we cannot predict how genetic information will be used in the future. These databases have safeguards to protect information while it is stored and used for research. If your child has a genetic condition, this information will be sent with only a code number and personal identifying information will *not* be included and will *not* be sent.

You will not receive any results produced from your child's participation in the national databases unless it is considered medically relevant. If you no longer want your child's data in these databases, you can choose to withdraw your consent at anytime with no penalty. However, data that has already been sent to researchers cannot be retrieved from them.

Will researchers seek approval from you to do future studies involving the specimens?

A committee called the Institutional Review Board (IRB) protects the rights and welfare of research participants in current and future research.

For your child's data to be used in a future research study, the IRB may require that you be re-contacted and asked for your consent. You have the right, at that future time, to refuse to allow your child to participate. This refusal will *not* affect your or your child's medical care or result in loss of benefits to which you are or your child is entitled. In other cases, the IRB may determine that future research on your child's specimen is acceptable without re-contacting you. For example, your child's uniquely coded specimen and sequence data may be useful for other genetic research studies *not* directly related to genomic sequencing in children.

You may opt-out of future genetic research studies unrelated to this consent form by initialing:

_____I do *not* want my child's sample or data to be used in future genetic studies unrelated to those described in this consent form

Can you withdraw from participation in this study?

You can withdraw from this study at any time, without penalty by contacting the researchers listed on the front page of this form. We will then destroy any remaining samples. If you withdraw after you have consented for your child's results to be entered into the UNC electronic medical record, this report cannot be removed and will remain a permanent part of the medical record. Analyses that are complete or in progress when you withdraw will continue to be used in the study.

What will happen if you are or your child is injured by this research?

All research involves a chance that something bad might happen to participants. This may include the risk of personal injury. In spite of all safety measures, your child might develop a reaction or injury from having the sample collected. UNC-Chapel Hill has *not* set aside funds to pay for any such reactions or injuries, or for the related medical care. However, by signing this form, you do *not* give up any of your or your child's legal rights.

Will there be any cost to you for participating in NC NEXUS?

You will *not* be charged for the visits or the sequencing done as part of the study.

Will you receive anything for your participation?

We will not pay you nor your child for allowing the samples to be taken or for coming to the visits. You will receive parking vouchers and a $20 VISA card for completing each questionnaire for a total of $80.

What if you have questions about your rights as a research participant?
The IRB reviews all research on human volunteers in order to protect your rights and welfare. If you have questions or concerns about your rights as a research participant you may contact, the IRC at 919-966-3113 or to IRB_subjects@unc.edu. You do not have to use your name.

Participant Agreement:
I have read the information provided above and have asked all the questions I have at this time. I voluntarily agree to my and my child's participation in **the N**orth **C**arolina **N**ewborn **Ex**ome **S**equencing as **U**niversal **S**creening **(NC NEXUS); Principal Investigators:** Cynthia Powell, MD and Jonathan S. Berg, MD, PhD

_____ _____
Signature of Research Participant's Parent or Guardian Date

_____
Printed Name of Research Participant's Parent or Guardian and Relationship

_____ _____
Signature of Research Participant's Parent or Guardian Date

_____
Printed Name of Research Participant's Parent or Guardian and Relationship

_____ _____
Signature of Research Team Member Obtaining Consent Date

_____
Printed Name of Research Team Member Obtaining Consent

The NC NEXUS Study

The North **C**arolina **N**ewborn **Ex**ome **S**equencing
for **U**niversal **S**creening Study

Making the Right Decision for You and Your Family

[picture]

NC Nexus
North Carolina Newborn Exome Sequencing for Universal Screening

You are being invited to take part in a research study. This brochure gives you some basic facts about the study and tells you how you can learn more.

This brochure will help you learn more about NC NEXUS, including:

- The purpose of the study
- Genomic sequencing
- What you will be asked to do if you decide to participate in NC NEXUS

After reading this brochure, if you want to learn more about taking part in the study, a member of our study team can help you take the next step.

The NC NEXUS Study

What is the purpose of this study?

- Right after birth, all babies in the United States are tested for at least 30 health conditions. This test is called newborn screening.
- Doctors do newborn screening to find these health conditions and treat them early.
- If these conditions are not treated, they may cause problems that affect how a child develops. Some of these conditions may be very serious and can even lead to death. All of these conditions are very rare.
- The health conditions screened for in North Carolina are listed here: http://www.babysfirsttest.org/newborn-screening/states/north-carolina
- The NC NEXUS study wants to find out if genomic sequencing does a better job of finding these same health conditions and hundreds of others.
- [NEWBORN COHORT ONLY] The genomic sequencing done in NC NEXUS will not replace the newborn screening your child has at birth.
- The study also hopes to learn:
  - What parents think about when deciding if they want to have genomic sequencing for their child
  - The types of things that parents want to learn from genomic sequencing
  - If parents find it helpful to learn their child's genomic sequencing results
  - If a decision guide is useful to parents making these decisions

[picture]

"

What is genomic sequencing and how does it work?

- Genomic sequencing is a way to study a person's genetic makeup, or DNA.
- This test looks for differences in a person's DNA that could cause health problems.
- Because it looks at thousands of genes, genomic sequencing can find much more information than the current newborn screening test.
- NC NEXUS researchers are using genomic sequencing to find health conditions that newborns and young children *might* have.
- They are also looking at whether the test can lead to better treatment for any health conditions it finds.

What happens if you decide to learn more about this study and take part?

- If both parents are involved in the child's life, and both are available, they both need to agree to take part in the study. If a dad or mom has sole custody of the child, he or she can decide to take part in the study without the other parent. Please discuss this with a member of our study team if you have questions.
- You will be asked to answer a few questions to make sure you are eligible to take part in the study.
- You will read an information sheet that describes what would happen in the next step of the study.
- We will ask you for your phone number so we can talk to you by phone and answer any questions.
- After this phone call, if you agree to take part in the next step of the study, you will answer some other questions online.
- We will send you a link to the online decision guide. You can complete it at home. If both parents are in the study, they need to listen to and use the online decision guide together. If you do not have Internet access at your home, you can come to UNC Hospitals to use the decision guide.
- This decision guide will tell you more about genomic sequencing and what it means for your child. It will explain how genomic sequencing works to find the same health conditions newborn screening does, along with other conditions like them. Then, the decision guide will help you decide if you want genomic sequencing.
- After completing the decision guide, if you decide you want to have genomic sequencing for newborn screening conditions and others like them, or if you want to talk to a genetic counselor before deciding, you will come to UNC Hospitals to meet with one of our genetic counselors.
- A genetic counselor understands the role that genetics can play in health and will answer any questions about the study.
- If you decide you do not want to take part in the study, we will ask you to complete a few more questions online.

What happens after you decide to have genomic sequencing for newborn screening conditions?

- After your baby is born, you will come to the UNC Hospitals with your child at a convenient time for you. OR You will come to the UNC Hospitals at a time convenient for you, and bring your child.
- At that visit, a small sponge will be lightly rubbed inside your child's mouth to get saliva (spit) that will be used for genomic sequencing.
- You will learn the results for health condition found with current newborn screening, along with more than a hundred other conditions.
- For most families who take part in the study, none of these very rare health conditions will be found.
- You will take an online survey.
- Some parents will have the choice to learn additional other genetic information about their child. We do not know if learning this additional information will help your child medically, if it is not helpful, or if it is harmful for you to learn. The parents who will be asked to make decisions about this additional information will be chosen by a random drawing.
- If you are chosen in the drawing and want to take part in the next step of the study, you will use another decision guide to help you decide if learning about other information is right for you and your family. You will also speak to a genetic counselor who can answer your questions.
- No matter which group you are in, if genomic sequencing finds that your child has a genetic cause for a health condition:
  - A second test will be done to make sure that the results of the first test are correct.
  - A genetic counselor and a doctor will meet with you to discuss the results.
  - You will be referred for medical or other services that your child needs.
- You can stop taking part in the study at any point if you do not want to continue. Your child will still receive care from doctors as he or she usually would.
- There is no cost to you for the study visits or the genomic sequencing done during the time you are taking part in the study. Each parent will get a $20 check after each online survey is completed. You will also get parking vouchers for the study visits.

Is the NC NEXUS Study Right for You and Your Family?

There are lots of things to think about when deciding to learn more or take part in a study like NC NEXUS. Here are some things that other parents thought about when making their decision. Remember, right now you are only being asked to agree to learn more about the study. Later on, we will help you decide if you want your child to have genomic sequencing as part of the study.

[Picture and quote here— "It's important for me to learn more about genomic sequencing. I'm the type of person that just wants all the information."]

Reasons why you might want to learn more and be a part of the NC NEXUS study

- You want to learn more about what genomic sequencing is and how it works.
- You are curious about using an online decision guide that will give you more information about the study.
- You can choose whether to have genomic sequencing for your child and to learn about health conditions like those found with the current newborn screening test. OR You can choose whether to have genomic sequencing for your child. The results may help doctors better understand your child's health condition.
- Agreeing to learn more about the study does not mean you have agreed to have genetic sequencing for your child.
- You are interested in having genomic sequencing for your child, but you want to make sure that it is the right decision for you and your family.

If these reasons are important to you, then you may want to learn more about the NC NEXUS study and decide to take part in it.

[Picture and quote here] “I don’t want to learn anything more about the study. I don’t think participating will be helpful or is right for my family right now.”

Reasons why you might not want to learn more about the NC NEXUS study

- You are satisfied with knowing your child will have current newborn screening. OR You are satisfied with the information you currently have about your child’s health condition. You do not think other information would be helpful.
- You do not want to learn more about genetics and health or genomic sequencing.
- You do not want to use an online decision guide to get more information about the study.
- You do not have the time to come to UNC Hospitals to take part in the study.
- You already decided that you do not want to take part in the study and feel that is the right decision for you and your family.

If these reasons are important to you, then you may decide you do not want to learn more about the NC NEXUS study.

Should My Family Learn More about the NC NEXUS Study?

Make the decision that is best for you and your family. There are no right or wrong choices.

Here are some questions to help you decide:

Yes	No	
❒	❒	Would you like to learn more about genetics and genomic sequencing?
❒	❒	Are you interested in learning more about the genetic health conditions that genomic sequencing may find in your child?
❒	❒	Do you have time to learn more about the NC NEXUS Study?
❒	❒	Would you like to have the choice to have genomic sequencing for your child?
❒	❒	Do you think you might like to be in the NC NEXUS Study?

If you have more ***Yes*** answers than ***No*** answers, you and your family may be ready to learn more about this study and then to decide if you would like to take part in the study.

If you have more ***No*** answers than ***Yes*** answers, taking part in this study may not be right for you and your family.

Please talk to a member of our study team about these and any other questions to help you decide if you would like to learn more about the NC NEXUS study. Remember that even if you

decide to learn more about the study and then decide to take part in genetic sequencing for your child, you can stop taking part in the study at *any* point in time.

To find out more about the NC NEXUS study:

WEBSITE TOLL FREE NUMBER

This brochure was developed with support from the National Institutes of Health's Eunice Kennedy Shriver National Institute of Child Health and Human Development and the National Human Genome Research Institute.

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

RTI INTERNATIONAL

NC NEXUS – Online Decision Aid 1 – Shooting Script
Version: 05/19/2015

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.1	ALL			The North Carolina Newborn Exome Sequencing for Universal Screening Study (NC NEXUS)	Login user name and password fields. Enter button.		(1) Welcome/Login
D1.2	ALL	Welcome to the NC NEXUS decision guide. This decision guide will help you learn more about the NC NEXUS Study, including: • The purpose of the study • How genes can affect your child's health. • Genomic sequencing, and • The types of results that might be found. The guide will also help you decide if you want to have genomic sequencing for your child.	Welcome	*Text onscreen:* What will you learn about with this decision guide? (headline) • Purpose of the NC NEXUS study • How genes can affect your child's health • Genomic sequencing • Results that might be found *NOTE:* Each bullet appears on screen in time with narration.	Next button Replay button		(3) General content, text list

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.3	ALL	Before getting started, let's look at the navigation controls you can use to move through the decision guide. If you need to pause for a moment and come back, click the play/pause button. If you want to listen to information on the screen again, click the replay button. On some screens you will be asked questions. One way to answer is with a slider scale. Click and drag the slider, moving it to the desired position. Then click the submit button. Other questions will ask you to sort items. Click and drag each item into the desired box. When you are done sorting the items, click the submit button. Lastly, some questions will ask you to select "yes" or "no." You can pick your answers either by touching the screen or by clicking your selection. Here is the next button to move forward. If you're ready to begin, please click the next button.		*Text on screen:* How to use this online decision guide. (headline) *Note:* Visual demo showing navigation controls and types of response scales. Animate and highlight control buttons as they are talked about in the narration.	Next button Replay button		(2) How to use the website

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.4	ALL	What is NC NEXUS? NC NEXUS is a research study that offers you the option to have genomic sequencing for your child. One goal of NC NEXUS is to find out how well genomic sequencing finds over 30 health conditions that all babies in North Carolina are tested for at birth. This test is called *newborn screening*. Genomic sequencing might also find hundreds of other important health conditions that are not part of newborn screening, but are otherwise similar to them.	NC NEXUS is a research study offering genomic sequencing for your child	*Text on screen:* What is NC NEXUS? (headline) Then images to illustrate following each of the three points.	Next button Replay button		(4) General content, image
D1.5	ALL	The NC NEXUS study team hopes to learn • How parents like you decide if they want to have genomic sequencing for their child		*Text on screen:* What is the goal of NC NEXUS? (headline) • To learn how parents make	Next button Replay button		(3) General content, text list

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		• The types of things that parents want to learn from genomic sequencing • Whether parents find it helpful to learn their child's genomic sequencing results, and • Whether this decision guide helps parents make informed choices about genomic sequencing		decisions about genomic sequencing • What parents want to learn from genomic sequencing • If parents find genomic sequencing results helpful • If parents find this decision guide helpful [*NOTE:* Each bullet appears on screen in time with narration. Unless that is overly complex]			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.6	ALL	What is newborn screening? Newborn screening is testing done when a baby is born to find serious health conditions before a child becomes sick. The health conditions found by newborn screening can cause disability or even death if they are not treated early.	Newborn screening tests for serious health conditions.	*Text on screen:* What is newborn screening? (headline) Newborn screening looks for conditions that cause serious health problem: [*NOTE:* This is a list of signs, symptoms and/or outcomes for many of the conditions tested for with newborn screening. Visuals may be useful here to get at the seriousness of the conditions.] • Intellectual disability • Delayed physical development • Hearing loss	Next button Replay button		(4) General content,

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				• Heart and breathing problems • Seizures • Coma • Early death			
D1.7	ALL	Most of the conditions are rare, which means that few newborns will ever have them. About 13 out of every 10,000 babies born in the United States have a condition that can be found by newborn screening.	Most conditions that are part of newborn screening are rare.	What is newborn screening? (headline) Close shot of 13 baby icons in row; fast zoom out to reveal the 13 babies are part of a grid of 10,000 baby icons.	Next button Replay button		(4) General content, images animated

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.8.Newborn	IF NEWBORN COHORT	The conditions that are part of newborn screening have treatments. If a child has one of these conditions, finding out early can help keep him or her from getting sick. It might even save the child's life. If you decide to have genomic sequencing as part of the NC NEXUS study you would still have regular newborn screening when your baby is born.	Newborn screening conditions are treatable	What is newborn screening? (headline) *Image*: baby at doctor's office?	Next button Replay button		(4) General content, images
D1.8.Diagnosed	IF DIAGNOSED COHORT	All the conditions found through newborn screening have treatments. If a child has one of these conditions, finding out early can help keep him or her from getting sick. It might even save the child's life.	Newborn screening conditions are treatable	What is newborn screening? (headline) *Image*: baby at doctor's office?	Next button Replay button		(4) General content, images
D1.9	ALL	What is genomic sequencing? Each cell in a person's body contains a copy of his or her *DNA*. DNA provides the instructions a person's body needs to grow and function. These instructions are divided into genes. Just like how the order of words in a sentence is important for understanding what you read, the	DNA contains the instructions your child's body needs to develop and function.	What is genomic sequencing? (headline) Illustration of double helix, preferably one that labels the nucleotide bases A, C, T, and G	Next button Replay button		(4) General content, images

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		order in a person's DNA is important for telling the body's cells what to do.					
D1.10	ALL	Differences in a person's DNA can cause people to have different forms of the same gene. Most often these gene differences will have no effect on health, but some gene differences can lead to health problems. Genomic sequencing is a way to look for differences in your child's DNA that could cause rare but serious health problems.	Genomic sequencing is a way to look for gene differences that might cause health problems.	What is genomic sequencing? (headline) Pan across two flattened strings of DNA A,C,T,and Gs arranged one above the other. Most letters in the two sequences are identical, but every so often a letter is different; highlight the differences as they arrive at center of screen.	Next button Replay button		(4) general content, images
D1.11	ALL	What Can Genomic Sequencing Tell You About Your Child? In the NC NEXUS study, genomic sequencing will look for gene differences that cause the same health conditions that are found through newborn screening, plus more than a hundred health conditions like them.		What can genomic sequencing tell you about your child? (headline) Images used to illustrate newborn screening; heel prick, blood spot card. The images should somehow convey the similarity between conditions detectable	Next button Replay button		(4) General content, images

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				by current newborn screening and conditions detectable by genomic sequencing.			
D1.12.Newborn	IF NEWBORN COHORT	Researchers are still trying to understand how useful genomic sequencing is compared to other tests that tell people about their health. The NC NEXUS study team wants to learn if genomic sequencing can improve current newborn screening. They also want to see if genomic sequencing can be used to find conditions that are not part of current newborn screening, but could be in the future. These are rare conditions that affect children early in life and can be improved with early treatment.		What can genomic sequencing tell you about your child? (headline)	Next button Replay button		(4) General content, image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.12.Diagnosed	IF DIAGNOSED COHORT	Researchers are still working to understand how useful genomic sequencing is compared to other tests that tell people about their health. The NC NEXUS study team wants to learn if genomic sequencing can find gene differences that cause the condition that your child currently has. They also want to see if genomic sequencing can be used to find conditions that are not part of current newborn screening, but could be in the future. These are rare conditions that affect children early in life and can be improved with early treatment.		What can genomic sequencing tell you about your child? (headline)	Next button Replay button		(4) General content, image
D1.13	ALL	What is a medically actionable childhood condition? In addition to the more than 30 health conditions that are part of current newborn screening, the NC NEXUS study team will look for over a hundred serious conditions that are like them. • These conditions usually begin during childhood and are medically actionable; that is, they • Can be improved with early treatment,	Medically actionable childhood conditions begin during childhood and can be improved with treatment.	What is a medically actionable childhood condition? (headline) Show pictures that indicate medical treatment. Medically actionable childhood conditions… • Begin during childhood • Can be improved with early treatment	Next button Replay button		(3) General content, image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		• Have treatments with benefits that typically outweigh the risks, and • Are well-understood by doctors.		• Benefit of treatment outweighs risks • Are well understood by doctors			
D1.14	ALL	Pompe disease is a condition that is medically actionable. Pompe disease affects about 1 out of every 40,000 people in the United States and usually begins in the first few months after birth. Children who have Pompe disease have weak muscles so they are not able to do things like hold their heads up or crawl at the same age as other babies. Other signs of Pompe disease include an enlarged liver and heart problems. If untreated, Pompe disease often leads to heart failure and death in the first year of life.	Pompe disease is an example of a medically actionable childhood condition not currently part of newborn screening.	What is a medically actionable childhood condition? (headline)	Next button Replay button		(4) general content
D1.15	ALL	What can genomic sequencing tell you about medically actionable childhood conditions?	NC NEXUS will use genomic sequencing to look for gene	What can genomic sequencing tell you about medically actionable childhood	Next button Replay button		(3) or (4) General content

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		The NC NEXUS team will look for gene differences that are known to cause specific health conditions. For some medically actionable childhood conditions, these gene differences determine how the condition will affect a child. For other conditions, these gene differences are not the *only* thing that determines how the condition will affect a child, but they are known to play an important role in a child developing the condition. Looking for these gene differences in your child's DNA can tell if he or she is more likely to get certain health conditions. Still, knowing for sure when a child will show signs and how severe they will be is often difficult because other genetic and environmental factors also play a part in most conditions and how they develop.	differences that lead to specific health conditions. For some conditions, these gene differences are the only thing that matters; for other conditions, gene differences are not the *only* cause.	conditions? (headline) We need a visual that somehow depicts environmental and genetic conditions			
D1.16	ALL	How common is it for genomic sequencing to find gene differences that lead to medically actionable childhood conditions? It is not known for sure how often genomic sequencing will find gene differences that lead to a medically actionable childhood condition. This is one of the things the NC NEXUS study will try to find out. The best estimate is that sequencing will find gene	The NC NEXUS study team wants to find out how often genomic sequencing will find gene differences that lead to a health problem.	How common is it for genomic sequencing to find gene differences that lead to medically actionable childhood conditions? (headline)	Next button Replay button		(3) or (4) General content

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		differences that cause conditions like those found with current newborn screening in less than 1% of children.		Visual to depict that it is unsure exactly how likely it is that a gene difference will be found, but less than 1 out of 100 children tested.			
D1.17.Newborn	IF NEWBORN COHORT	What will happen if you decide to have genomic sequencing for your child? • If you decide you want your child to have genomic sequencing in NC NEXUS, you will come to the UNC Hospitals after your baby is born. The visit will take approximately one hour. • We will ask for your consent to participate in the study by signing a consent form. • If you choose to have genomic sequencing for your child, a small sponge will be lightly rubbed inside your child's mouth to get saliva (spit) that will be used for genomic sequencing. • You will learn results related to medically actionable childhood conditions that are found with current newborn screening and many other conditions like them.		What happens if you choose to have genomic sequencing for your child? (headline) • 1 hour visit to UNC Hospitals • Sign a consent form • Cheek swab to collect DNA sample • Learn genomic sequencing results related to medically actionable childhood conditions	Next button Replay button		(3) General content, text list

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		• All parents in the study will complete several online surveys.		• Complete online surveys			
D1.17.Diagnosed	IF DIAGNOSED COHORT	What will happen if you decide to have genomic sequencing for your child? • If you decide you want your child to have genomic sequencing in NC NEXUS, you will come to the UNC Hospitals with your child. The visit will take approximately one hour. • We will ask for your consent to participate in the study by signing a consent form. • If you choose to have genomic sequencing for your child, a small sponge will be lightly rubbed inside your child's mouth to get saliva (spit) that will be used for genomic sequencing. • You will learn results related to medically actionable childhood conditions that are found with current newborn screening and many other conditions like them. • All parents in the study will complete several online surveys.		What happens if you choose to have genomic sequencing for your child? (headline) • 1 hour visit to UNC Hospitals • Sign a consent form • Cheek swab to collect DNA sample • Learn genomic sequencing results related to medically actionable childhood conditions • Complete online surveys	Next button Replay button		(3) General content, text list

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template

D1.18	ALL	• If genomic sequencing finds that your child has any gene differences that cause these health conditions:
		  • The results will be confirmed with another test.
		  • A genetic counselor and a doctor will meet with you to discuss the results.
		  • You will be referred for medical or other services your child needs for those conditions.
				What if genomic sequencing finds gene differences that cause a health condition? (headline)
				
				• Results will be confirmed with another test
				• A genetic counselor and a doctor will discuss the results with you.
				• Your child will be referred for medical or other services for those conditions
					Next button
					
					Replay button
							(3) General content, text list

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.19.Single	IF SINGLE	• You will not be charged for the study visits or genomic sequencing. Each parent will be given a $20 Visa card after each survey is completed. You will also get parking vouchers for study visits. At any point in the study, you can stop participation if you don't want to continue. Your child would still receive regular care from doctors as they usually would.		What else should you know if you choose to have genomic sequencing for your child? (headline)	Next button Replay button		(3) or (4) General content
D1.19.Couple	IF COUPLE	• You will not be charged for the study visits or genomic sequencing. You will be given a $20 Visa card after each survey is completed. You will also get parking vouchers for study visits. At any point in the study, you can stop participation if you don't want to continue. Your child would still receive regular care		What else should you know if you choose to have genomic sequencing for your child? (headline)	Next button Replay button		(3) or (4) General content

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		from doctors as they usually would.					

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.20	ALL	Which way are you leaning? If you had to decide right now about having genomic sequencing for your child as part of NC NEXUS, which way are you leaning? Leaning away from my child having genomic sequencing Uncertain Leaning toward my child having genomic sequencing		Which way are you leaning? (headline) If you had to decide right now about having genomic sequencing for your child as part of NC NEXUS, which way are you leaning? Interactive scale anchored by leaning away…leaning toward. Leaning away------- Uncertain------- Leaning Toward	Interactive response scale; Submit button Next button;	Capture numerical value associated with position on scale. Treat as scale ranging from 0-100, where anchor points are 0 = left-most position, leaning away 50= center position, Uncertain 100=right-most position, leaning toward Intermediate values captured as integers.	(5) Leaning yes/no screen

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
						Capture time in milliseconds spent on this screen	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.21.Newborn. Single	IF NEWBORN COHORT & SINGLE	What matters most to you when deciding if your child should have genomic sequencing as part of NC NEXUS? There are lots of things to think about when deciding if you want your child to have genomic sequencing as part of NC NEXUS. Are the following reasons important or unimportant to you?		What matters most to you? (Headline) Reasons for your child to have genomic sequencing as part of NC NEXUS • Knowing your child has a genetic condition may help him or her get early treatment and support services. • Knowing your child has a genetic condition may help you and your family be prepared if he or she develops the condition. • Genomic sequencing in NC NEXUS may help doctors understand genetic health conditions better.	Sorting task for users to move boxes with 'reasons for' into <u>two</u> bins labeled 'Important' and 'Unimportant' 2 interactive textboxes that allows users to write in 2 additional 'reasons for' that is not listed; write-in textbox is also sortable Allow participants to continue to next screen only after at least one statement is moved into a sorting category. Submit button Next button	Capture which bin (i.e., important, unimportant) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it not sorted into any box (i.e., values for each statement: important,	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				• Genomic sequencing in NC NEXUS may help scientists make better tools for finding serious health conditions before people get sick. • You would rather not wait to see if any problems occur to find out if your child may to have a genetic condition. • *Are there any other reasons you can think of? Please type them here.* Move at least one statement to continue.		unimportant , not sorted)	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.21.Diagnosed .Single	IF DIAGNOSED COHORT & SINGLE	What matters most to you when deciding if your child should have genomic sequencing as part of NC NEXUS? There are lots of things to think about when deciding if you want your child to have genomic sequencing as part of NC NEXUS. Are these reasons important or unimportant to you?		What matters most to you? (Headline) Reasons for your child to have genomic sequencing as part of NC NEXUS • Genomic sequencing in NC NEXUS may help doctors understand your child's health condition better. • Genomic sequencing for your child in NC NEXUS may provide information about the risk for others in your family of having a child with the same condition. • Knowing the genetic cause of your child's health condition could	Sorting task for users to move boxes with 'reasons for' into two bins labeled 'Important' and 'Unimportant' 2 interactive textboxes that allows users to write in 2 additional 'reasons for' that is not listed; write-in textbox is also sortable Allow participants to continue to next screen only after at least one statement is moved into a sorting category. Submit button Next button	Capture which bin (i.e., important, unimportant) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it not sorted into any box (i.e., values for each statement: important,	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				help your family plan for the future. • Genomic sequencing in NC NEXUS may help scientists make better tools for finding serious health conditions before people get sick. • You want to learn everything you can about your child's health condition. • *Are there any other reasons you can think of? Please type them here.* Move at least one statement to continue.		unimportant, not sorted)	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.21.Newborn. Couple	IF NEWBORN COHORT & COUPLE	What matters most to you when deciding if your child should have genomic sequencing as part of NC NEXUS? There are lots of things to think about when deciding if you want your child to have genomic sequencing as part of NC NEXUS. Are the following reasons important or unimportant to you? If you and your partner disagree about the importance of a reason, you can move it into the box labelled "We disagree."		What matters most to you? (Headline) Reasons for your child to have genomic sequencing as part of NC NEXUS • Knowing your child has a genetic condition may help him or her get early treatment and support services. • Knowing your child has a genetic condition may help you and your family be prepared if he or she develops the condition. • Genomic sequencing in NC NEXUS may help doctors understand genetic health conditions better.	Sorting task for users to move boxes with 'reasons for' into three bins labeled 'Important,' 'Unimportant,' and 'We disagree' 2 interactive textboxes that allows users to write in 2 additional 'reasons for' that is not listed; write-in textbox is also sortable Allow participants to continue to next screen only after at least one statement is moved into a sorting category. Submit button Next button	Capture which bin (i.e., important, unimportant, disagree) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it not sorted into any box (i.e., values for each statement:	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				• Genomic sequencing in NC NEXUS may help scientists make better tools for finding serious health conditions before people get sick. • You would rather not wait to see if any problems occur to find out if your child may to have a genetic condition. • *Are there any other reasons you can think of? Please type them here.* Move at least one statement to continue.		important, unimportant, disagree, not sorted)	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.21.Diagnosed .Couple	IF DIAGNOSED COHORT & COUPLE	What matters most to you when deciding if your child should have genomic sequencing as part of NC NEXUS? There are lots of things to think about when deciding if you want your child to have genomic sequencing as part of NC NEXUS. Are these reasons important or unimportant to you? If you and your partner disagree about the importance of a reason, you can move it into the box labelled "We disagree."		What matters most to you? (Headline) Reasons for your child to have genomic sequencing as part of NC NEXUS • Genomic sequencing in NC NEXUS may help doctors understand your child's health condition better. • Genomic sequencing for your child in NC NEXUS may provide information about the risk for others in your family of having a child with the same condition. • Knowing the genetic cause of your child's health condition could	Sorting task for users to move boxes with 'reasons for' into three bins labeled 'Important,' 'Unimportant,' and 'We disagree' 2 interactive textboxes that allows users to write in 2 additional 'reasons for' that is not listed; write-in textbox is also sortable Allow participants to continue to next screen only after at least one statement is moved into a sorting category. Submit button Next button	Capture which bin (i.e., important, unimportant, disagree) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it not sorted into any box (i.e., values for each statement:	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				help your family plan for the future. • Genomic sequencing in NC NEXUS may help scientists make better tools for finding serious health conditions before people get sick. • You want to learn everything you can about your child's health condition. • *Are there any other reasons you can think of? Please type them here.* Move at least one statement to continue.		important, unimportant, disagree, not sorted)	
D1.22.Newborn. Single	IF NEWBORN COHORT& SINGLE	Are these reasons important or unimportant to you?		What matters most to you? (Headline) Reasons for your child <u>not</u> to have genomic sequencing as part of NC NEXUS.	Sorting task for users to move boxes with 'reasons against' into <u>two</u> bins labeled 'Important' and 'Unimportant'	Capture which bin (i.e., important, unimportant) user sorts each 'reason against'	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				• Waiting for genomic sequencing results may cause you to worry or feel anxious. • You do not feel prepared to learn that your child may have a genetic condition. • Knowing that the NC NEXUS study team will have your child's genomic sequencing results makes you uncomfortable. • You are satisfied with knowing that your child will have standard newborn screening. • You would rather wait to see if your child has any	Interactive textbox that allows users to write in a 'reason against' that is not listed; write-in textbox is also sortable Allow participants to continue to next screen only after at least one statement is moved into a sorting category. Submit button Next button	Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it not sorted into any box (i.e., values for each statement: important, unimportant, not sorted)	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				problems before having genetic testing. • *Are there any other reasons you can think of? Please type them here.* Move at least one statement to continue.			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.22.Diagnosed .Single	IF DIAGNOSED COHORT & SINGLE	Are these reasons important or unimportant to you?		What matters most to you? (Headline) Reasons for your child not to have genomic sequencing as part of NC NEXUS. • Waiting for genomic sequencing results may cause you to worry or feel anxious. • You do not feel prepared to learn that your child may have another health problem. • Knowing that the NC NEXUS study team will have your child's genomic sequencing results makes you uncomfortable. • You are satisfied with the medical	Sorting task for users to move boxes with 'reasons against' into two bins labeled 'Important' and 'Unimportant' Interactive textbox that allows users to write in a 'reason against' that is not listed; write-in textbox is also sortable Allow participants to continue to next screen only after at least one statement is moved into a sorting category. Submit button Next button	Capture which bin (i.e., important, unimportant) user sorts each 'reason against' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it not sorted into any box (i.e., values for each statement: important,	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				care your child receives and don't think other information would be helpful. • You would rather wait to see if your child has any problems before having genetic testing • *Are there any other reasons you can think of? Please type them here.* Move at least one statement to continue.		unimportant, not sorted)	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.22.Newborn. Couple	IF NEWBORN COHORT & COUPLE	Are these reasons important or unimportant to you? Or do you and your partner disagree?		What matters most to you? (Headline) Reasons for your child not to have genomic sequencing as part of NC NEXUS. • Waiting for genomic sequencing results may cause you to worry or feel anxious. • You do not feel prepared to learn that your child may have a genetic condition. • Knowing that the NC NEXUS study team will have your child's genomic sequencing results makes you uncomfortable. • You are satisfied with knowing that	Sorting task for users to move boxes with 'reasons for' into three bins labeled 'Important,' 'Unimportant,' and 'We disagree' 2 interactive textboxes that allows users to write in 2 additional 'reasons against' that is not listed; write-in textbox is also sortable Allow participants to continue to next screen only after at least one statement is moved into a sorting category. Submit button Next button	Capture which bin i.e., important, unimportant, disagree) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it not sorted into any box (i.e., values for each statement:	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				your child will have standard newborn screening. • You would rather wait to see if your child has any problems before having genetic testing. • *Are there any other reasons you can think of? Please type them here.* Move at least one statement to continue.		important, unimportant, disagree, not sorted)	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.22.Diagnosed .Couple	IF DIAGNOSED COHORT & COUPLE	Are these reasons important or unimportant to you? Or do you and your partner disagree?		What matters most to you? (Headline) Reasons for your child <u>not</u> to have genomic sequencing as part of NC NEXUS. • Waiting for genomic sequencing results may cause you to worry or feel anxious. • You do not feel prepared to learn that your child may have another health problem. • Knowing that the NC NEXUS study team will have your child's genomic sequencing results makes you uncomfortable. • You are satisfied with the medical	Sorting task for users to move boxes with 'reasons for' into <u>three</u> bins labeled 'Important,' 'Unimportant,' and 'We disagree' 2 interactive textboxes that allows users to write in 2 additional 'reasons against' that is not listed; write-in textbox is also sortable Allow participants to continue to next screen only after at least one statement is moved into a sorting category. Submit button Next button	Capture which bin (i.e., important, unimportant, disagree) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it not sorted into any box (i.e., values for each statement:	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				care your child receives and don't think other information would be helpful. • You would rather wait to see if your child has any problems before having genetic testing • *Are there any other reasons you can think of? Please type them here.* Move at least one statement to continue.		important, unimportant, disagree, not sorted)	
D1.23.Newborn. Single	IF NEWBORN COHORT & SINGLE	Here are the reasons for and against genomic sequencing for your child that matter most to you.		What matters most to you? (Headline) Two boxes on screen. One is labelled "Reasons for your child to have	Visually present whether user sorted 'reasons for' as important on screen D1.21.Newborn.Single and 'reasons against' as		(7) Values clarification, review

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				genomic sequencing as part of NC NEXUS." In this box, list the reasons that the user sorted into the 'important' box from screen D1.21.Newborn.Single "Reasons for your child not to have genomic sequencing as part of NC NEXUS" In this box, list the reasons that the user sorted into the 'important' box from screen D1.22.Newborn.Single	important on screen D1.22.Newborn.Single Any statement that was not sorted into a category is not displayed on review screen. Replay button Next button		

D1.23.Diagnosed.Single	IF DIAGNOSED COHORT & SINGLE	Here are the reasons for and against genomic sequencing for your child that matter most to you.		What matters most to you? (Headline) Two boxes on screen. One is labelled "Reasons for your child to have genomic sequencing as part of NC NEXUS." In this box, list the reasons that the user sorted into the 'important' box from screen D1.21.Diagnosed.Single "Reasons for your child not to have genomic sequencing as part of NC NEXUS" In this box, list the reasons that the user sorted into the 'important' box from screen D1.22.Diagnosed.Single	Visually present whether user sorted 'reasons for' as important on screen D1.21.Diagnosed.Single and 'reasons against' as important on screen D1.22.Diagnosed.Single Any statement that was not sorted into a category is not displayed on review screen. Replay button Next button		(7) Values clarification, review

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.23.Newborn.Couple	IF NEWBORN COHORT & COUPLE	Here are the reasons for and against genomic sequencing for your child that matter most to you.		What matters most to you? (Headline) Three boxes on screen. One is labelled "Reasons for your child to have genomic sequencing as part of NC NEXUS." In this box, list the reasons that the user sorted into the 'important' box from screen D1.21.Newborn.Couple "Reasons for your child not to have genomic sequencing as part of NC NEXUS" In this box, list the reasons that the user sorted into the 'important' box from screen D1.22.Newborn.Couple "Reasons that you and your partner	Visually present whether user sorted 'reasons for' as important on screen D1.21.Newborn.Couple, the 'reasons against' as important on screen D1.22.Newborn.Couple, or any reasons sorted into 'we disagree' on D1.21.Newborn.Couple or D1.22.Newborn.Couple Any statement that was not sorted into a category is not displayed on review screen. Replay button Next button		(7) Values clarification, review

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				disagree about" In this box, list the reasons that the user sorted into the 'We disagree' box from screen D1.21.Newborn.Couple or D1.22.Newborn.Couple			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.23.Diagnosed.Couple	IF DIAGNOSED COHORT & COUPLE	Here are the reasons for and against genomic sequencing for your child that matter most to you.		What matters most to you? (Headline) Three boxes on screen. One is labelled "Reasons for your child to have genomic sequencing as part of NC NEXUS." In this box, list the reasons that the user sorted into the 'important' box from screen D1.21.Diagnosed.Couple "Reasons for your child not to have genomic sequencing as part of NC NEXUS" In this box, list the reasons that the user sorted into the 'important' box from screen D1.22.Diagnosed.Couple "Reasons that you and your partner	Visually present whether user sorted 'reasons for' as important on screen D1.21.Diagnosed.Couple, the 'reasons against' as important on screen D1.22.Diagnosed.Couple, or any reasons sorted into 'we disagree' on D1.21.Diagnosed.Couple or D1.22.Diagnosed.Couple Replay button Next button		(7) Values clarification, review

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				disagree about" In this box, list the reasons that the user sorted into the 'We disagree' box from screen D1.21.Diagnosed.Couple or D1.22.Diagnosed.Couple			
D1.24.Single	IF SINGLE	You should make the decision that is best for you and your family. There are no right or wrong choices.		Questions to Help You Decide(headline) Yes No	Check boxes/buttons for users to select yes or no for each question	Capture y/n answers to each question;	(9) Questions to help decide, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		Here are some questions that can help you decide if you want your child to have genomic sequencing: • Will having genomic sequencing for your child as part of NC NEXUS help you learn things that are important to you? • Do you have enough information to make a decision about having genomic sequencing for your child? • Are you prepared to learn genomic sequencing results for medically actionable childhood conditions? • Are you interested in learning if your child has gene differences that can cause medically actionable childhood conditions? • Are you confident you can make the decision that is right for you and your family?		❒ ❒ Will genomic sequencing help you learn things that are important to you? ❒ ❒ Do you have enough information to make a decision about	Submit button Next button;	Capture time in milliseconds spent on this screen	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				having genomic sequencing for your child? ❒ ❒ Are you prepared to learn genomic sequencing results for medically actionable childhoo			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				d conditions? ❒ ❒ Are you interested in learning if your child has gene differences that can cause medically actionable childhood cond			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				itions? ❒ ❒ Are you confident you can decide?			
D1.24.Couple	IF COUPLE	Here are some questions that can help you decide if you want your child to have genomic sequencing: • Will having genomic sequencing for your child as part of NC NEXUS help you learn things that are important to you? • Do you have enough information to make a decision about having genomic sequencing for your child? • Are you prepared to learn genomic sequencing results for medically actionable childhood conditions? • Are you interested in learning if your child has gene differences that can cause		Questions to Help You Decide (headline) Yes No ❒ ❒ Will genomic sequencing help you learn things that are important to you?	Check boxes/buttons for users to select yes or no for each question Submit button Next button;	Capture y/n answers to each question; Capture time in milliseconds spent on this screen	(9) Questions to help decide, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		medically actionable childhood conditions? • Are you and your partner confident you can make the decision that is right for you and your family?		❒ ❒ Do you have enough information to make a decision about having genomic sequencing for your child? ❒ ❒ Are you prepared to learn			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				genomic sequencing results for medically actionable childhood conditions? ❒ ❒ Are you interested in learning if your child has gene differenc			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				es that can cause medically actionable childhood conditions? ❒ ❒ Are you and your partner confident you can decide?			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.25.Single	IF SINGLE	If you answered *Yes* to more of the questions above, maybe you are ready for your child to have genomic sequencing. If you answered *No* to more, maybe this is not the right decision for your family at this time. Or you might still need more time or information to decide. You should make the decision that is best for you and your family. There are no right or wrong choices.		Questions to Help You Decide (headline) • Will genomic sequencing help you learn things that are important to you? • Do you have enough information to make a decision about having genomic sequencing for your child? • Are you prepared to learn genomic sequencing results for medically actionable childhood conditions?	Visually show whether user selected yes/no for each question from screen 'D1.24.Single' Replay button Next button		(10) Questions to help decide, review

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				• Are you interested in learning if your child has gene differences that can cause medically actionable childhood conditions? • Are you confident you can decide? If you answered *Yes* to more of the questions above, maybe you are ready for your child to have genomic sequencing. If you answered *No* to more, maybe this is not the right decision for your family at this time. Or you might still need more time or information to decide.			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.25.Couple	IF COUPLE	If you answered *Yes* to more of the questions above, maybe you are ready for your child to have genomic sequencing. If you answered *No* to more, maybe this is not the right decision for your family at this time. Or you might still need more time or information to decide. You should make the decision that is best for you and your family. There are no right or wrong choices.		Questions to Help You Decide (headline) • Will genomic sequencing help you learn things that are important to you? • Do you have enough information to make a decision about having genomic sequencing for your child? • Are you prepared to learn genomic sequencing results for medically actionable childhood conditions?	Visually show whether user selected yes/no for each question from screen 'D1.24.Couple' Replay button Next button		(10) Questions to help decide, review

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				• Are you interested in learning if your child has gene differences that can cause medically actionable childhood conditions? • Are you and your partner confident you can decide? If you answered *Yes* to more of the questions above, maybe you are ready for your child to have genomic sequencing. If you answered *No* to more, maybe this is not the right decision for your family at this time. Or you might still need more time or information to decide.			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.26.Single	IF SINGLE	You have a decision to make at this time. Remember, even if you decide to schedule a study visit, you can change your mind and stop participation in this study at any point in time. • No, I do not want my child to have genomic sequencing at this time for conditions like those found in newborn screening. I do not want to schedule a study visit. • I'm not sure if I want my child to have genomic sequencing or not, but I want to schedule a study visit with a genetic counselor at UNC Hospitals to discuss the decision. • Yes, I want my child to have genomic sequencing for conditions like those found in newborn screening. I want to schedule a study visit with a genetic counselor at UNC Hospitals.		Making a Decision about Genomic Sequencing (headline) You have a decision to make at this time. Remember, even if you decide to schedule a study visit, you can change your mind and stop participation in this study at any point in time. Interactive scale anchored by… • No, I do not want my child to have genomic sequencing at this time. • I'm not sure • Yes, I want genomic sequencing for my child.	Interactive response scale (slider); Submit button Next button;	Capture selection-yes/no/not sure Capture numerical value associated with position on scale. Treat as scale ranging from 0-100. In addition to capturing integer values in dataset, values will also be used for conditional piping on screens"D1.27…" 3-point categorical values, where	(11) Decision choices

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
						anchor points are 0-33 = left third, No 34-66= center third, Not sure 67-100= right third, Yes Capture time in milliseconds spent on this screen	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.26.Couple	IF COUPLE	You have a decision to make at this time. Remember, even if you decide to schedule a study visit, you can change your mind and stop participation in this study at any point in time. • No, we do not want our child to have genomic sequencing at this time for conditions like those found in newborn screening. We do not want to schedule a study visit. • We're not sure if we want my child to have genomic sequencing or not, but we want to schedule a study visit with a genetic counselor at UNC Hospitals to discuss the decision. • Yes, we want our child to have genomic sequencing for conditions like those found in newborn screening. We want to schedule a study visit with a genetic counselor at UNC Hospitals.		Making a Decision about Genomic Sequencing (headline) You have a decision to make at this time. Remember, even if you decide to schedule a study visit, you can change your mind and stop participation in this study at any point in time. Interactive scale anchored by… • No, we do not want our child to have genomic sequencing at this time. • We're not sure • Yes, we want genomic sequencing for our child.	Interactive response scale (slider); Submit button Next button;	Capture selection-yes/no/not sure Capture numerical value associated with position on scale. Treat as scale ranging from 0-100. In addition to capturing integer values in dataset, values will also be used for conditional piping on screens"D1. 27…" 3-point categorical values, where anchor points are	(11) Decision choices

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
						0-33 = left third, No 34-66= center third, Not sure 67-100= right third, Yes Capture time in milliseconds spent on this screen	
D1.27.Single.No	IF D1.26.Single=No & SINGLE	What happens next? You have decided not to have genomic sequencing for your child.		What happens next? (headline) • You will be asked to complete an	Replay button Next/Exit button		(12) Closing

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		Within the next week or so, you will be asked to complete an online survey about this decision because understanding why you made this decision is important. You will be sent a $20 VISA card for completing the survey. After completing this survey, you will end your participation in the NC NEXUS study.		online survey • You will be sent a $20 Visa card for completing the survey • Your participation in the NC NEXUS study will end Thank you!			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.27.Couple.No	IF D1.26.Couple= No & COUPLE	What happens next? You and your partner have decided not to have genomic sequencing for your child. Within the next week or so, you and your partner will both be asked to complete an online survey about this decision because understanding why you made this decision is important. You will each be sent a $20 VISA card for completing the survey. After completing the survey, you will end your participation in the NC NEXUS study.		What happens next? (headline) • You will be asked to complete an online survey • You will each be sent a $20 Visa card after you complete the survey • Your participation in the NC NEXUS study will end Thank you!	Replay button Next/Exit button		(12) Closing

D1.27.Single.MaybeYes	IF D1.26.Single=Not sure OR IF D1.26.Single=Yes & SINGLE	What happens next? • A member of the NC NEXUS study team will contact you to schedule a study visit at UNC Hospitals. • At the study visit, you will meet with a genetic counselor to discuss why you may or may not want to have genomic sequencing for your child. This visit will last about 1 hour. • You will then be asked if you want to consent to having genomic sequencing for your child. If you come to the study visit, it does <u>not</u> mean you have to consent to genomic sequencing. • If you choose to have genomic sequencing for your child, you will sign a consent form and continue your participation in the NC NEXUS study. You will learn your child's genomic sequencing results for medically actionable childhood conditions, like those found with newborn screening. You will be asked to complete three online questionnaires over the next several months. You will be sent a $20 Visa card for each survey		What happens next? (headline) • Set a time to visit the UNC Hospitals • Meet with a genetic counselor to discuss genomic sequencing for your child • Decide if you want to consent to genomic sequencing for your child	Replay button Exit/Close button		(12) Closing

		you complete. You will also be sorted into one of two groups by a random drawing. One group will have the choice to learn other genetic information about their child, and will use another decision guide to help decide if learning this additional information is right for them and their families. The other group will not have the choice to learn these additional genomic sequencing results. • If you choose not to have genomic sequencing for your child at that time, you will complete an online survey asking about this decision and then your participation in the NC NEXUS study will end. You will be sent a $20 VISA card after completing the survey.					

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D1.27.Couple.MaybeYes	IF D1.26.Couple=Not sure OR IF D1.26.Couple=Yes & COUPLE	What happens next? • A member of the NC NEXUS study team will contact you to schedule a study visit at UNC Hospitals. • At the study visit, you and your partner will meet with a genetic counselor to discuss why you may or may not want to have genomic sequencing for your child. This visit will last about 1 hour. • You will then be asked if you want to consent to having genomic sequencing for your child. If you come to the study visit, it does not mean you have to consent to genomic sequencing. • If you choose to have genomic sequencing for your child, both you and your partner will sign a consent form and continue your participation in the NC NEXUS study. You will learn your child's genomic sequencing results for medically actionable childhood conditions, like those found with newborn screening. You and your partner will each be asked		What happens next? (headline) • Set a time to visit the UNC Hospitals • Meet with a genetic counselor to discuss genomic sequencing for your child • Decide if you want to consent to genomic sequencing for your child	Replay button Next/Exit button		(12) Closing

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		to complete three online questionnaires over the next several months. You will each be sent a $20 Visa card for each survey you complete. You will also be sorted into one of two groups by a random drawing. One group will have the choice to learn other genetic information about their child, and will use another decision guide to help decide if learning this additional information is right for them and their families. The other group will not have the choice to learn these additional genomic sequencing results. • If you and your partner choose not to have genomic sequencing for your child at that time, you will complete an online survey asking about this decision and then your participation in the NC NEXUS study will end. You will each be sent a $20 VISA card after completing the survey.					

NC NEXUS – Online Decision Aid 2 – Shooting Script
Version: 05/19/2015

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.1	IF EXPERIMENT ARM			The North Carolina Newborn Exome Sequencing for Universal Screening Study (NC NEXUS)	Login user name and password fields. Enter button.		(1) Welcome/Login
D2.2	IF EXPERIMENT ARM	Welcome back to the NC NEXUS decision guide. This part of the decision guide will help you: • Learn about three kinds of additional genomic sequencing results in the NC NEXUS study. The guide will also help you decide if you want the NC NEXUS study team to look at your child's genomic sequencing results and tell you about findings in any of the additional categories.	Welcome	*Text onscreen:* What will this decision guide help you do? (headline) • Learn about additional genomic sequencing results • Decide if you want to learn your child's additional genomic sequencing results	Replay button Next button		(3) General content, text list

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.3	IF EXPERIMENT ARM	What are additional genomic sequencing results? When you decided to have genomic sequencing for your child, you chose to receive results for conditions that are found with current newborn screening and other conditions like them. Now, you can also decide if you want to learn additional genomic sequencing results for three types of rare genetic health conditions: 1. Conditions for which your child is a *carrier*. Being a carrier for a health condition means your child would not have the health problem, but might pass on a gene difference that	NC NEXUS has 3 kinds of additional genomic sequencing results.	What are additional genomic sequencing results? (headline) NC NEXUS has 3 kinds of additional genomic sequencing results: (NOTE: Show 3 'bins', labelled as follows) 1. Carrier status 2. Medically actionable adult conditions 3. Non-medically actionable childhood conditions	Replay button Next button		(3) (4) general content, text image combined

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		causes the condition to their children. 2. *Medically actionable adult conditions*. These are health conditions that usually begin in adulthood for which there are treatments that can help, and 3. *Non-medically actionable childhood conditions*. These are health conditions that usually begin in childhood or the teen years for which there are no medical treatments that improve the condition.					
D2.4	IF EXPERIMENT ARM	Carrier Status What does it mean to be a carrier?	Carriers have two copies of a gene. One	What does it mean to be a carrier? (headline)	Replay button Next button		(4) general content, image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		Genes are passed on in families from one generation to the next. We all have two copies of most genes. One copy is from the mother and the other is from the father. If your child has two copies of a gene but only *one* copy contains gene differences that cause a health condition, then he or she is a ***carrier*** for that condition.	copy contains a gene difference that causes a health condition.	Note: Show image of inheritance chart, with some kind of emphasis on the carriers. Ben – I linked to an example here			
D2.5	IF EXPERIMENT ARM	What can carrier results tell you about your child? The conditions that your child might be a carrier for differ greatly from one to the next. Some may be preventable and others may not be. If your child is a carrier, he or she will not usually ever have any signs of the health condition. But your child might pass on	Carriers do not usually have the health condition, but may pass on a gene difference that causes it in their children.	What can carrier results tell you about your child? (headline)	Replay button Next button		(4) general content, image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		gene differences that cause the condition in his or her children.					
D2.6	IF EXPERIMENT ARM	How common is it for genomic sequencing to find carrier results? On average, everyone is a carrier for about 3 to 5 gene differences that could cause health conditions in their children. Genomic sequencing in the NC NEXUS study cannot find every gene difference that would show if your child is a carrier. If a gene difference is not found in a specific gene, it does not mean that your child is not a carrier. Instead, it means that your child is less likely to be a carrier for a gene difference that causes that condition.	Everyone is a carrier for around 3 to 5 gene differences that cause health conditions	How common is it for genomic sequencing to find carrier results? (headline) Visual to depict that on average, everyone is a carrier for 3 to 5 gene differences that cause health conditions.	Replay button Next button		(4) general content, image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.7	IF EXPERIMENT ARM	What would knowing your child is a carrier mean for you? Learning a child is a carrier of a gene difference that causes a health condition means that the mother or the father is also a carrier of the same gene difference. Most people do not know they are a carrier until they have a child who develops a condition. If both you and your partner are carriers, then you may have a child with the health condition. Genomic sequencing through the NC NEXUS study cannot confirm whether you and your partner are carriers.	If your child is a carrier for a specific gene difference, then you or your partner are too.	What would knowing your child is a carrier mean for you? (headline) Visual of inheritance chart. Would be good if motion graphic: Close shot on carrier. Pan up the family tree to dad and mom (in this case, both are carriers). Then pan down to affected sibling.	Replay button Next button		(4) general content, image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.8	IF EXPERIMENT ARM	What happens if your child is a carrier? If genomic sequencing finds that your child is a carrier for any gene differences that cause health conditions: • These results will be confirmed with another test. • A genetic counselor and a doctor will meet with you to discuss the results. • You will be given information about genetic tests you can take to learn your carrier status.		What happens if your child is a carrier? (headline) If genomic sequencing finds that your child is a carrier • Results will be confirmed with another test • A genetic counselor and a doctor will discuss the results with you. • You will learn about genetic tests you can take learn your own carrier status	Replay button Next button		(3) General content, text list

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.9	IF EXPERIMENT ARM	Which way are you leaning? If you had to decide about learning your child's carrier results right now, which way are you leaning? After you have reviewed the information for the other types of health conditions, you will be able to decide whether you want to learn sequencing results for one or more of them.		Which way are you leaning? Carrier status(headline) If you had to decide about learning your child's carrier results right now, which way are you leaning? Interactive scale anchored by leaning away…leaning toward. Leaning away------- Uncertain------- Leaning Toward	Interactive response scale; Submit button Next button;	Capture numerical value associated with position on scale. Treat as continuous scale ranging from 0-100, where anchor points are 0 = left-most position, leaning away 50= center position, Uncertain 100=right-most position, leaning toward Intermediate values captured as integers.	(5) Leaning yes/now screen

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
						Capture time in milliseconds spent on this screen	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.10.Single	IF EXPERIMENT ARM & SINGLE	What matters most to you when deciding if you should learn your child's carrier results? There are lots of things to think about when deciding whether you want to learn your child's carrier results. Are these reasons important or unimportant to you?		What matters most to you? Carrier status (headline) Reasons to learn your child's carrier results • You want information about your family risk for some inherited health conditions • You want to know which rare genetic health conditions your child may pass on in his or her own children. • You are curious to know if your child is a carrier. • You want to learn if you or your partner are likely to be carriers. • You could help scientists better understand how parents respond to	Sorting task for users to move boxes with 'reasons for' into <u>two</u> bins labeled 'Important' and 'Unimportant' 2 interactive textboxes that allows users to write in 2 additional 'reasons for' that is not listed; write-in textbox is also sortable Allow participants to continue to next screen only after at least one statement is moved into a sorting category. Submit button	Capture which bin (i.e., important, unimportant) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it not sorted into any box (i.e., values for each statement: important,	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				learning a child's carrier results. • *Are there any other reasons you can think of? Please type them here.* Move at least one statement to continue.	Next button	unimportant, not sorted)	
D2.10.Couple	IF EXPERIMENT ARM &	What matters most to you when deciding if you should learn your child's carrier results?		What matters most to you? Carrier status (headline)	Sorting task for users to move boxes with 'reasons for' into three bins labeled	Capture which bin (i.e., important, unimportant) user sorts	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
	COUPLE	There are lots of things to think about when deciding whether you want to learn your child's carrier results. Are these reasons important or unimportant to you? If you and your partner disagree about the importance of a reason, you can move it into the box labelled "We disagree."		Reasons to learn your child's carrier results • You want information about your family risk for some inherited health conditions • You want to know which rare genetic health conditions your child may pass on in his or her own children. • You are curious to know if your child is a carrier. • You want to learn if you or your partner are likely to be carriers. • You could help scientists better understand how parents respond to learning a child's carrier results. • *Are there any other reasons you can*	'Important,' 'Unimportant,' and 'We disagree' 2 interactive textboxes that allows users to write in 2 additional 'reasons for' that is not listed; write-in textbox is also sortable Allow participants to continue to next screen only after at least one statement is moved into a sorting category. Submit button Next button	each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it not sorted into any box (i.e., values for each statement: important, unimportant, not sorted)	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				think of? Please type them here. Move at least one statement to continue.			
D2. 11.Single	IF EXPERIMENT ARM & SINGLE	Are these reasons important or unimportant to you?		What matters most to you? Carrier status (headline) Reasons <u>not</u> to learn your child's carrier results • You are worried what the results would mean for your child and family • You think the decision to learn if your child is a carrier should be left to your child when he or she is an adult. • Knowing this information could lead you to worry or feel anxious.	Sorting task for users to move boxes with 'reasons against' into <u>two</u> bins labeled 'Important' and 'Unimportant' Interactive textbox that allows users to write in a 'reason against' that is not listed; write-in textbox is also sortable Allow participants to continue to next screen only after at least one	Capture which bin (i.e., important, unimportant) user sorts each 'reason against' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				• You think learning your child's carrier results may lead you to treat him or her differently. • The idea of learning your child's carrier results makes you uncomfortable, even if there is no particular reason why. • *Are there any other reasons you can think of? Please type them here.* Move at least one statement to continue.	statement is moved into a sorting category. Submit button Next button	into or if it not sorted into any box (i.e., values for each statement: important, unimportant, not sorted)	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2. 11.Couple	IF EXPERIMENT ARM & COUPLE	Are these reasons important or unimportant to you? Or do you and your partner disagree?		What matters most to you? Carrier status (headline) Reasons not to learn your child's carrier results • You are worried what the results would mean for your child and family • You think the decision to learn if your child is a carrier should be left to your child when he or she is an adult. • Knowing this information could lead you to worry or feel anxious. • You think learning your child's carrier results may lead you to treat him or her differently.	Sorting task for users to move boxes with 'reasons for' into three bins labeled 'Important,' 'Unimportant,' and 'We disagree' 2 interactive textboxes that allows users to write in 2 additional 'reasons against' that is not listed; write-in textbox is also sortable Allow participants to continue to next screen only after at least one statement is moved into a sorting category.	Capture which bin (i.e., important, unimportant, disagree) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it not sorted into any box (i.e., values for each statement:	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				• The idea of learning your child's carrier results makes you uncomfortable, even if there is no particular reason why. • *Are there any other reasons you can think of? Please type them here.* Move at least one statement to continue.	Submit button Next button	important, unimportant, disagree, not sorted)	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.12.Single	IF EXPERIMENT ARM & SINGLE	Here are the reasons for and against learning your child's carrier results that matter most to you.		What matters most to you? Carrier status ((Headline) Two boxes on screen. One is labelled "Reasons to learn your child's carrier results." In this box, list the reasons that the user sorted into the 'important' box from screen D2.10.Single "Reasons not to learn your child's carrier results" In this box, list the reasons that the user sorted into the 'important' box from screen D2.11.Single	Visually present whether user sorted 'reasons for' as important on screen D2.10.Single and 'reasons against' as important on screen D2.11.Single Any statement that was not sorted into a category is not displayed on review screen. Replay button Next button		(7) Values clarification, review

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.12.Couple	IF EXPERIMENT ARM & COUPLE	Here are the reasons for and against learning your child's carrier results that matter most to you.		What matters most to you? Carrier status ((Headline) Two boxes on screen. One is labelled "Reasons to learn your child's carrier results." In this box, list the reasons that the user sorted into the 'important' box from screen D2.10.Couple "Reasons not to learn your child's carrier results" In this box, list the reasons that the user sorted into the 'important' box from screen D2.11.Couple	Visually present whether user sorted 'reasons for' as important on screen D2.10.Couple and 'reasons against' as important on screen D2.11.Couple Any statement that was not sorted into a category is not displayed on review screen. Replay button Next button		(7) Values clarification, review
D2.13	IF EXPERIMENT ARM	What is a medically actionable adult condition? People who have a *medically actionable adult condition* will	Medically actionable adult conditions begin in adulthood and can be improved with treatment.	What is a medically actionable adult condition? (headline) Show pictures that indicate medical treatment for adult conditions.	Replay button Next button		(3) General content, image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		• usually not begin showing signs until he or she is an adult. Conditions that are medically actionable… • Can be improved with treatment, • Have treatments with benefits that typically outweigh the risks, and • Are well-understood by doctors.		Medically actionable adult conditions… • Begin in adulthood • Can be improved with treatment • Benefit of treatment outweighs risks • Are well-understood by doctors			
D2.14	IF EXPERIMENT ARM	An example of these conditions is Lynch syndrome. People with Lynch syndrome are more likely to get colon cancer, as well as several other types of cancer. Cancers caused by Lynch syndrome usually begin between the ages of 40 and 60 years old. These cancers can often can be prevented by early screening or surgery. About 5 to 15 out of every 10,000 people in	Lynch syndrome is an example of a medically actionable adult condition.	What is a medically actionable adult condition? (headline)	Replay button Next button		(4) general content

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		the United States have Lynch syndrome.					
D2.15	IF EXPERIMENT ARM	What can genomic sequencing tell you about medically actionable adult conditions? The NC NEXUS team will look for gene differences that are known to cause specific health conditions. These gene differences are usually not the *only* cause, but they are known to play a part in whether a child will get the condition in the future. Looking for these gene differences in your child's DNA can tell if he or she is more likely to get certain health conditions. Still,	NC NEXUS will uses genomic sequencing to look for gene differences that lead to specific health conditions. These gene differences are not the *only* cause.	What can genomic sequencing tell you about medically actionable adult conditions? (headline) We need a visual that somehow depicts the conditions.	Replay button Next button		(3) or (4) General content

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		knowing for sure if a child will show signs and how severe they will be is often difficult because other genetic and environmental factors also play a part in most conditions.					
D2.16	IF EXPERIMENT ARM	How common is it for genomic sequencing to find a gene difference that leads to a medically actionable adult condition? It is not known for sure how often genomic sequencing will find gene differences that cause medically actionable adult conditions. This is one of the things the NC NEXUS study will try to find out. The best estimate is that sequencing will find one of these gene differences in about 2% or 3% of children.	The NC NEXUS study team wants to find out how often genomic sequencing will find gene differences that lead to a health problem.	How common is it for genomic sequencing to find a gene difference that leads to a medically actionable adult condition? (headline) Visual to depict that it is unsure exactly how likely it is that a gene difference will be found, but about 2 or 3 out of 100 children. Maybe a risk array.	Replay button Next button		(3) or (4) General content

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.17	IF EXPERIMENT ARM	What would finding a gene difference that leads to a medically actionable adult condition mean for your child? Learning that your child has a gene difference that causes a medically actionable adult condition will not affect your child's health right now. But it could help your child's doctors in the future recommend ways to prevent or delay a health condition that would likely begin in adulthood. Some people think it is wrong for parents to learn whether their children have gene differences that cause health conditions in adulthood because it takes away the choice from the children to decide to learn these things themselves. One		What do genomic sequencing results for medically actionable adult conditions mean for your child? (headline)	Replay button Next button		(3) or (4) General content

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		possible risk is that your child could face discrimination based on this type of finding.					
D2.18	IF EXPERIMENT ARM	What would finding a gene difference that leads to a medically actionable adult condition mean for you? Many medically actionable adult conditions are passed on in such a way that finding a gene difference in a child could mean that one of the parents has the condition. If your child has a gene difference that causes a medically actionable adult condition, you might think about having testing for yourself. In this way, your child's genomic sequencing results could lead you to receive early treatment or prevention services	If your child has a gene difference that causes a medically actionable adult condition, you could mean that one of the parents will have the condition.	What do genomic sequencing results for medically actionable adult conditions mean for you? (headline)	Replay button Next button		(3) or (4) general content

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		before any symptoms appear.					
D2.19	IF EXPERIMENT ARM	What happens if my child has a gene difference that causes a medically actionable adult condition? If genomic sequencing finds that your child has any gene differences that cause a medically actionable adult condition: • These results will be confirmed with a second test • A genetic counselor and a doctor will meet with you to discuss the results and how your child should be followed up as an adult. • You will be given information about testing options for yourself.		What happens if my child has a gene difference that causes a medically actionable adult condition? (headline) If genomic sequencing finds gene differences that cause a health condition • Results will be confirmed with another test • A genetic counselor and a doctor will discuss the results with you. • You will be given information about testing options for yourself	Replay button Next button		(3) general content, text list

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.20	IF EXPERIMENT ARM	Which way are you leaning? If you had to decide about learning your child's results for medically actionable adult conditions right now, which way are you leaning?		Which way are you leaning? Medically actionable adult conditions (headline) If you had to decide about learning your child's results for medically actionable adult conditions right now, which way are you leaning? Interactive scale anchored by leaning away…leaning toward. Leaning away--------- Uncertain--------- Leaning Toward	Interactive response scale; Submit button Next button;	Capture numerical value associated with position on scale. Treat as continuous scale ranging from 0-100, where anchor points are 0 = left-most position, leaning away 50= center position, Uncertain 100=right-most position, leaning toward Intermediate values captured as integers.	(5) Leaning yes/now screen

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
						Capture time in milliseconds spent on this screen	
D2.21.Single	IF EXPERIMENT ARM & SINGLE	What matters most to you when deciding if you should learn your child's results for medically actionable adult conditions? There are lots of things to think about when deciding whether you want to learn your child's sequencing results for medically actionable adult conditions. Are these reasons important or unimportant to you?		What matters most to you? Medically actionable adult conditions (Headline) Reasons to learn your child's genomic sequencing results for medically actionable adult conditions • Your child's future doctors might be helped by knowing this information when he or she is an adult • The results may help you prepare your child for the future. • You want to know if you or your partner are at greater risk for	Sorting task for users to move boxes with 'reasons for' into two bins labeled 'Important' and 'Unimportant' 2 interactive textboxes that allows users to write in 2 additional 'reasons for' that is not listed; write-in textbox is also sortable Allow participants to continue to next screen only after at least one statement is moved into a	Capture which bin (i.e., important, unimportant) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it not sorted	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				certain health problems. • You or your partner might benefit from early intervention for a health condition. • You could help scientists better understand how the health condition impacts a child before symptoms appear. • *Are there any other reasons you can think of? Please type them here.* Move at least one statement to continue.	sorting category. Submit button Next button	into any box (i.e., values for each statement: important, unimportant, not sorted)	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.21.Couple	IF EXPERIMENT ARM & COUPLE	What matters most to you when deciding if you should learn your child's results for medically actionable adult conditions? There are lots of things to think about when deciding whether you want to learn your child's sequencing results for medically actionable adult conditions. Are these reasons important or unimportant to you? If you and your partner disagree about the importance of a reason, you can move it into the box labelled "We disagree."		What matters most to you? Medically actionable adult conditions (Headline) Reasons to learn your child's genomic sequencing results for medically actionable adult conditions • Your child's future doctors might be helped by knowing this information when he or she is an adult • The results may help you prepare your child for the future. • You want to know if you or your partner are at greater risk for certain health problems • You or your partner might benefit from early intervention	Sorting task for users to move boxes with 'reasons for' into three bins labeled 'Important,' 'Unimportant,' and 'We disagree' 2 interactive textboxes that allows users to write in 2 additional 'reasons for' that is not listed; write-in textbox is also sortable Allow participants to continue to next screen only after at least one statement is moved into a sorting category.	Capture which bin (i.e., important, unimportant, disagree) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it not sorted into any box (i.e., values for each statement:	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				for a health condition. • You could help scientists better understand how the health condition impacts a child before symptoms appear. • *Are there any other reasons you can think of? Please type them here.* Move at least one statement to continue.	Submit button Next button	important, unimportant, disagree, not sorted)	
D2.22.Single	IF EXPERIMENT ARM & SINGLE	Are these reasons important or unimportant to you?		What matters most to you? Medically actionable adult conditions (Headline) Reasons <u>not</u> to learn your child's genomic sequencing results for	Sorting task for users to move boxes with 'reasons against' into <u>two</u> bins labeled	Capture which bin (i.e., important, unimportant) user sorts	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				medically actionable adult conditions • You think the decision to learn this information should be left to your child, when he or she is an adult. • The benefit of knowing these results will not apply to your child for many years. • Knowing this information could lead you to worry or feel anxious. • Learning this information could cause your child to have problems getting life insurance, disability insurance, or long-term care insurance as an adult.	'Important' and 'Unimportant' Interactive textbox that allows users to write in a 'reason against' that is not listed; write-in textbox is also sortable Allow participants to continue to next screen only after at least one statement is moved into a sorting category. Submit button Next button	each 'reason against' Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it not sorted into any box (i.e., values for each statement: important, unimportant, not sorted)	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				• You do not want to learn that you or your partner is more likely to have a gene difference that causes a medically actionable adult condition. • *Are there any other reasons you can think of? Please type them here.* Move at least one statement to continue.			
D2.22.Couple	IF EXPERIMENT ARM & COUPLE	Are these reasons important or unimportant to you? Or do you and your partner disagree?		What matters most to you? Medically actionable adult conditions (Headline) Reasons <u>not</u> to learn your child's genomic sequencing results for medically actionable adult conditions • You think the decision to learn this information should be left to	Sorting task for users to move boxes with 'reasons for' into <u>three</u> bins labeled 'Important,' 'Unimportant,' and 'We disagree' 2 interactive textboxes that allows users to write in 2	Capture which bin i.e., important, unimportant, disagree) user sorts each 'reason for' Capture text user types into interactive text box	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				your child, when he or she is an adult. • The benefit of knowing these results will not apply to your child for many years. • Knowing this information could lead you to worry or feel anxious. • Learning this information could cause your child to have problems getting life insurance, disability insurance, or long-term care insurance as an adult. • You do not want to learn that you or your partner is more likely to have a gene difference that causes a	additional 'reasons against' that is not listed; write-in textbox is also sortable Allow participants to continue to next screen only after at least one statement is moved into a sorting category. Submit button Next button	Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it not sorted into any box (i.e., values for each statement: important, unimportant, disagree, not sorted)	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				medically actionable adult condition. • *Are there any other reasons you can think of? Please type them here.* Move at least one statement to continue.			
D2.23.Single	IF EXPERIMENT ARM & SINGLE	Here are the reasons for and against learning genomic sequencing results for medically actionable adult condition that matter most to you.		What matters most to you? Medically actionable adult conditions (Headline) Two boxes on screen. One is labelled "Reasons to learn your child's results for medically actionable adult conditions." In this box, list the reasons that the user sorted into the 'important' box from screen D2.21.Single "Reasons not to learn your child's results for medically actionable	Visually present whether user sorted 'reasons for' as important on screen D2.21.Single and 'reasons against' as important on screen D2.22.Single Any statement that was not sorted into a category is not displayed on review screen. Replay button		(7) Values clarification, review

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				adult condition." In this box, list the reasons that the user sorted into the 'important' box from screen D2.22.Single	Next button		
D2.23.Couple	IF EXPERIMENT ARM & COUPLE	Here are the reasons for and against learning genomic sequencing results for medically actionable adult condition that matter most to you.		What matters most to you? Medically actionable adult conditions (Headline) Two boxes on screen. One is labelled "Reasons to learn your child's results for medically actionable adult conditions." In this box, list the reasons that the user sorted into the	Visually present whether user sorted 'reasons for' as important on screen D2.21.Couple and 'reasons against' as important on screen D2.22.Couple Any statement that was not sorted into a		(7) Values clarification, review

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				'important' box from screen D2.21.Couple "Reasons not to learn your child's results for medically actionable adult condition." In this box, list the reasons that the user sorted into the 'important' box from screen D2.22.Couple	category is not displayed on review screen. Replay button Next button		
D2.24	IF EXPERIMENT ARM	Non-Medically Actionable Childhood Conditions What is a non-medically actionable childhood condition? A person who has a *non-medically actionable childhood condition* • Usually begins showing signs before the age of 18.	Non-medically actionable childhood conditions begin early in a child's life and there are no medical treatments that can cure the condition.	What is a non-medically actionable childhood condition? (headline) Show pictures that indicate medical treatment. Medically actionable childhood conditions… • Begin early in a child's life • May have treatments that	Replay button Next button		(3) General content, image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		Non-medically actionable childhood conditions • May have some treatments that help symptoms, but… • There are no effective medical treatments to cure the condition.		help with symptoms • Cannot be cured with early treatment			
D2.25	IF EXPERIMENT ARM	Mowat-Wilson syndrome is a non-medically actionable childhood condition that affects many parts of the body. Signs of Mowat-Wilson syndrome include distinctive facial features and intellectual disability. Many children who have Mowat-Wilson syndrome can understand what others say, but only	Mowat-Wilson syndrome is an example of a non-medically actionable childhood condition.	What is a non-medically actionable childhood condition? (headline) Visuals depicting Mowat-Wilson syndrome signs/symptoms	Replay button Next button		(4) General content, image

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		learn to speak a few words themselves. Children with Mowat-Wilson syndrome are also not able to sit, stand, and walk at the same age as other children. About 1 to 2 out of every 100,000 babies born in the United States have Mowat-Wilson syndrome.					
D2.26	IF EXPERIMENT ARM	What can genomic sequencing tell you about non-medically actionable childhood conditions? The NC NEXUS team will look for gene differences that are known to cause specific non-medically actionable childhood conditions. In some cases, these gene differences can tell if your child is nearly certain to develop a health		What can genomic sequencing tell you about non-medically actionable childhood conditions? (headline) We need a visual that somehow depicts these conditions.	Replay button Next button		(3) or (4) General content

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		condition. In other cases, looking for these gene differences in your child's DNA will only tell you if he or she is more likely to get certain health conditions, but not for sure. Knowing for sure if a child will show signs and how severe they will be is often difficult because other genetic and environmental factors also play a part in most conditions.					
D2.27	IF EXPERIMENT ARM	How common is it for genomic sequencing to find a gene difference that leads to a non-medically actionable childhood condition? It is not known for sure how often genomic sequencing will find gene differences that cause non-medically actionable childhood conditions. This is one of the things the NC	The NC NEXUS study team wants to find out how often genomic sequencing will find gene differences that lead to a health problem.	How common is it for genomic sequencing to find a gene difference that leads to a non-medically actionable childhood condition? (headline) Visual to depict that it is unsure exactly how likely it is that a gene difference will be found, but less than 1 out of 100	Replay button Next button		(3) or (4) General content

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		NEXUS study will try to find out. The best estimate is that sequencing will find one of these gene differences in less than 1% of children.					
D2.28	IF EXPERIMENT ARM	What would it mean for your child if sequencing finds a gene difference that leads to a non-medically actionable childhood condition? Learning that your child has a gene difference that causes a non-medically actionable childhood condition will not allow your child's doctor to take specific steps to prevent it. That's because, right now, there are no definite ways to use the information to help protect your child's health. Parents may have different views on		What do genomic sequencing results for non-medically actionable childhood conditions mean for your child? (headline) Note. Visuals to depict that different parents will have differing views	Replay button Next button		(3) or (4) General content

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		whether or not learning this information about their child is harmful and distressing or valuable and helpful.					
D2.29	IF EXPERIMENT ARM	Some parents prefer not to learn about their child's gene differences. They may be concerned that the information will make them worry about their child's future health. Other parents might be concerned that uncertainty about whether the condition will develop will cause them to believe their child is sick even if he or she is healthy.	Knowing might cause some parents to worry excessively. Knowing might cause some parents might treat a healthy child like s/he is sick.	What do genomic sequencing results for non-medically actionable childhood conditions mean for your child? (headline) Note. Slide show-type visuals that illustrate each key point.	Replay button Next button		(3) or (4) General content
D2.30	IF EXPERIMENT ARM	Some parents might think it is useful to learn that their child has one of these gene differences. Even though these health conditions are not preventable right now, new treatments may become available in the future. Knowing that your child has a gene	Knowing might help parents prepare for the health condition and act fast if new treatments are developed.	What do genomic sequencing results for non-medically actionable childhood conditions mean for your child? (headline) Note. Slide show-type visuals that illustrate each key point.	Replay button Next button		(3) or (4) General content

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		difference that causes a non-medically actionable childhood condition may help you and your child's doctor prepare for the health condition if symptoms appear, refer your child to support services, and act more quickly if new treatments become available. Some diseases are difficult for doctors to diagnose, even after symptoms appear. Learning that your child has a gene variant that causes a non-medically actionable childhood condition may lower the number of tests your child's doctor would need to explain the symptoms.	Knowing might make it easier for a child's doctor to diagnose health condition if your child begins showing symptoms (shorten the "diagnostic odyssey")				

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.31	IF EXPERIMENT ARM	What happens if my child has a gene difference that causes a non-medically actionable childhood condition? If genomic sequencing finds that your child has any gene differences that cause a non-medically actionable childhood condition: • These results will be confirmed with a second test. • A genetic counselor and a		What happens if my child has a gene difference that causes a non-medically actionable childhood condition? (headline) If genomic sequencing finds gene differences that cause a health condition • Results will be confirmed with another test • A genetic counselor and a doctor will discuss the results with you. • You will be referred for services your child needs for the condition	Replay button Next button		(3) general content, text list

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		doctor will meet with you to discuss the results. • You will be referred for medical or other services your child needs for the condition.					

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.32	IF EXPERIMENT ARM	Which way are you leaning? If you had to decide about learning your child's results for non-medically actionable childhood conditions right now, which way are you leaning?		Which way are you leaning? Non-medically actionable childhood conditions (headline) If you had to decide about learning your child's results for non-medically actionable childhood conditions right now, which way are you leaning? Interactive scale anchored by leaning away…leaning toward. Leaning away--------- Uncertain--------- Leaning Toward	Interactive response scale; Submit button Next button;	Capture numerical value associated with position on scale. Treat as continuous scale ranging from 0-100, where anchor points are 0 = left-most position, leaning away 50= center position, Uncertain 100=right-most position, leaning toward Intermediate values captured as integers.	(5) Leaning yes/now screen

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
						Capture time in milliseconds spent on this screen	
D2.33.Single	IF EXPERIMENT ARM & SINGLE	What matters most to you when deciding if you should learn your child's genomic sequencing results for non-medically actionable childhood conditions?		What matters most to you? Non-medically actionable childhood conditions (Headline) Reasons to learn your child's genomic sequencing results for non-medically	Sorting task for users to move boxes with 'reasons for' into <u>two</u> bins labeled 'Important' and 'Unimportant' 2 interactive textboxes that	Capture which bin (i.e., important, unimportant) user sorts each 'reason for' Capture text user types	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		There are lots of things to think about when deciding if you want to learn your child's sequencing results for non-medically actionable childhood conditions. Are the following reasons important or unimportant to you?		actionable childhood conditions • This information may help your child's doctor diagnose a health problem if your child develops symptoms. • Children with a gene difference that causes a non-medically actionable childhood condition will be referred to support services. • Knowing this information could help you take advantage of new treatments if they become available. • You could help scientists better understand how these health	allows users to write in 2 additional 'reasons for' that is not listed; write-in textbox is also sortable Allow participants to continue to next screen only after at least one statement is moved into a sorting category. Submit button Next button	into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it not sorted into any box (i.e., values for each statement: important, unimportant, not sorted)	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				conditions impact children before symptoms appear. • Knowing this information may help find your chances of having other children with the same non-medically actionable childhood condition. • *Are there any other reasons you can think of? Please type them here.* Move at least one statement to continue.			
D2.33.Couple	IF EXPERIMENT ARM & COUPLE	What matters most to you when deciding if you should learn your child's genomic sequencing results for non-medically actionable childhood conditions?		What matters most to you? Non-medically actionable childhood conditions (Headline) Reasons to learn your child's genomic sequencing results for non-medically	Sorting task for users to move boxes with 'reasons for' into <u>three</u> bins labeled 'Important,' 'Unimportant,'	Capture which bin (i.e., important, unimportant, disagree) user sorts each 'reason for'	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		There are lots of things to think about when deciding if you want to learn your child's sequencing results for non-medically actionable childhood conditions. Are the following reasons important or unimportant to you? If you and your partner disagree about the importance of a reason, you can move it into the box labelled "We disagree."		actionable childhood conditions • This information may help your child's doctor diagnose a health problem if your child develops symptoms. • Children with a gene difference that causes a non-medically actionable childhood condition will be referred to support services. • Knowing this information could help you take advantage of new treatments if they become available. • You could help scientists better understand how these health	and 'We disagree' 2 interactive textboxes that allows users to write in 2 additional 'reasons for' that is not listed; write-in textbox is also sortable Allow participants to continue to next screen only after at least one statement is moved into a sorting category. Submit button Next button	Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it not sorted into any box (i.e., values for each statement: important, unimportant, disagree, not sorted)	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				conditions impact children before symptoms appear. • Knowing this information may help find your chances of having other children with the same non-medically actionable childhood condition. • *Are there any other reasons you can think of? Please type them here.* Move at least one statement to continue.			
D2.34.Single	IF EXPERIMENT ARM & SINGLE	Are the following reasons important or unimportant to you?		What matters most to you? Non-medically actionable childhood conditions (Headline) Reasons not to learn your child's genomic sequencing results for non-medically	Sorting task for users to move boxes with 'reasons against' into two bins labeled 'Important' and 'Unimportant'	Capture which bin (i.e., important, unimportant) user sorts each 'reason against'	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				actionable childhood conditions • There are no specific steps you and your child's doctor can take to prevent symptoms based on this information. • Learning this information might lead you to think your child is sick even when healthy. • Knowing this information could lead you to feel less connected to your child. • Learning this information could cause your child to have problems getting disability insurance or long-term care insurance. • You would rather not know this information because	Interactive textbox that allows users to write in a 'reason against' that is not listed; write-in textbox is also sortable Allow participants to continue to next screen only after at least one statement is moved into a sorting category. Submit button Next button	Capture text user types into interactive text box Capture time in milliseconds spent on this screen Capture which box each statement was sorted into or if it not sorted into any box (i.e., values for each statement: important, unimportant, not sorted)	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				it is not certain if or when symptoms would begin. • *Are there any other reasons you can think of? Please type them here.* Move at least one statement to continue.			
D2.34.Couple	IF EXPERIMENT ARM & COUPLE	Are the following reasons important or unimportant to you? Or do you and your partner disagree?		What matters most to you? Non-medically actionable childhood conditions (Headline) Reasons not to learn your child's genomic sequencing results for non-medically actionable childhood conditions • There are no specific steps you and your child's doctor can take to prevent symptoms based on this information. • Learning this information might	Sorting task for users to move boxes with 'reasons for' into three bins labeled 'Important,' 'Unimportant,' and 'We disagree' 2 interactive textboxes that allows users to write in 2 additional 'reasons against' that is not listed; write-in textbox is also sortable	Capture which bin i.e., important, unimportant, disagree) user sorts each 'reason for' Capture text user types into interactive text box Capture time in milliseconds spent on this screen	(6) Values clarification, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				lead you to think your child is sick even when healthy. • Knowing this information could lead you to feel less connected to your child. • Learning this information could cause your child to have problems getting disability insurance or long-term care insurance. • You would rather not know this information because it is not certain if or when symptoms would begin. • *Are there any other reasons you can think of? Please type them here.* Move at least one statement to continue.	Allow participants to continue to next screen only after at least one statement is moved into a sorting category. Submit button Next button	Capture which box each statement was sorted into or if it not sorted into any box (i.e., values for each statement: important, unimportant, disagree, not sorted)	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.35.Single	IF EXPERIMENT ARM & SINGLE	Here are the reasons for and against learning genomic sequencing results for non-medically actionable childhood condition that matter most to you.		What matters most to you? Non-medically actionable childhood conditions (Headline) Two boxes on screen. One is labelled "Reasons to learn your child's results for non-medically actionable childhood condition." In this box, list the reasons that the user sorted into the 'important' box from screen D2.33.Single "Reasons not to learn your child's results for medically actionable adult condition" In this box, list the reasons that the user sorted into the 'important' box from screen D2.34.Single	Visually present whether user sorted 'reasons for' as important on screen D2.33.Single and 'reasons against' as important on screen D2.34.Single Any statement that was not sorted into a category is not displayed on review screen. Replay button Next button		(7) Values clarification, review

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.35.Couple	IF EXPERIMENT ARM & COUPLE	Here are the reasons for and against learning genomic sequencing results for non-medically actionable childhood condition that matter most to you.		What matters most to you? Non-medically actionable childhood conditions (Headline) Two boxes on screen. One is labelled "Reasons to learn your child's results for non-medically actionable childhood condition." In this box, list the reasons that the user sorted into the 'important' box from screen D2.33.Couple "Reasons not to learn your child's results for medically actionable adult condition" In this box, list the reasons that the user sorted into the 'important' box from screen D2.34.Couple	Visually present whether user sorted 'reasons for' as important on screen D2.33.Couple and 'reasons against' as important on screen D2.34.Couple Any statement that was not sorted into a category is not displayed on review screen. Replay button Next button		(7) Values clarification, review

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.36.Single	IF EXPERIMENT ARM AND SINGLE	Here are some questions that will help you decide if you want to learn one or more kinds of additional genomic sequencing results: • Will learning additional genomic sequencing results help you learn things that are important to you? • Do you have enough information to make a decision about learning one or more of the three kinds of additional sequencing results? • Are you prepared to learn one or more kinds of additional results from your child's genomic sequencing? • Are you interested in learning one or more kinds of additional genomic sequencing results?		Questions to Hel p You Decide about Learning Additional Genomic Sequencing Results (headline) Yes No ❒ ❒ Will learning additional genomic sequencing results help you learn things that are important to you? ❒ ❒ Do you have enough information	Check boxes/buttons for users to select yes or no for each question Submit button Next button;	Capture y/n answers to each question; Capture time in milliseconds spent on this screen	(8) Questions to help decide, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		• Are you confident you can make the decision that is right for you and your family?		to make a decision about learning one or more of the three kinds of additional sequencing results? ❒ ❒ Are you prepared to learn one or more kinds of additional results from your child's			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				genomic sequencing? ❒ ❒ Are you interested in learning one or more kinds of additional genomic sequencing results? ❒ ❒ Are you confident you can decide?			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.36.Couple	IF EXPERIMENT ARM AND COUPLE	Here are some questions that will help you decide if you want to learn one or more kinds of additional genomic sequencing results: • Will learning additional genomic sequencing results help you learn things that are important to you? • Do you have enough information to make a decision about learning one or more of the three kinds of additional sequencing results? • Are you prepared to learn one or more kinds of additional results from your child's genomic sequencing? • Are you interested in learning one or more kinds of additional genomic sequencing results?		Questions to Hel p You Decide about Learning Additional Genomic Sequencing Results (headline) Yes No ❒ ❒ Will learning additional genomic sequencing results help you learn things that are important to you? ❒ ❒ Do you have enough information	Check boxes/buttons for users to select yes or no for each question Submit button Next button;	Capture y/n answers to each question; Capture time in milliseconds spent on this screen	(8) Questions to help decide, input

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		• Are you and your partner confident you can make the decision that is right for you and your family?		to make a decision about learning one or more of the three kinds of additional sequencing results? ❒ ❒ Are you prepared to learn one or more kinds of additional results from your child's			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				genomic sequencing? ❒ ❒ Are you interested in learning one or more kinds of additional genomic sequencing results? ❒ ❒ Are you and your partner confident you can decide?			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.37.Single	IF EXPERIMENT ARM AND SINGLE	If you answered ***Yes*** to more of the questions above, maybe you are ready to learn one or more kinds of additional genomic sequencing results. If you answered ***No*** to more, maybe this is not the right decision for your family at this time. You should make the decision that is best for you and your family. There are no right or wrong choices.		Making a Decision about Learning Additional Genomic Sequencing Results (headline) • Will learning additional genomic sequencing results help you learn things that are important to you? • Do you have enough information to make a decision about learning one or more of the three kinds of additional sequencing results? • Are you prepared to learn one or more kinds of additional results from your child's genomic sequencing? • Are you interested in learning one or	Visually show whether user selected yes/no for each question from screen 'D2.36.Single' Replay button Next button		(9) Questions to help decide, review

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				more kinds of additional genomic sequencing results? • Are you confident you can decide? If you answered ***Yes*** to more of the questions above, maybe you are ready to learn one or more kinds of additional genomic sequencing results. If you answered ***No*** to more, maybe this is not the right decision for your family at this time.			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.37.Couple	IF EXPERIMENT ARM AND COUPLE	If you answered ***Yes*** to more of the questions above, maybe you are ready to learn one or more kinds of additional genomic sequencing results. If you answered ***No*** to more, maybe this is not the right decision for your family at this time. You should make the decision that is best for you and your family. There are no right or wrong choices.		Making a Decision about Learning Additional Genomic Sequencing Results (headline) • Will learning additional genomic sequencing results help you learn things that are important to you? • Do you have enough information to make a decision about learning one or more of the three kinds of additional sequencing results? • Are you prepared to learn one or more kinds of additional results from your child's genomic sequencing? • Are you interested in learning one or	Visually show whether user selected yes/no for each question from screen 'D2.36.Couple' Replay button Next button		(9) Questions to help decide, review

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				more kinds of additional genomic sequencing results? • Are you and your partner confident you can decide? If you answered ***Yes*** to more of the questions above, maybe you are ready to learn one or more kinds of additional genomic sequencing results. If you answered ***No*** to more, maybe this is not the right decision for your family at this time.			

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.38.Single	IF EXPERIMENT ARM & Single	You may choose to learn additional genomic sequencing results for all three kinds of conditions, only one or two of them, or none of them. All of these options are up to you and, if you want, you can change your mind even after you have made your decision and not learn the results. How interested are you in learning your child's genomic sequencing results for carrier status?		How interested are you in learning your child's genomic sequencing results for carrier status? (Headline) Interactive scale anchored by… • Definitely not interested in learning carrier status results • I'm not sure • Definitely interested in learning carrier status results	Interactive response scale (slider); Submit button Next button;	Capture numerical value associated with position on scale. Treat as scale ranging from 0-100, where anchor points are 0 = left-most position, Definitely not interested 50= center position, Not sure 100=right-most position, Definitely interested Intermediate values captured as integers.	(12) Interest inventory

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
						Capture time in milliseconds spent on this screen	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.39.Single	IF EXPERIMENT ARM & Single	How interested are you in learning your child's genomic sequencing results for *medically actionable adult conditions*?		How interested are you in learning your child's genomic sequencing results for medically actionable adult conditions? (Headline) Interactive scale anchored by… • Definitely <u>not</u> interested in learning results for medically actionable adult conditions • I'm not sure • Definitely interested in learning results for medically actionable adult conditions	Interactive response scale (slider); Submit button Next button;	Capture numerical value associated with position on scale. Treat as scale ranging from 0-100, where anchor points are 0 = left-most position, Definitely not interested 50= center position, Not sure 100=right-most position, Definitely interested Intermediate values captured as integers.	(12) Interest inventory

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
						Capture time in milliseconds spent on this screen	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.40.Single	IF EXPERIMENT ARM & Single	How interested are you in learning your child's genomic sequencing results for non-*medically actionable childhood conditions*?		How interested are you in learning your child's genomic sequencing results for non-medically actionable childhood conditions? (Headline) Interactive scale anchored by… • Definitely <u>not</u> interested in learning results for non-medically actionable childhood conditions • I'm not sure • Definitely interested in learning results for non-medically	Interactive response scale (slider); Submit button Next button;	Capture numerical value associated with position on scale. Treat as scale ranging from 0-100, where anchor points are 0 = left-most position, Definitely not interested 50= center position, Not sure 100=right-most position, Definitely interested Intermediate values captured as integers.	(12) Interest inventory

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				actionable childhood conditions		Capture time in milliseconds spent on this screen	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.38.Couple	IF EXPERIMENT ARM & COUPLE	How interested are you in learning your child's genomic sequencing results for carrier status?		How interested are you in learning your child's genomic sequencing results for carrier status? (Headline) Interactive scale anchored by… • Definitely not interested in learning carrier status results • We're not sure • Definitely interested in learning carrier status results *Note:* We cannot agree	Interactive response scale (slider); Submit button Next button;	Capture numerical value associated with position on scale. Treat as scale ranging from 0-100, where anchor points are 0 = left-most position, Definitely not interested 50= center position, Not sure 100=right-most position, Definitely interested Intermediate values captured as integers.	(12) Interest inventory

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
						Capture time in milliseconds spent on this screen	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.39.Couple	IF EXPERIMENT ARM & COUPLE	How interested are you in learning your child's genomic sequencing results for *medically actionable adult conditions*?		How interested are you in learning your child's genomic sequencing results for medically actionable adult conditions? (Headline) Interactive scale anchored by… • Definitely <u>not</u> interested in learning results for medically actionable adult conditions • We're not sure • Definitely interested in learning results for medically actionable adult conditions	Interactive response scale (slider); Submit button Next button;	Capture numerical value associated with position on scale. Treat as scale ranging from 0-100, where anchor points are 0 = left-most position, Definitely not interested 50= center position, Not sure 100=right-most position, Definitely interested Intermediate values captured as integers.	(12) Interest inventory

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
						Capture time in milliseconds spent on this screen	

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
D2.40.Couple	IF EXPERIMENT ARM & COUPLE	How interested are you in learning your child's genomic sequencing results for non-*medically actionable childhood conditions*?		How interested are you in learning your child's genomic sequencing results for non-medically actionable childhood conditions? (Headline) Interactive scale anchored by… • Definitely <u>not</u> interested in learning results for non-medically actionable childhood conditions • We're not sure • Definitely interested in learning results for non-	Interactive response scale (slider); Submit button Next button;	Capture numerical value associated with position on scale. Treat as scale ranging from 0-100, where anchor points are 0 = left-most position, Definitely not interested 50= center position, Not sure 100=right-most position, Definitely interested Intermediate values captured as integers.	(12) Interest inventory

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
				medically actionable childhood conditions		Capture time in milliseconds spent on this screen	
D2.41	IF EXPERIMENT ARM	What happens next? • At your next study visit, you will meet with a genetic counselor to discuss		What happens next? (headline) • Meet with a genetic counselor to	Replay button Exit/Close button		(11) Closing

Screen	Cohort	Script/Audio	Key phrase	Visual Notes	User Interface Notes	Data Capture	Screen Template
		why you may or may not want to learn your child's additional genomic sequencing results. • You will then be asked if you want to consent to learning your child's additional genomic sequencing results for *carrier status*, *medically actionable adult conditions*, and *non-medically actionable childhood conditions.* • At that time, you may choose to learn additional genomic sequencing results for all three kinds of conditions, only one or two of them, or none of them. All of these options are up to you.		talk about learning your child's additional genomic sequencing results. • Decide if you want to consent to learning any of your child's additional genomic sequencing results.			

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POLICY STATEMENT

Ethical and Policy Issues in Genetic Testing and Screening of Children

abstract

FREE

The genetic testing and genetic screening of children are commonplace. Decisions about whether to offer genetic testing and screening should be driven by the best interest of the child. The growing literature on the psychosocial and clinical effects of such testing and screening can help inform best practices. This policy statement represents recommendations developed collaboratively by the American Academy of Pediatrics and the American College of Medical Genetics and Genomics with respect to many of the scenarios in which genetic testing and screening can occur. *Pediatrics* 2013;131:620–622

COMMITTEE ON BIOETHICS, COMMITTEE ON GENETICS, AND THE AMERICAN COLLEGE OF MEDICAL GENETICS AND GENOMICS SOCIAL, ETHICAL, AND LEGAL ISSUES COMMITTEE

KEY WORDS

genetic testing, genetic screening, newborn screening, predictive testing, disclosure, carrier identification

ABBREVIATIONS

AAP—American Academy of Pediatrics
ACMG—American College of Medical Genetics and Genomics

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BACKGROUND

In 1953, Watson and Crick described the DNA double helix. Fifty years later, the full sequence of the human genome was published. Our knowledge of genetics grows rapidly, as does consumer interest in undergoing genetic testing. Statements about genetic testing of children in the United States written in the past 2 decades need to be updated to consider the ethical issues arising with new technologies and expanded uses of genetic testing and screening.[1,2] The growing literature on the psychosocial and clinical effects of such testing and screening can help inform us about best practices.

Genetic testing and screening of minors are commonplace. Every year, ~4 million infants in the United States undergo newborn screening for metabolic, hematologic, and endocrine abnormalities for which early treatment may prevent or reduce morbidity or mortality.

Outside of newborn screening, genetic testing of children is less commonly performed. Diagnostic genetic testing may be performed on a child with signs or symptoms of a potential genetic condition or for treatment decisions made on the basis of results of pharmacogenetic assays. Genetic testing may also be performed on an asymptomatic child with a positive family history for a specific genetic condition, particularly if early treatment may affect morbidity or mortality. The American Academy of Pediatrics (AAP) and the American College of Medical Genetics and Genomics (ACMG) provide the following recommendations regarding genetic testing and screening of minors. An accompanying technical report provides ethical explanations and empirical data in support of these recommendations (http://www.nature.com/gim/journal/vaop/ncurrent/full/gim2012176a.html).[3]

GENERAL RECOMMENDATIONS

1. Decisions about whether to offer genetic testing and screening should be driven by the best interest of the child.
2. Genetic testing is best offered in the context of genetic counseling. Genetic counseling can be performed by clinical geneticists, genetic counselors, or any other health care provider with appropriate training and expertise. The AAP and ACMG support the expansion of educational opportunities in human genomics and genetics for medical students, residents, and practicing pediatric primary care providers.

DIAGNOSTIC TESTING

3. In a child with symptoms of a genetic condition, the rationale for genetic testing is similar to that of other medical diagnostic evaluations. Parents or guardians should be informed about the risks and benefits of testing, and their permission should be obtained. Ideally and when appropriate, the assent of the child should be obtained.[4]
4. When performed for therapeutic purposes, pharmacogenetic testing of children is acceptable, with permission of parents or guardians and, when appropriate, the child's assent. If a pharmacogenetic test result carries implications beyond drug targeting or dose-responsiveness, the broader implications should be discussed before testing.

NEWBORN SCREENING

5. The AAP and ACMG support the mandatory offering of newborn screening for all children. After education and counseling about the substantial benefits of newborn screening, its remote risks, and the next steps in the event of a positive screening result, parents should have the option of refusing the procedure, and an informed refusal should be respected.

CARRIER TESTING

6. The AAP and ACMG do not support routine carrier testing in minors when such testing does not provide health benefits in childhood. The AAP and ACMG advise against school-based testing or screening programs, because the school environment is unlikely to be conducive to voluntary participation, thoughtful consent, privacy, confidentiality, or appropriate counseling about test results.
7. For pregnant adolescents or for adolescents considering reproduction, genetic testing and screening should be offered as clinically indicated, and the risks and benefits should be explained clearly.

PREDICTIVE GENETIC TESTING

8. Parents or guardians may authorize predictive genetic testing for asymptomatic children at risk of childhood-onset conditions. Ideally, the assent of the child should be obtained.
9. Predictive genetic testing for adult-onset conditions generally should be deferred unless an intervention initiated in childhood may reduce morbidity or mortality. An exception might be made for families for whom diagnostic uncertainty poses a significant psychosocial burden, particularly when an adolescent and his or her parents concur in their interest in predictive testing.
10. For ethical and legal reasons, health care providers should be cautious about providing predictive genetic testing to minors without the involvement of their parents or guardians, even if a minor is mature. Results of such tests may have significant medical, psychological, and social implications, not only for the minor but also for other family members.

HISTOCOMPATIBILITY TESTING

11. Tissue compatibility testing of minors of all ages is permissible to benefit immediate family members but should be conducted only after thorough exploration of the psychosocial, emotional, and physical implications of the minor serving as a potential stem cell donor. A donor advocate or similar mechanism should be in place from the outset to avert coercion and safeguard the interests of the child.[5]

ADOPTION

12. The rationale for genetic testing of children in biological families should apply for adopted children and children awaiting placement for adoption. If a child has a known genetic risk, prospective adoptive parents must be made aware of this possibility. In rare cases, it may be in a child's best interest to undergo predictive genetic testing for a known risk before adoption to ensure the child's placement with a family capable of and willing to accept the child's potential medical and developmental challenges. In the absence of such indications, genetic testing should not be performed as a condition of adoption.

DISCLOSURE

13. At the time of genetic testing, parents or guardians should be encouraged to inform their child of the test results at an appropriate age. Parents or guardians should be advised that, under most circumstances, a request by a mature adolescent for test results should be honored.
14. Results from genetic testing of a child may have implications for the parents and other family

members. Health care providers have an obligation to inform parents and the child, when appropriate, about these potential implications. Health care providers should encourage patients and families to share this information and offer to help explain the results to the extended family or refer them for genetic counseling.

15. Misattributed paternity, use of donor gametes, adoption, or other questions about family relationships may be uncovered "incidentally" whenever genetic testing is performed, particularly when testing multiple family members. This risk should be discussed, and a plan about disclosure or nondisclosure should be in place before testing.

DIRECT-TO-CONSUMER TESTING

16. The AAP and ACMG strongly discourage the use of direct-to-consumer and home kit genetic testing of children because of the lack of oversight on test content, accuracy, and interpretation.

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Ethical and Policy Issues in Genetic Testing and Screening of Children

COMMITTEE ON BIOETHICS, COMMITTEE ON GENETICS, AND, THE AMERICAN COLLEGE OF MEDICAL GENETICS AND, GENOMICS SOCIAL, ETHICAL and AND LEGAL ISSUES COMMITTEE

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SUPPLEMENT ARTICLE

Newborn Screening: Toward a Uniform Screening Panel and System—Executive Summary

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The authors have indicated they have no financial relationships relevant to this article to disclose.

ABSTRACT

The Maternal and Child Health Bureau commissioned the American College of Medical Genetics to outline a process of standardization of outcomes and guidelines for state newborn screening programs and to define responsibilities for collecting and evaluating outcome data, including a recommended uniform panel of conditions to include in state newborn screening programs. The expert panel identified 29 conditions for which screening should be mandated. An additional 25 conditions were identified because they are part of the differential diagnosis of a condition in the core panel, they are clinically significant and revealed with screening technology but lack an efficacious treatment, or they represent incidental findings for which there is potential clinical significance. The process of identification is described, and recommendations are provided.

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Key Words
newborn, screening, genetics, public health, panel, congenital

Abbreviations
HRSA—Health Resources and Services Administration
AAP—American Academy of Pediatrics
ACMG—American College of Medical Genetics
MS/MS—tandem mass spectrometry

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In the United States, newborn screening is a highly visible and important state-based public health program that began >40 years ago. States and territories mandate newborn screening of all infants born within their jurisdiction for certain disorders that may not otherwise be detected before developmental disability or death occurs. Newborns with these disorders typically appear normal at birth. Appropriate compliance with the medical management prescribed can allow most affected newborns to develop normally. As the model for public health-based population genetic screening, newborn screening is recognized nationally as an essential program that aims to ensure the best outcomes for the nation's newborn population.

There are no national newborn screening standards, aside from the *Standard on Blood Collection on Filter Paper* published by the National Committee for Clinical Laboratory Standards[1] and guidance from the Council of Regional Networks for Genetic Services, funded by the Health Resources and Services Administration (HRSA), and limited advice is available from national advisory committees and national medical or public health professional organizations regarding newborn screening policies and conditions to be included in screening mandates. The level of state resources available (personnel, equipment, and service capacity); programs' interpretations of available evidence concerning given conditions (incidence, treatability, and impact); availability or expense of new screening methods; and public advocacy by families, health care professionals, and state legislators have often led to divergence among states regarding which conditions should be mandated for newborn screening. This divergence has resulted in significant disparities in screening services available to infants. In 2000, the American Academy of Pediatrics (AAP) Newborn Screening Task Force[1] indicated that greater uniformity among programs would benefit families, professionals, and public health agencies.

The public health system faces many challenges as newborn screening capabilities continue to evolve. The health care service infrastructure is limited with respect to the interconnections among primary care professionals and subspecialists, particularly in rural areas, a problem complicated by the number and diversity of very rare conditions identified in newborn screening programs. There are geographic limitations in the availability of specific expertise for many of the rare conditions, and considerable needs exist throughout the health care system in the areas of training and education about the disorders detected through newborn screening programs. Furthermore, improvements in the newborn screening system and expansion of the number of conditions for which screening is offered have costs, and these costs and the associated benefits seem to accrue independently of the public and private health care delivery systems, which complicates their integration. Many states provide the programs necessary to ensure that screening and diagnosis occur, but they are limited in their ability to ensure long-term management, including the provision of the necessary treatment and services. In addition, new technologies have brought 3 major challenges to newborn screening: (1) expansion of the knowledge base regarding the causes and therefore the treatment or potential treatment of genetic diseases; (2) rapid expansion of diverse technologies, such as multiplex platforms, that may be used in screening; and (3) increased use of tiered testing strategies to enhance the positive predictive value of an initial abnormal result.

The lack of newborn screening program uniformity for infants, the changing dynamics of emerging technology, and the complexity of genetics necessitate assessment of the state of the art in newborn screening and views on future directions such programs could take. In 1999, the AAP Newborn Screening Task Force[2] recommended that "HRSA should engage in a national process involving government, professionals, and consumers to advance the recommendations of this Task Force and assist in the development and implementation of nationally recognized newborn screening system standards and policies."

In response to this need, the Maternal and Child Health Bureau of HRSA commissioned the American College of Medical Genetics (ACMG) to outline a process of standardization of outcomes and guidelines for state newborn screening programs and to define responsibilities for collecting and evaluating outcome data, including a recommended uniform panel of conditions to include in state newborn screening programs. It was expected that the analytical endeavor and subsequent recommendations would be definitive and that the subsequent recommendations would be based on the best scientific evidence and analysis of that evidence. ACMG was asked specifically to develop recommendations to address (1) a uniform condition panel (including implementation methods), (2) model policies and procedures for state newborn screening programs (with consideration of a national model), (3) model minimal standards for state newborn screening programs (with consideration of national oversight), (4) a model decision matrix for consideration of state newborn screening program expansion, and (5) consideration of the value of a national process for quality assurance and oversight. This report is a response to the HRSA/Maternal and Child Health Bureau request.

DEVELOPING A UNIFORM SCREENING PANEL

As indicated, the AAP task force was concerned particularly about the lack of uniformity between the state-based newborn screening programs and the need for "nationally recognized newborn screening system standards and policies." There are few existing systems that allow for the assessment of conditions to determine their

appropriateness for newborn screening. In addition to the original Wilson-Jungner criteria,[3] some states (eg, Nebraska and Washington) have developed such evaluation criteria and systems; other countries (eg, Australia and Belgium) have developed them as well. However, most use criteria that either are difficult to quantify or do not allow conditions to be comparatively ranked adequately. Most are inadequate with respect to the handling of conditions that have similar or overlapping disease markers or may be detected through the use of multiplex technologies but may vary in their analytical and clinical features.

METHODS

Expert Group Development and Process

ACMG convened a group, the Newborn Screening Expert Group, that included participants with expertise in various areas of subspecialty medicine and primary care, health policy, law, ethics, and public health, consumers, and several ad hoc work groups. As an initial step in the process, the expert group developed a set of guiding principles for its work. The establishment of these principals was followed by the development of criteria with which conditions were to be evaluated and the identification of the conditions to be evaluated. A steering committee oversaw the work of this group. The 2 work groups were formed to provide more in-depth analysis in 2 specific areas, that is the uniform panel and its criteria and the diagnosis and follow-up system.

The expert group used a 2-tiered approach for assessing and ranking conditions. In the first tier, with the specific evaluation criteria, conditions were analyzed by recognized experts and other interested individuals to develop a quantification of opinion. In the second tier, the quantification data were subjected to an analysis of the evidence base for each specific screening criterion score. Basic principles developed to guide the decision-making process were factored with the 2 levels of analysis to yield a set of core conditions. Further, additional conditions that are clinically significant that can be revealed during establishment of the diagnosis due to relationships with screening analytes used to identify core conditions or the technology used to screen were identified and referred to as secondary condtions.

Establishing Principles

The following basic principles were developed as a framework for defining the criteria with which to evaluate conditions and to make recommendations. (1) Universal newborn screening is an essential public health responsibility that is critical for improving the health outcomes of affected children. (2) Newborn screening policy development should be driven primarily by the interests of affected newborns, with secondary consideration being given to the interests of unaffected newborns, families, health professionals, and the public. (3) Newborn screening is more than testing. It is a coordinated comprehensive system consisting of education, screening, follow-up contact, diagnosis, treatment and management, and program evaluation. (4) The medical home and the public and private components of the screening programs should be in close communication, to ensure confirmation of test results and appropriate follow-up evaluation and care of identified newborns. (5) Recommendations about the appropriateness of conditions for newborn screening should be based on evaluation of scientific evidence and expert opinion. (6) To be included as a primary target condition in a newborn screening program, a condition should meet the following minimal criteria: it can be identified at a time (24–48 hours after birth) at which it would not ordinarily be detected clinically; a test with appropriate sensitivity and specificity is available for it; and there are demonstrated benefits of early detection, timely intervention, and efficacious treatment of the condition. (7) The primary targets of newborn screening should be conditions that meet the criteria listed in principle 6. The newborn screening program also should report any other results of potential clinical significance. (8) Centralized health information data collection is needed for longitudinal assessment of disease-specific screening programs. (9) Total quality management should be applied to newborn screening programs. (10) Newborn screening specimens are valuable health resources. Every program should have policies in place to ensure confidential storage and appropriate use of specimens. (11) Public awareness, coupled with professional training and family education, is a significant program responsibility that must be part of the complete newborn screening system.

Choosing Conditions

The conditions chosen for evaluation were included for ≥1 of several reasons, as follows. They are included in private, state, or national newborn screening programs. They are revealed coincidentally by some of the technologies used in newborn screening. They were identified by members of the expert group as worthy of consideration. They were identified by disease-specific advocacy organizations. They are included in the differential diagnosis of screening results for another condition. In the course of information collection, all conditions were subject to reconsideration. Eighty-four conditions were chosen for consideration.

Developing Evaluation Criteria and Their Comparative Values

The uniform panel working group developed the criteria with which conditions were to be evaluated; these were modified subsequently by the expert group. Criteria were divided into 3 main categories that covered aspects of the condition, that is, (1) clinical characteristics (eg, incidence, burden of disease if not treated, and pheno-

type in the newborn); (2) analytical characteristics of the screening test (eg, availability and features of the platform); and (3) diagnosis, treatment, and management of the condition in acute and chronic forms (this criterion includes the availability of health professionals experienced in diagnosis, treatment, and management).

Within each of these categories, several component criteria were developed (resulting in a total of 19 criteria) for assignment of the comparative value or score. The scoring system recognizes the strengths and limitations found for each condition and summarizes them in a ranking system. Therefore, a low score in a particular area does not necessarily mean that screening for that condition will never be conducted. In fact, low scores could be overruled by scientific evidence of new advances in testing and treatment and should be recognized as opportunities for targeted research endeavors and subsequent reconsideration of the condition for inclusion.

The criteria that were developed to differentiate the appropriateness of conditions for newborn screening include some that have a highly objective scientific basis and others that are associated with more subjective aspects. To the extent possible, the expert group relied on the scientific literature to provide the information on which the recommendations are based. However, some criteria have significant subjective aspects that require the consideration of more than just scientific and expert opinion. For example, issues of cost were considered but were not viewed as central in the analysis of the scientific literature. Cost is an example of a subjective criterion because it is a contextual concern and can be measured only against the value of the outcome.

Collecting Data

The first tier of the analysis was accomplished through the development of a data collection instrument containing the evaluation screening criteria. A survey was conducted to allow for the input of a wide range of individuals and organizations with interest in newborn screening. The data collection instrument included methods not only to collect information from experts but also to quantify that expert opinion regarding features of the conditions under consideration for inclusion in a uniform condition panel.

Before wide distribution, the data collection instrument was pilot tested. Potentially ambiguous language was identified and clarified, and scores were adjusted modestly to reflect the evolving priorities of the expert group. After modification, the data collection instrument was made widely available through passive efforts (eg, Listserv lists of interest groups such as the Genetic Alliance, Association of Public Health Laboratories, and Association of State and Territorial Health Officials) and active efforts (eg, direct approaches to experts on the conditions under evaluation and/or to support groups for particular conditions under evaluation). In this way, it was possible to acknowledge broad views that were of a more-subjective nature, such as the simplicity of the treatment (parents and individuals with the disorder in question often differed significantly from experts when scoring items such as simplicity of treatment). The results led to a preliminary listing of conditions and their placement in 1 of 3 categories, that is, high scoring, moderately scoring but part of the differential diagnosis of a high-scoring condition, or low scoring and not appropriate for newborn screening at this time. The responses of ≥ 3 recognized experts for each condition were compared with responses of all respondents regarding that condition, and results were found to be consistent.

Survey results were analyzed statistically. Respondents were characterized to ensure that they were broadly representative of the population. With the recognition that not all who responded have expertise or experience in all aspects of newborn screening for a specific condition, methods were used that allowed data to be aggregated for each criterion for each condition, rather than using the total score for a condition. A mean score for each criterion for each condition was based only on the responses provided for the criterion. Respondents were allowed to insert a "U" if an answer was unknown. The sum of the means was used for the total score assigned to a condition, because the sum of means tends to acknowledge dissenting views more clearly than does the sum of medians.

It is recognized that this relatively open survey process limited the views of experts while considering the views of those less knowledgeable about the individual conditions. However, analyses provided by scientific experts showed that their views were in close agreement with those of most respondents.

Establishing and Integrating the Evidence Base

In the second tier of the assessment, the evidence base for the conditions was established and an algorithm through which conditions were reassessed was developed. Each condition was considered with respect to the available scientific evidence, such as systematic reviews of reference lists (including Medline, PubMed, and others), books, Internet sources, professional guidelines, clinical evidence, and cost/economic evidence and modeling, for each criterion. The categorization was adjusted in accordance with the evidence. The analysis of the evidence base from the scientific literature included details about the screening tests, the efficacy of treatments, and the adequacy of the knowledge base for the condition. Disease-specific fact sheets were developed to describe this evidence.

At least 2 recognized experts examined the evidence on the fact sheet for all criterion scores for the conditions and assigned a level of evidence for each criterion score,

making the scoring system part of a fuller evidence-based analysis. Therefore, the evaluation of the evidence for the scores in the second tier of analysis is part of a broader assessment of the scientific literature related to the conditions, tests, and treatments. In addition to validating the evidence gleaned from the literature and other sources, the experts assigned a level of quality to the studies from which the evidence was drawn. Adjustments based on the evidence were made primarily on the basis of the accuracy of the information. When significant differences were found between the data collected through the survey and the evidence base, the differences were acknowledged and addressed in each of the fact sheets. Only rarely were adjustments required to align the literature evidence with the views of the survey respondents.

RESULTS

In the first tier of assessment, nearly 300 individuals from the United States and other countries completed the data collection instrument. Many respondents provided information on multiple conditions, yielding information on nearly 4000 individual disease-specific responses. The data are displayed in Table 1 and Fig 1, where the sums of the means are displayed for all conditions. Medium-chain acyl-CoA dehydrogenase deficiency, congenital hypothyroidism, and phenylketonuria were the highest-scoring conditions in this evaluation system, followed by biotinidase deficiency, sickle cell anemia, and congenital adrenal hyperplasia. A number of other conditions that scored in the upper third were also found to have an efficacious treatment and sufficient natural history information to be considered appropriate for newborn screening. Most conditions in the middle third of scores were also included in the differential diagnosis of ≥ 1 of the higher-scoring conditions. Almost all conditions in the bottom third of scores either lacked a screening test that had been validated in a general newborn population or were deficient in meeting several of the assigned evaluation criteria. Because of limited involvement of infectious disease experts, the expert group chose to defer decision-making on infectious diseases.

A score of 1200 on the data collection instrument was found to provide a logical point of separation between a group of high-scoring conditions (1200–1799 of a possible 2100) and another group of low-scoring (<1000) conditions. A group of conditions with intermediate scores (1000–1199) was identified, all of which were part of the differential diagnosis of a high-scoring core condition but without an efficacious treatment or without a well-understood natural history.

With the use of expert opinion and the validated evidence base, each condition that had been assigned previously to a category on the basis of quantified scores was reconsidered on the basis of the scientific evidence regarding an available screening test, an efficacious treatment, an adequate understanding of the natural history, whether the condition was part of the differential diagnosis of another condition, and whether the screening test results were related to a clinically significant condition. These categories were referred to as the core panel, secondary targets (conditions that are part of the differential diagnosis of a core panel condition), and not appropriate for newborn screening (either no newborn screening test is available or there is poor performance with respect to multiple other evaluation criteria).

DISCUSSION

The basis for decision-making started with whether a screening test is available, which was then overlaid with the overall quantified expert opinion analysis gathered with the data collection information tool. The process of quantifying this expert opinion was informed by literature review and expert validation.

In the first tier of analysis, conditions with scores of >1200 met key criteria and were preliminarily considered appropriate for inclusion in a core newborn screening panel. Conditions scoring <1000 were not considered appropriate for inclusion in the core newborn screening panel at this time. As noted previously, the expert group determined that laboratories should report any result revealed coincidentally in the course of newborn screening that might be clinically significant. In general, the screening test has been optimized for the detection of primary target conditions. Optimizing the technology for a primary target condition does not necessarily optimize the detection of all possible conditions. These conditions are often revealed through diagnostic testing because they are part of the differential diagnosis of a core condition, as occurs with tandem mass spectrometry (MS/MS)-identified cases, but they may be apparent in the screening laboratory because of the technologies used in screening (eg, hemoglobinopathies detected with high-pressure liquid chromatography/isoelectric focusing). Therefore, the expert group designated a category of secondary targets, which included conditions for which results should be made available to health care professionals and/or families by the screening laboratory or for which results are determined during the diagnostic phase of the screening program and provided to families in the course of diagnosis and follow-up care. Most conditions placed in the secondary target category are part of the differential diagnosis of a condition in the core panel. Inclusion in the secondary target category allows for the collection of cases on a national level for additional investigation to understand the disease process and for development of treatment modalities. Regardless of whether programs choose to integrate all such conditions into their broader newborn screening programs, it will be important for them to

TABLE 1 **Scores for All Conditions (Sorted in Descending Order of Sum of Mean Scores)**

Condition	Abbreviation	Sum of Mean Scores	Percentile
Medium-chain acyl-CoA dehydrogenase deficiency	MCAD	1799	1.00
Congenital hypothyroidism	CH	1718	0.99
Phenylketonuria	PKU	1663	0.98
Neonatal hyperbilirubinemia (kernicterus)	HPRBIL	1584	0.96
Biotinidase deficiency	BIOT	1566	0.95
Sickle cell anemia (hemoglobin SS disease)	Hb SS	1542	0.94
Congenital adrenal hyperplasia (21-hydroxylase deficiency)	CAH	1533	0.93
Isovaleric acidemia	IVA	1493	0.89
Very long-chain acyl-CoA dehydrogenase deficiency	VLCAD	1493	0.89
Maple syrup disease	MSUD	1493	0.89
Classic galactosemia	GALT	1473	0.88
Hemoglobin S/β-thalassemia	Hb S/βTh	1455	0.87
Hemoglobin S/C disease	Hb S/C	1453	0.86
Long-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency	LCHAD	1445	0.84
Glutaric acidemia type I	GA I	1435	0.83
3-Hydroxy-3-methyglutaric aciduria	HMG	1420	0.82
Trifunctional protein deficiency	TFP	1418	0.81
Multiple carboxylase deficiency	MCD	1386	0.80
Benign hyperphenylalaninemia	H-PHE	1365	0.78
Methylmalonic acidemia (mutase deficiency)	MUT	1358	0.77
Homocystinuria (attributable to cystathionine β-synthase deficiency)	HCY	1357	0.76
3-Methylcrotonyl-CoA carboxylase deficiency	3MCC	1355	0.75
Hearing loss	HEAR	1354	0.73
Methylmalonic acidemia (Cbl A,B)	Cbl A,B	1343	0.72
Propionic acidemia	PROP	1333	0.71
Carnitine uptake defect	CUD	1309	0.69
Galactokinase deficiency	GALK	1286	0.69
Glucose-6-phosphate dehydrogenase deficiency	G6PD	1286	0.67
β-Ketothiolase deficiency	BKT	1282	0.66
Citrullinemia	CIT	1266	0.65
Argininosuccinic acidemia	ASA	1263	0.64
Tyrosinemia type I	TYR I	1257	0.63
Short-chain acyl-CoA dehydrogenase deficiency	SCAD	1252	0.61
Tyrosinemia type II	TYR II	1249	0.60
Glutaric acidemia type II	GA2	1224	0.59
Medium/short-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency	M/SCHAD	1223	0.58
Cystic fibrosis	CF	1200	0.57
Variant hemoglobinopathies (including hemoglobin E)	Var Hb	1199	0.55
Human HIV infection	HIV	1193	0.54
Defects of biopterin cofactor biosynthesis	BIOPT(BS)	1174	0.53
Medium-chain ketoacyl-CoA thiolase deficiency	MCKAT	1170	0.52
Carnitine palmitoyltransferase II deficiency	CPT II	1169	0.51
Methylmalonic acidemia (Cbl C,D)	Cbl C,D	1166	0.49
Argininemia	ARG	1151	0.48
Tyrosinemia type III	TYR III	1149	0.47
Defects of biopterin cofactor regeneration	BIOPT(REG)	1146	0.46
Malonic acidemia	MAL	1143	0.45
Carnitine/acylcarnitine translocase deficiency	CACT	1141	0.43
Isobutyryl-CoA dehydrogenase deficiency	IBG	1134	0.42
2-Methyl-3-hydroxybutyric aciduria	2M3HBA	1132	0.41
Carnitine palmitoyltransferase I deficiency (liver)	CPT IA	1131	0.40
2-Methylbutyryl-CoA dehydrogenase deficiency	2MBG	1124	0.39
Hypermethioninemia	MET	1121	0.37
Dienoyl-CoA reductase deficiency	DE RED	1119	0.36
Galactose epimerase deficiency	GALE	1066	0.35
3-Methylglutaconic aciduria	3MGA	1057	0.34
Severe combined immunodeficiency	SCID	1047	0.33
Congenital toxoplasmosis	TOXO	1041	0.31
Familial hypercholesterolemia (heterozygote)	FHC	1038	0.30
Carnitine palmitoyltransferase I deficiency (muscle)	CPT IB	1009	0.29
Citrullinemia type II	CIT II	1001	0.28

TABLE 1 **Continued**

Condition	Abbreviation	Sum of Mean Scores	Percentile
Ornithine transcarbamylase deficiency	OTC	942	0.27
Guanidinoacetate methyltransferase deficiency	GAMT	922	0.24
Wilson disease	WD	922	0.24
Diabetes mellitus, insulin dependent	IDDM	891	0.23
Neuroblastoma	NB	864	0.22
Arginine:glycine amidinotransferase deficiency	AGAT	861	0.20
Turner syndrome	TURNER	847	0.19
Adenosine deaminase deficiency	ADA	841	0.18
Carbamoylphosphate synthetase deficiency	CPS	833	0.17
α1-Antitrypsin deficiency	A1AT	819	0.16
Congenital cytomegalovirus infection	CMV	779	0.14
Duchenne and Becker muscular dystrophy	DMD	776	0.12
Fragile X syndrome	FX	776	0.12
Congenital disorder of glycosylation type Ib	CDG Ib	766	0.11
Smith-Lemli-Opitz syndrome	SLO	759	0.10
Biliary atresia	BIL	744	0.08
Hurler-Scheie syndrome	MPS-1H	707	0.07
X-linked adrenoleukodystrophy	ALD	705	0.06
Fabry disease	FABRY	661	0.05
Creatine transport defect	CR TRANS	646	0.04
Lysosomal storage diseases	LSD	638	0.02
Pompe disease	POMPE	613	0.01
Krabbe disease	KRABBE	447	0.00

have the diagnostic confirmatory results for all such cases, because the results have a direct impact on the calculation of false-positive rates of screening for the core panel conditions.

After conditions were preliminarily categorized on the basis of their data collection instrument scores, the evidence base, as reflected in fact sheets developed for each condition, was assessed. If a clinically significant condition in the core panel did not have the scientific evidence to support the availability of an efficacious treatment, then it was moved to the secondary target category. Similarly, if it was determined that an understanding of the natural history of the condition was insufficient to justify primary screening, then the condition was moved to the secondary target category. When test results identified carriers of the conditions definitively, the handling of carrier information was moved into the secondary target category.

Figure 2 demonstrates the decision-making algorithm. It is important to note that the algorithm presumes an ongoing review of conditions to determine their continued or newly identified appropriateness for newborn screening as new tests and treatments evolve. The data collection instrument used in this project provides an assessment of only one aspect of a broader decision-making process required for establishing a newborn screening uniform panel. An ongoing analysis of the scientific evidence must be overlaid on the quantified expert opinion.

Clearly, the first decision to screen is based on the availability of a sensitive specific screening test that can be performed in the 24- to 48-hour period after birth. A total of 29 conditions are considered appropriate for newborn screening because they have a screening test, an efficacious treatment, and adequate knowledge of natural history (Table 2). The conditions best meeting all of the criteria established by the expert group are medium-chain acyl-CoA dehydrogenase, congenital hypothyroidism, and phenylketonuria. Among conditions assigned to the core panel are 9 organic acidurias, 6 amino acidurias, 5 disorders of fatty acid oxidation, 3 hemoglobinopathies associated with a hemoglobin S allele, and 6 other conditions. Twenty-three of the 29 conditions in the core panel are identified with multiplex technologies such as MS/MS.

On the basis of the evidence, 6 of the 35 conditions placed initially in the core panel were moved into the secondary target category, which expanded to 25 conditions that are part of the differential diagnosis of a core panel condition. Knowledge of these secondary targets (ie, from newborn screening or follow-up test results) can be clinically important to the family. In addition to the 54 conditions identified in Table 2, the expert group identified 27 conditions that were not considered appropriate for newborn screening, either because they met few evaluation criteria or because they lacked a screening test.

There were limitations. Conditions with limited evidence reported in the scientific literature were more difficult to evaluate with the data collection instrument. For example, some conditions have been reported for

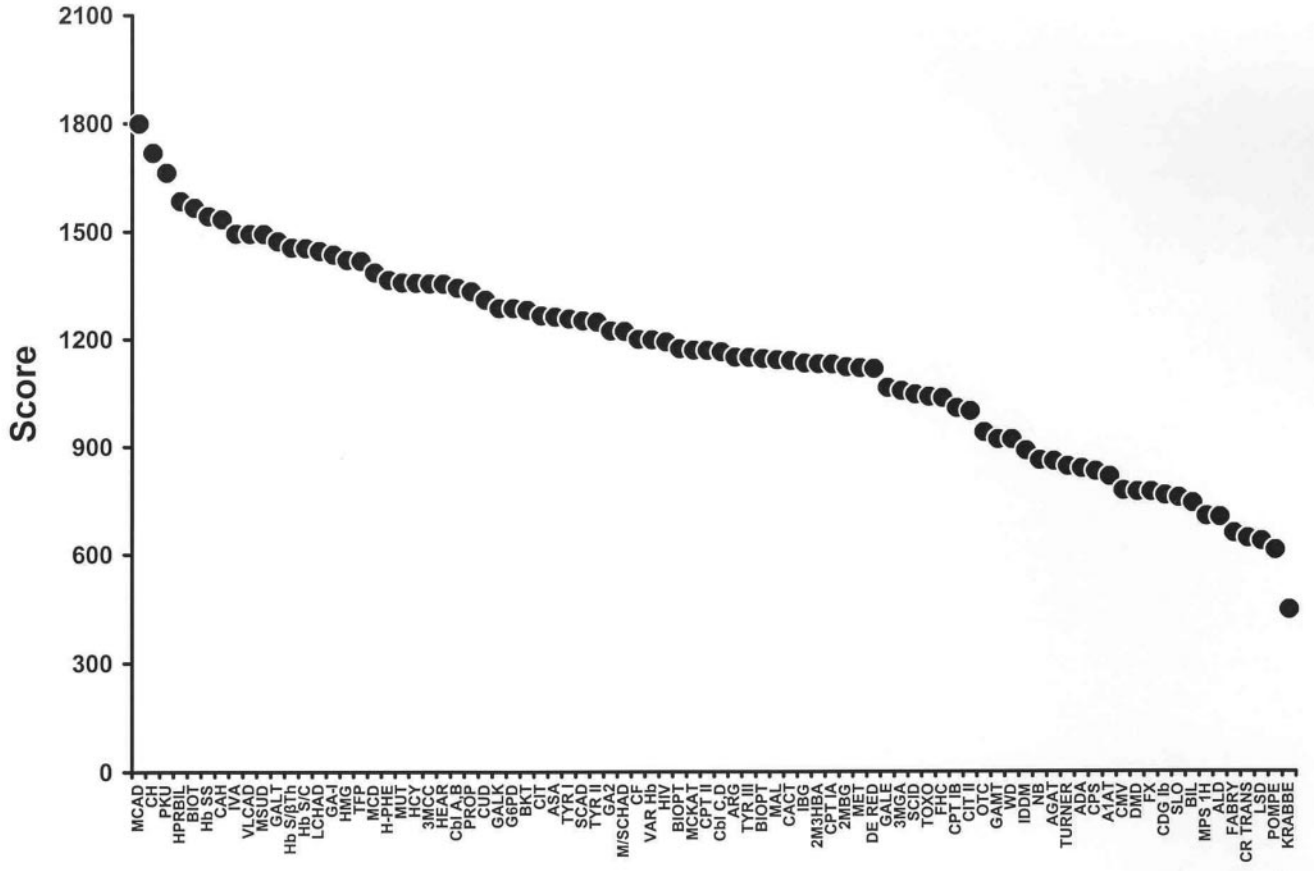

FIGURE 1

Scoring according to test availability. This scoring separates conditions that have an acceptable, validated, population-based screening test from those that do not. Abbreviations for conditions are as listed in Table 1.

≤10 families in the world. Many conditions were found to occur in multiple forms, distinguished by age of onset, severity, or other features. Furthermore, unless a condition was already included in newborn screening programs, a potential for bias was apparent in the information related to some criteria. The power of the statistical analyses and the blending of 2 forms of evaluation also presented limitations. The data collection process in the first tier of the analysis was limited also by the significant variability in the numbers of individuals responding for the different conditions. Because of limitations in the scientific evidence for these rare diseases, there was significant reliance on the opinions of experts on the conditions. There were many conditions that scored close to other conditions, and it is unlikely that the statistical power provided in these analyses was sufficient to discriminate accurately among the conditions in a ranking system. Nevertheless, groups of scores were assessed, and natural separations between groups became apparent. In such circumstances, expert opinion, with reasoning that applied first principles of genetic medicine to the evidence and to the quality of the data, determined the placement of the conditions in particular categories.

PROGRAM EVALUATION, COST-EFFECTIVENESS, AND FUTURE NEEDS OF THE NEWBORN SCREENING SYSTEM

The Newborn Screening System

Because the appropriate functioning of the system is critical to realizing improved outcomes, the components of a screening system were examined by the expert group during the project. (Information was obtained from program reports submitted to the National Newborn Screening and Genetics Resource Center and is based on information available as of October 2003.) The goal of the evaluation was to determine the extent to which states have addressed the many aspects of components of this system and to recommend performance standards to improve the quality of the system. The ability to ensure appropriate diagnosis and management is considered to be primarily a system responsibility. Limitations and significant variability were identified in the components of prenatal education, screening, follow-up services, diagnosis, treatment, and program management. For example, financing across state and county lines is constrained by state-based Medicaid rules; service delivery is fragmented on a categorical or disease basis; there is insufficient support to bridge geo-

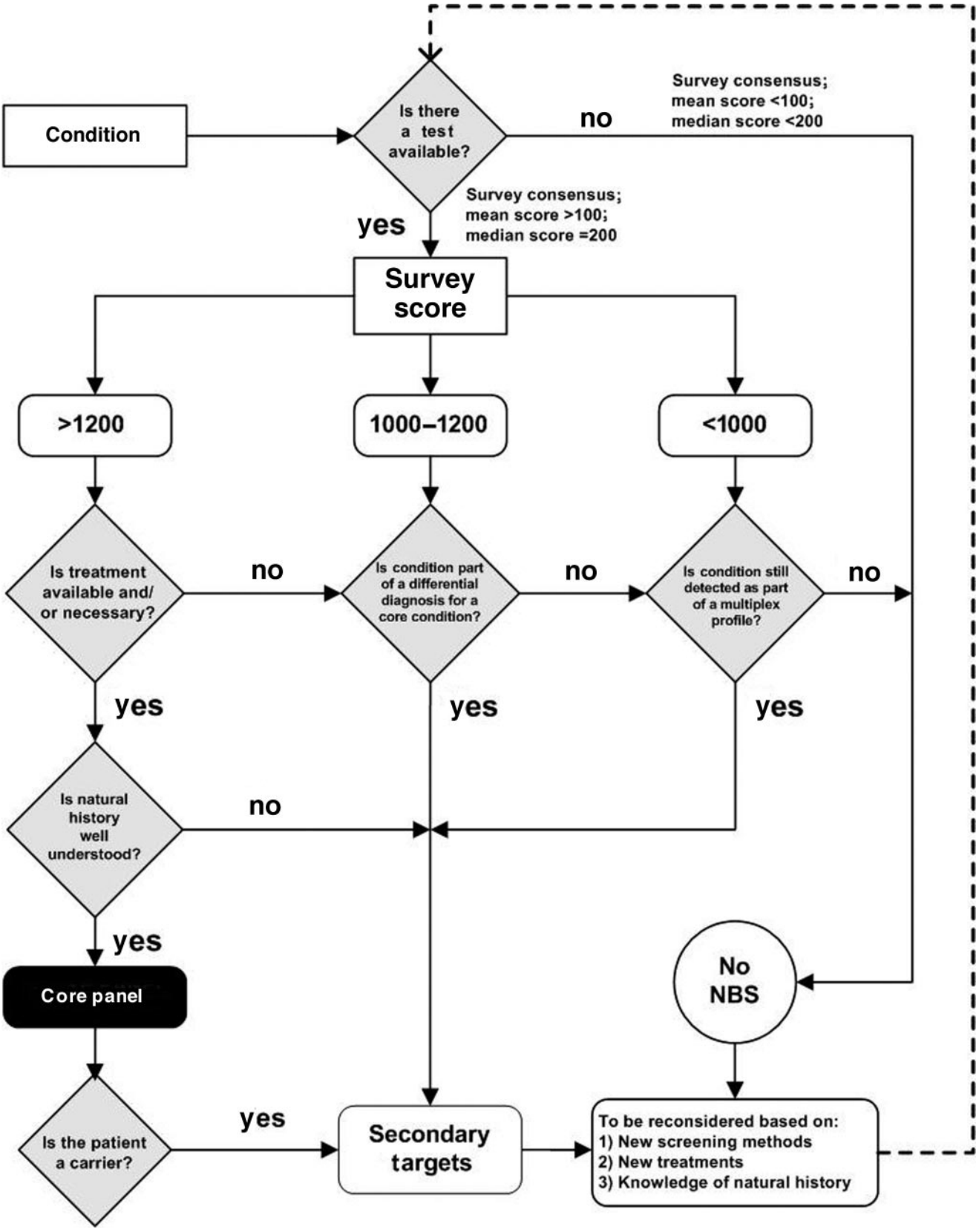

FIGURE 2
Condition evaluation and decision-making algorithm. NBS indicates newborn screening.

TABLE 2 **Core Panel and Secondary Targets in Newborn Screening Panel**

OA	FAO	AA	Hemoglobinopathies	Other
Core panel				
IVA	MCAD	PKU	Hb SS[a]	CH
GA I	VLCAD	MSUD	Hb S/βTh[a]	BIOT
HMG	LCHAD	HCY[a]	Hb S/C[a]	CAH[a]
MCD	TFP	CIT		GALT
MUT[a]	CUD	ASA		HEAR
3MCC[a]		TYR I[a]		CF
Cbl A,B[a]				
PROP				
BKT				
Secondary targets				
Cbl C,D[a]	SCAD	H-PHE	Var Hb[a]	GALK[a]
MAL	GA2	TYR II		GALE
IBG	M/SCHAD	BIOPT(BS)		
2M3HBA	MCKAT	ARG		
2MBG	CPT II	TYR III		
3MGA	CACT	BIOPT(REG)		
	CPT IA	MET		
	DE RED	CIT II		

OA indicates disorders of organic acid metabolism; FAO, disorders of fatty acid metabolism; AA, disorders of amino acid metabolism. Abbreviations used for conditions are listed in Table 1.
[a] Conditions for which specific discussions of unique issues are found in the text.

graphic barriers; it is difficult to identify experienced health care professionals for complex care (eg, centers of excellence for genital reconstructive surgery for congenital adrenal hyperplasia or confirmation of metabolic diagnoses); there is misinterpretation of privacy regulations (eg, the Health Information Portability and Accountability Act); there is underuse and lack of uniformity of information technology; collaborative management and care are often constrained by systems of reimbursement for services; state sovereignty sometimes dictates individual approaches; and there is variability in financing of screening programs.[4]

There are national and state roles in addressing these limitations, and states must retain their significant roles and responsibilities. They have clear authority with regard to oversight and evaluation, as well as enforcement. There is a need to integrate the various systems of health care coverage and payment through flexible comprehensive financing of services. Service coordination at state and local levels must be considered, as well as program integration with the State Children's Health Insurance Program, early intervention programs, Title V programs, and similar services.

It is apparent, however, that all state programs could benefit from a more-robust national role in newborn screening. Because so many of the conditions screened for among newborns or under consideration for screening are rare, most states that undertake evaluations of the scientific basis for screening of conditions must rely on the same, relatively small group of patients identified throughout the world. There is a potential national role in providing scientific evaluation of conditions and defining core condition panels. This would allow states to apply the best science to their own considerations when determining their roles in expanded screening.

Practice guidelines also could be developed at a national level by interested organizations. The expert group identified a clear gap between the information available and the information needed by primary care professionals to facilitate an immediate response in the event of a screen-positive case. In response, the expert group developed an action sheet for each core condition and secondary target, to facilitate immediate responses on the part of primary care professionals with respect to the expected steps in diagnosis and follow-up care.

There are also potentially expanded national roles in oversight, data collection, and program evaluation, as well as development of educational materials to support newborn screening. Depending on the overall incidence of particular conditions, regional collaborative groups such as those funded by HRSA could coordinate access to health care professionals, serve as coordinators and repositories for data collection, provide long-term follow-up capability when resources and expertise are limited, facilitate transition (and access) from pediatric to adult care, and provide education.

The distribution of primary, secondary, and tertiary services is based largely on the incidence of a condition and the complexity of its short- and long-term diagnosis and management. For more common conditions with easier diagnosis and follow-up management, there is likely to be sufficient local health care expertise for patient care. As incidence decreases and complexity increases, particularly for rare metabolic diseases, services become more difficult to access. Developing resources to ensure that health care professionals are available locally, regionally, and nationally will be important to ensuring access to high-quality services.

Cost-Effectiveness Analysis

A basic cost-effectiveness assessment project was performed to inform the decision-making process. The assessment focused primarily on a scientific analysis of conditions and the features that should be considered when deciding whether they should be included in a newborn screening program, because costs often are the basis on which such decisions are made.

Costs and benefits related to screening for particular conditions or groups of conditions were evaluated after mapping them over major disease outcomes (eg, life expectancy, cerebral palsy/stroke, seizures, developmental delay, hearing loss, and vision loss). Costs were obtained from the literature, and benefits were determined from expected outcomes with and without early treatment or intervention. The results of these analyses indicated that most newborn screening programs improve outcomes and reduce overall costs. Furthermore, technologies such as MS/MS or high-pressure liquid

chromatography save money because of their multiplexing capabilities and low screening false-positive rates. The identification of potentially affected individuals at such an early age leads to many years over which the benefits accrue and aggregate over costs.

CONCLUSIONS

Significant variability in the conditions for which newborns are screened led to this project to assess the scientific and medical evidence and the views of various individuals and interest groups associated with the conditions being considered. Throughout this undertaking, scientific literature and expert opinion formed the basis for information collection and assessment. The expert panel considered a range of information, from disease-specific information to the full breadth of the newborn screening system, in evaluating 84 conditions. There was an effort to overlay the evidence, where available, on expert opinion. The process of quantifying this expert opinion was informed by literature review and expert validation. It is important to acknowledge that there was limited scientific evidence available on the rare disorders considered by the expert panel. Furthermore, because there was limited activity in the area of coordinated data collection and analysis, it seemed unlikely that robust scientific evidence would be available in the near future. Therefore, reliance on experts and their ability to apply first principles[5,6] was required.

Guiding principals for newborn screening and criteria were established for evaluating conditions. The conditions being considered were assigned initially, through expert analysis, to 1 of 3 categories, depending on how they met the screening criteria. The categories were core panel, secondary targets (conditions that are part of the differential diagnosis of a core panel condition), and not appropriate for newborn screening (either no newborn screening test is available or there is poor performance with respect to multiple other evaluation criteria). Each condition was then evaluated to determine the extent to which the scientific evidence supports the availability of a test and a treatment, whether the natural history of the condition is well understood, and whether the information provided by testing indicates the possible presence of the condition or of a carrier state.

The expert panel identified 29 conditions for which screening should be mandated. An additional 25 conditions were identified because they are part of the differential diagnosis of a condition in the core panel, they are clinically significant and revealed with screening technology but lack an efficacious treatment (eg, some identified with MS/MS technology), or they represent incidental findings for which there is potential clinical significance (hemoglobinopathies). The expert group thought it was important that such findings be communicated to the health care service community and to families. In addition, the view that the technologies used in newborn screening should be maximized is inherent in the recommendation that all clinically significant information discovered through newborn screening should be provided to the relevant health care professionals and/or the family. The expert group recommends that state newborn screening programs mandate screening for all core panel conditions defined in this article; mandate reporting of all secondary target conditions defined herein and reporting of any abnormal results that may be associated with clinically significant conditions, including definitive identification of carrier status; maximize the use of multiplex technologies; and consider that the range of benefits realized through newborn screening includes treatments that go beyond an infant's death or morbidity.

The full breadth of the newborn screening system was assessed, including a brief review of its cost-effectiveness. Numerous barriers to implementation of an optimal screening and follow-up program were identified. Recommended actions to overcome these barriers include establishment of a national role in the scientific evaluation of conditions and the technologies with which they are screened, standardization of case definitions and reporting procedures, enhanced oversight of hospital-based screening activities, long-term data collection and surveillance, and consideration of the financial needs of programs.

The recommendations are as follows. (1) Programs should continue to improve the components of the system beyond the initial screening, communicate results, and ensure that affected newborns enter short-term follow-up care. (2) Reporting procedures should be standardized. (3) Reports of confirmatory results should be obtained. (4) There should be improved oversight (eg, Joint Commission on Accreditation of Hospital Organizations) of hospital-based screening activities, to improve tracking of screen-positive cases. (5) There should be more uniformity in the definition of the performance standards (eg, repeat test versus second test) monitored and reported by programs. (6) The quality assurance programs involving the diagnostic and follow-up system should be enhanced. (7) National oversight and authority, with appropriate resources, should be provided. (8) Systems should be in place for collection of data about individuals identified as screen-positive in newborn screening programs.

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ERRATA

American College of Medical Genetics Newborn Screening Expert Group. Newborn Screening: Toward a Uniform Screening Panel and System—Executive Summary. PEDIATRICS 2006;117:S296–S307.

An error appeared in the article by the American College of Medical Genetics Newborn Screening Expert Group titled "Newborn Screening: Toward a Uniform Screening Panel and System-Executive Summary" that was published in the May 2006 e-supplement issue of *Pediatrics* (doi: 10.1542/peds.2005-2633I). The editors of the report were not listed on the title page. The editors are Michael S. Watson, PhD, Marie Y. Mann, MD, MPH, Michele A. Lloyd-Puryear, MD, PhD, Piero Rinaldo, MD, PhD, and R. Rodney Howell, MD. We regret the error, which has been corrected in the online edition of *Pediatrics*.

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Sweetman L, Millington DS, Therrell BL, et al. Naming and Counting Disorders (Conditions) Included in Newborn Screening Panels. PEDIATRICS 2006;117:S308–S314.

An error appeared in the article by Sweetman et al, titled "Naming and Counting Disorders (Conditions) Included in Newborn Screening Panels" published in the May 2006 e-supplement of *Pediatrics* (doi: 10.1542/peds.2005-2633J). An Acknowledgement should read: "The authors wish to thank Piero Rinaldo, MD, PhD, for his technical assistance, expertise, and careful review and editing of this article." We regret the error, which has been corrected in the online edition of *Pediatrics*.

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Davis TC, Humiston SG, Arnold CL, et al. Recommendations for Effective Newborn Screening Communication: Results of Focus Groups with Parents, Providers, and Experts. PEDIATRICS 2006; 117:S326–S340.

An error appeared in the article by Davis et al, titled "Recommendations for Effective Newborn Screening Communication: Results of Focus Groups with Parents, Providers, and Experts" published in the May 2006 e-supplement of *Pediatrics* (doi: 10.1542/peds.2005-2633M). Donna Williams was omitted from the list of authors. The author list currently reads: "Terry C. Davis, Sharon G. Humiston, Connie L. Arnold, Joseph A. Bocchini, Jr, Pat F. Bass, III, Estela M. Kennen, Anna Bocchini, Penny Kyler, and Michele Lloyd-Puryear." The author list should read: "Terry C. Davis, Sharon G. Humiston, Connie L. Arnold, Joseph A. Bocchini, Jr, Pat F. Bass, III, Estela M. Kennen, Anna Bocchini, Donna Williams, Penny Kyler, and Michele Lloyd-Puryear." We regret the error, which has been corrected in the online edition of *Pediatrics*.

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Newborn Screening: Toward a Uniform Screening Panel and System—Executive Summary

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Actionable exomic incidental findings in 6503 participants: challenges of variant classification

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Recommendations for laboratories to report incidental findings from genomic tests have stimulated interest in such results. In order to investigate the criteria and processes for assigning the pathogenicity of specific variants and to estimate the frequency of such incidental findings in patients of European and African ancestry, we classified potentially actionable pathogenic single-nucleotide variants (SNVs) in all 4300 European- and 2203 African-ancestry participants sequenced by the NHLBI Exome Sequencing Project (ESP). We considered 112 gene-disease pairs selected by an expert panel as associated with medically actionable genetic disorders that may be undiagnosed in adults. The resulting classifications were compared to classifications from other clinical and research genetic testing laboratories, as well as with in silico pathogenicity scores. Among European-ancestry participants, 30 of 4300 (0.7%) had a pathogenic SNV and six (0.1%) had a disruptive variant that was expected to be pathogenic, whereas 52 (1.2%) had likely pathogenic SNVs. For African-ancestry participants, six of 2203 (0.3%) had a pathogenic SNV and six (0.3%) had an expected pathogenic disruptive variant, whereas 13 (0.6%) had likely pathogenic SNVs. Genomic Evolutionary Rate Profiling mammalian conservation score and the Combined Annotation Dependent Depletion summary score of conservation, substitution, regulation, and other evidence were compared across pathogenicity assignments and appear to have utility in variant classification. This work provides a refined estimate of the burden of adult onset, medically actionable incidental findings expected from exome sequencing, highlights challenges in variant classification, and demonstrates the need for a better curated variant interpretation knowledge base.

[Supplemental material is available for this article.]

Whole genome and exome tests are increasingly applied in clinical medicine. The American College of Medical Genetics and Genomics (ACMG) has recommended identification and return of incidental findings (IFs) from a minimum set of 56 actionable genes when a genomic test is performed (Green et al. 2013), unless patients opt out (American College of Medical Genetics and Genomics 2014). Some clinical laboratories return a broader set of IFs.

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However, there are limited data on frequency of such actionable pathogenic variants, and a standardized level of evidence for determining the pathogenicity of these variants has not been identified. We previously reviewed the primary literature for possible actionable, high penetrance pathogenic single-nucleotide variants (SNVs) in 114 genes in 500 European- and 500 African-ancestry participants randomly selected from the NHLBI Exome Sequencing Project (ESP) and posted on the exome variant server (EVS; http://evs.gs.washington.edu/EVS/) (Dorschner et al. 2013). We now extend these analyses to the 5503 additional participants in the ESP and revise from 114 to 112 genes associated with medically actionable genetic disorders that may remain undiagnosed in adults. These data give a more precise estimate of the frequency of such actionable findings in individuals of European or African ancestry. Such an estimate will allow a better understanding of the implications, including cost, of recommendations to return IFs from genomic tests.

Lack of consensus criteria for pathogenicity classification of variants is an ongoing issue in genomic medicine. It is common for clinicians to disagree with classifications from clinical laboratories. Therefore, we compare the results of our variant classification system to the classification of these variants by different clinical and research laboratories. A goal of these analyses is to investigate consistency in variant classification using criteria from different classification systems and to understand the features of these approaches that lead to discrepant pathogenicity assignments.

Results

Characteristics of the variants reviewed

Variants in 112 genes paired with medically actionable phenotypes of interest were reviewed in the 6503 participants from the NHLBI ESP. The variant classification criteria and categories are presented in Table 1A and Table 1B, respectively. There were 615 distinct variants annotated in the Human Gene Mutation Database (HGMD) as disease causing in these 6503 participants' exomes: 224 were identified in the original 1000 participants (500 European ancestry and 500 African ancestry) in Dorschner et al. (2013) and 391 additional variants in the remaining 5503 (4300 European-ancestry and 2203 African-ancestry) participants. This is attributable to the most common variants being identified in the first set analyzed, leaving fewer novel variants in the second set. Of the 615 unique variants, 116 (18.9%) variants had a minor allele frequency (MAF) greater than the allowable estimated disorder allele frequency and were not compatible with a highly penetrant disorder. On review of the literature, none of these 116 variants was classified as pathogenic or likely pathogenic by reviewers.

Most variants were observed more than once. Of the 599 variants in genes associated with dominant disorders, 44% (261/599) were seen only once. These singleton variants represented 51 (79.7%) of the 64 pathogenic or likely pathogenic variants in genes associated with dominant disorders. This frequency represented a significant excess of rare pathogenic variants relative to those variants observed more than once ($P = 4.6 \times 10^{-9}$). The distribution of the MAF of these variants for disorders inherited in an autosomal dominant pattern by classification is summarized in Table 2. The highest ancestry-specific MAF is a strong predictor of variant classification, excluding the likely benign class and variants in genes associated with recessive disorders ($P = 0.01$).

Variants classified as pathogenic or likely pathogenic

We used stringent criteria to classify variants as pathogenic or likely pathogenic given that we are addressing potential IFs. The details of the variant classification framework and review process are described in Methods. Variant classifications are summarized in Table 3 and the classification for each variant is given in Supplemental Table 1. We found 32 unique variants in 16 genes in all 6503 participants when considering the "pathogenic" variants from those annotated as disease causing in HGMD. The genes with these variants are summarized in Table 4 and the individual variants are listed in Supplemental Table 2. Pathogenic variants were found in 36/6503 (0.6%) of the ESP participants. Thirty-one participants had pathogenic variants in ACMG genes, whereas five had pathogenic variants in non-ACMG genes. Note that four of these 36 individuals were compound heterozygotes for two pathogenic variants assumed to be in *trans* in genes associated with disorders inherited in an autosomal recessive pattern. None of these 36 participants had more than one pathogenic or likely pathogenic variants in genes associated with dominant disorders. One individual was heterozygous for a pathogenic variant in *ATP7B*; however, this individual is not counted in the total number of participants with pathogenic results because carrier status was not considered reportable. Pathogenic variants were found in 30/4300 (0.7%) European-ancestry participants versus 6/2203 (0.3%) African-ancestry participants. No pathogenic variants were found in the 208 participants of Ashkenazi Jewish ancestry.

Table 1. Variant classification criteria[a,b] and variant classification categories

(A) Variant classification criteria

Allele frequency of variant	A1. Below cutoff[c] A2. Above cutoff
Segregation[d]	B1. In ≥2 unrelated families B2. In one family B3. No segregation studies
Number of affected unrelated individuals	C1. Identified in ≥3 unrelated affected individuals[e] or a significant difference in cases versus controls[f] C2. Identified in <3 unrelated affected individuals
De novo events in a trio[g]	D1. ≥1 event D2. No events
Function	E1. Protein truncation[h] where protein truncation is known to cause disorder
Other	F1. Seen only in combination with a known pathogenic variant for a dominant disorder

(B) Variant classification categories

Pathogenic	Likely pathogenic	Uncertain significance	Likely benign
A1 plus	A1 plus	A1 plus	A2 +/or F1
B1 or B2 + C1 or B2 + D1 or A1 + E1	B2 or C1 or D1	B3 or C2 or D2	

[a]Cautiously interpret functional evidence for all variant categories.
[b]Classify based on amino acid change, regardless of nucleotide change.
[c]Based on disorder frequency and inheritance pattern.
[d]Defined as probability of consistent sharing in the family of ≤1/16.
[e]If plausible based on frequency of disorder.
[f]For common variants.
[g]Mutation identified as de novo dominant in an affected offspring of unaffected parents (with known paternity).
[h]For example, nonsense, missplice, initiation codon.

Table 2. Highest ancestry-specific minor allele frequency (MAF) in EVS of HGMD disease-causing variants in dominant genes by variant classification

	Pathogenic	Likely pathogenic	Uncertain significance	Likely benign
N	29	34	395	135
Mean (range)	0.015 (0.012–0.035%)	0.023 (0.012–0.068%)	0.075 (0.012–1.037%)	0.206 (0.012–1.407%)

We found 38 unique variants in 23 genes when evaluating the "likely pathogenic" variants from those annotated as disease causing in HGMD for all 6503 participants. The genes with these variants are summarized in Table 4, and the individual variants are listed in Supplemental Table 1. A total of 65/6503 (1.0%) ESP participants had likely pathogenic mutations. Three individuals were compound heterozygous for one pathogenic and one likely pathogenic variant assumed to be in *trans* in genes associated with disorders inherited in an autosomal recessive pattern. A total of 53/65 (81.5%) had likely pathogenic variants in genes for which pathogenic variants are recommended for return by the ACMG report. Of these 65 participants with likely pathogenic variants, 52 (80%) were of European ancestry and 13 (20%) of African ancestry. Four of the European ancestry individuals with likely pathogenic variants also had Ashkenazi ancestry.

Variants classified as expected pathogenic

"Disruptive" expected pathogenic variants were defined as truncating and missplice-causing variants in the EVS that are not identified by HGMD as disease causing. The classification process for these variants included identifying those within the part of the transcript that likely lead to nonsense-mediated mRNA decay and investigating if truncating and missplice-causing variants are known to cause the associated phenotype of interest. There were 11 of these expected "disruptive" variants that were not listed in HGMD as disease-causing variants (Supplemental Table 3). There was no significant difference in distribution of expected pathogenic variants between ancestry groups; of the 12 participants with such variants, six were in the African-ancestry group and six in the European-ancestry group ($P = 0.12$), although power was limited.

A flowchart summarizing the number of HGMD disease-causing variants and non-HGMD expected disruptive variants reviewed, and the classifications of these variants in ACMG and non-ACMG genes, is presented in Figure 1.

Ancestry differences in identification of pathogenic or likely pathogenic variants

The number of pathogenic or likely pathogenic variants in individuals of African and European ancestry was compared. Among all 6503 subjects, the participants of African ancestry had fewer pathogenic or likely pathogenic variants annotated in HGMD (Table 3) than those of European ancestry, consistent with the prior analysis of 1000 participants. Only 19 (18.8%) of the 101 participants with likely pathogenic or pathogenic variants were in individuals of African ancestry, again significantly less than the proportion (2203/6503, 33.8%) that would be expected at random under the null hypothesis (binomial test $P = 0.0004$). This result replicates the previously reported deficit of HGMD derived pathogenic or likely pathogenic variants among African-ancestry individuals.

Median time and concordance in double review of variants

The time spent for the literature review and categorization step for each HGMD disease-causing variant by the initial reviewer was recorded. The median recorded time was 37 min (range: 1–175 min). This time did not include the time to generate the list of potential variants, collect the references, or resolve variants by secondary review.

Several quality control exercises were undertaken including the examination of 156 of the 615 disease-causing HGMD variants by a second reviewer. In addition, all variants initially classified as pathogenic or likely pathogenic (79) were reanalyzed by an experienced reviewer. Of the 156 variants that were initially double reviewed, 83 (53%) of the classifications were discrepant. Of the 79 variants initially classified as pathogenic and likely pathogenic that underwent blinded expert review, 56% (44/79) were reclassified. Nearly all of these reclassifications (42/44) were from the pathogenic or likely pathogenic classification to the variant of uncertain significance (VUS) classification. A repeated error was counting EVS participants as a person who is affected with the disorder and has the variant, even though the phenotypes of those participants were unknown. This was compounded when papers had reported the variants in EVS without relevant phenotype information for specific disorders (e.g., cardiomyopathy). As a result, all articles summarizing EVS data were identified, and the named variants were re-reviewed to be sure that papers referring to EVS data were not included in the calculation of the number of affecteds carrying the variant of interest. Geneticists of all experience levels made classification errors.

Variant classifications were compared with those collected through the Sharing Clinical Reports Project (SCRP). There was complete agreement (45/45) between classifications from the SCRP and those made by our reviewers. The classification of variants reviewed by both this project and the Partners Laboratory for Molecular Medicine (LMM) were also compared and agreement was high (97/99, 98%) (Supplemental Table 4). A summary of the evidence supporting the two

Table 3. Summary of number of participants with variant classifications in 112 genes and the 56 ACMG genes

	European ancestry $N = 4300$ (ACMG)[a]	African ancestry $N = 2203$ (ACMG)
Pathogenic variants from HGMD	30 (0.7%) [26 (0.6%)]	6 (0.3%) [5 (0.2%)]
Likely pathogenic variants from HGMD	52 (1.2%) [41 (1.0%)]	13 (0.6%) [12 (0.5%)]
Novel disruptive variants	6 (0.1%) [3 (0.07%)]	6 (0.3%) [6 (0.3%)]
Total	88 (2.0%) [70 (1.67%)]	25 (1.1%) [23 (1.0%)]

[a]The second, square-bracketed value indicates the summary considering only the 56 ACMG gene-disease pairs versus the 112 considered by authors.

Table 4. Pathogenic and likely pathogenic variants

	Associated phenotype	Pathogenic variants (participants)	Likely pathogenic variants (participants)	Expected disruptive variants (participants)
ACMG genes				
BRCA1 or *BRCA2*	Breast/ovarian cancer	7 (7)	0 (0)	3 (3)
MSH6, PMS2, CHD1	GI cancer	4 (4)	1 (2)	2 (3)
LDLR	Hypercholesterolemia	4 (6)	7 (12)	0 (0)
LMNA1, MYBPC3, DSG2, MYH7, MYL2, MYL3, PKP2, TNNI3, TNNT2	Cardiomyopathy	4 (4)	14 (24)	2 (2)
RYR1	Malignant hyperthermia	4 (5)	1 (2)	0 (0)
KCNQ1, SCN5A	Arrhythmia	1 (1)	3 (7)	0 (0)
RET	Multiple endocrine neoplasia	1 (1)	0 (0)	0 (0)
TP53	Li-Fraumeni syndrome	1 (1)	2 (6)	0 (0)
DSC2, DSP	Arrhythmogenic right ventricular dysplasia	0 (0)	0 (0)	2 (2)
ACMG gene total		26 (29)	28 (53)	9 (10)
Non-ACMG genes				
SERPINA1	Lung disease	2 (4[a])	2 (3[b])	0 (0)
PROC	Protein C deficiency	1 (1)	2 (2)	0 (0)
PROS	Protein S deficiency	0 (0)	0 (0)	1 (1)
ATP7B	Wilson disease	1 (3[c])	0 (0)	0 (0)
ENG, ACVRL1	Hereditary hemorrhagic telangiectasia	1 (1)	1 (1)	0 (0)
FLCN	Birt-Hogg-Dube	1 (1)	0 (0)	0 (0)
DMD	Cardiomyopathy	0 (0)	1 (1)	0 (0)
KCNE1, KCNE2	Arrhythmia	0 (0)	2 (4)	0 (0)
SLC7A9	Cystinuria	0 (0)	1 (1[c])	0 (0)
HMBS	Porphyria	0	1 (1)	0 (0)
PTCH1	Basal cell nevus syndrome	0 (0)	0 (0)	1 (1)
Non-ACMG gene total		6 (7)	10 (12)	2 (2)
Grand total		32 (36)	38 (65)	11 (12)

[a]Participant was compound heterozygote for two pathogenic variants.
[b]Participant was compound heterozygote for one pathogenic variant and one likely pathogenic variant.
[c]Participant was heterozygous for a pathogenic variant or a likely pathogenic variant and does not count toward the total number of participants.

different classifications is also presented. These few discrepancies are due, in part, to differences in classification criteria, including Partners LLM's inclusion of in silico predictions and use of functional data as supporting pieces of evidence, as well as reducing the significance of a variant when it has been reported in a person with an alternate explanation of the disorder. These results demonstrate that discrepant variant classifications may result even when the same public resources are used when different types of evidence are given different weights.

In addition, six variants were randomly selected within groups of varying pathogenicity assignments and were classified blindly by five research and clinical laboratories within the Clinical Sequencing Exploratory Research (CSER) consortium (http://www.genome.gov/27546194) according to their routine laboratory procedures. These variants along with each laboratory's classification are listed in Table 5. Complete agreement was attained only for the truncating variant; in contrast, for one variant, classification ranged from pathogenic to variant of uncertain significance. One laboratory appeared to have a lower threshold for calling variants likely pathogenic than the others. This difference in threshold raises concern for the consistency of variant assignments across laboratories. An investigation of the basis for discrepant classifications (data not shown) found that discrepancies appeared to result from differences in how cosegregation was used, how functional and in silico evidence were weighted, as well as in differences in resources used among reviewers.

Association of in silico pathogenicity scores with pathogenicity assignment

To address the utility of incorporating in silico pathogenicity scores into the interpretation of variants, Genomic Evolutionary Rate Profiling (GERP) and Combined Annotation Dependent Depletion (CADD) scores were compared across pathogenicity assignments (Table 6; Fig. 2). As previously noted, these scores were not used in our classifications. High CADD and GERP scores were found in all pathogenicity categories. Low and negative scores were seen more often in the likely benign class, while all variants in the pathogenic class had GERP $\geq$ 2.95 and CADD $\geq$ 12.37.

Discussion

We have analyzed exomes of 6503 ESP participants for variants in 112 medically actionable genes. We found that some 2.0% of adults of European ancestry and 1.1% of adults of African ancestry can be expected to have actionable highly penetrant pathogenic (including novel expected pathogenic) or likely pathogenic single-nucleotide variants (SNVs). If we analyze only pathogenic variants in the subset of genes that are included in the ACMG recommendations for reporting of IFs (Green et al. 2013), the proportion of individuals with returned IFs would be 0.7% in European-ancestry participants and 0.5% in African-ancestry participants.

Our classification of these reviewed variants is expected to be useful to clinical geneticists who commonly consult the EVS to determine allele frequencies and when evaluating SNV pathoge-

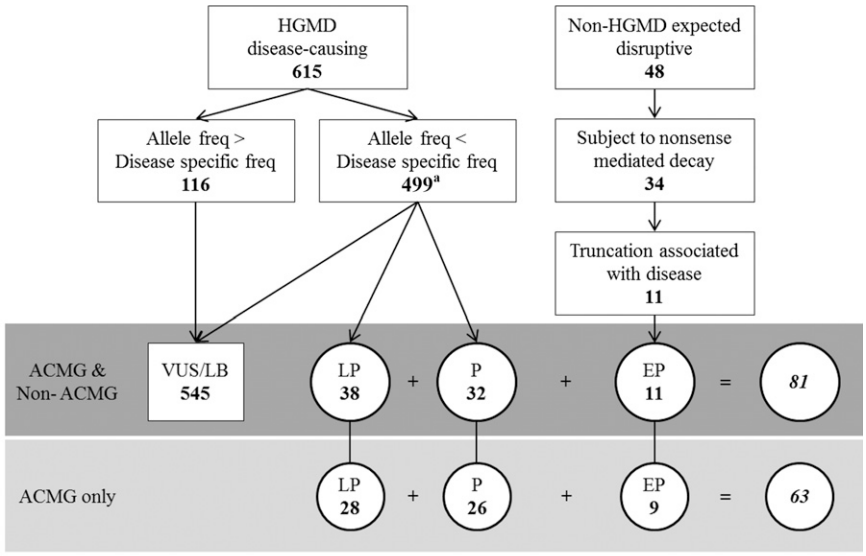

Figure 1. Variants reviewed and classifications in actionable ACMG and non-ACMG genes: (P) pathogenic; (LP) likely pathogenic; (VUS) variant of uncertain significance; (LB) likely benign; (EP) expected pathogenic.

nicity. Based on the few differences in our classification and those of Partners LMM, we may be overcalling variants that are truly VUS as likely pathogenic, as all of our differences are of this type. These data are based on contemporary variant databases and such databases are expected to include more classified variants over time. Our variant classifications in Supplemental Table 1 are annotated for which variants might be moved to a lower class if the base position's evolutionary conservation were incorporated into our criteria. The use of conservation would not affect the classifications of any variants we classified as pathogenic.

Notably, these data do not suggest that <2% of individuals will have abnormal genomic tests. Abnormal tests will also include results for the primary indication, copy number variants, and nonactionable disorders, none of which are considered in these analyses. Further, we consider only high penetrance variants. The results presented here reflect expected actionable IFs from SNVs identified in exome sequencing.

This report is consistent with the proportion of participants with pathogenic variants estimated for the non-Ashkenazi ancestry participants in ClinSeq, despite differences in criteria for classification. Considering 37 cancer risk genes, ClinSeq found four of 475 non-Ashkenazi (0.8%) participants had pathogenic variants, whereas four of 97 participants of Ashkenazi ancestry (4.1%) did (Johnston et al. 2012); seven of these eight variants were in *BRCA1* or *BRCA2*. We considered 34 of the 37 genes included by ClinSeq. ClinSeq reported no pathogenic variants in the remaining three genes: *RB1*, *WT1*, and *CDKN2A*. Examining only those 34 genes in the non-Ashkenazi ancestry group separately, our proportion of participants with pathogenic variants did not differ with those of ClinSeq (both $P = 0.78$). Alternatively, for those of Ashkenazi ancestry, our data was not comparable to that of ClinSeq due to our lack of insertion and deletion (indel) data. *BRCA1* or *BRCA2* founder mutations, all indels, have been reported to be found in 2.4% of 3742 Ashkenazi ancestry women (Hartge et al. 1999).

The proportion of participants with pathogenic and likely pathogenic variants reported here is slightly lower than that reported in our previous work with 1000 EVS samples (Dorschner et al. 2013). The revised estimates are likely due to (1) imprecision related to the smaller sample size, and (2) the absence of double review of all pathogenic and likely pathogenic variants in the original paper. After correction following double review, 2.4% of the original 500 European-ancestry participants and 0.8% of the original 500 African-ancestry participants had pathogenic or likely pathogenic SNVs.

The estimates reported here of ~2.0% of adults of European ancestry and 1.1% of adults of African ancestry having high penetrance actionable variants are substantially different from the estimate of 5% of participants (14 variants in 27 of 543 participants, with each variant only seen in a single family) expected to have a pathogenic variant in one of the 56 ACMG genes (but not limited to ACMG gene-phenotype pairs) recently published by Lawrence et al. (2014). These authors attributed differences in their estimates and those published in our prior paper on 1000 participants (Dorschner et al. 2013) to a variety of factors; however, it appears that their inclusion of family data, extension of phenotypes, and differences in classification criteria were the major factors. First, nearly half of the variants they reported as pathogenic (13/28) were the second occurrence of a variant in the same family, with the double counting yielding a higher estimate than might be found in unrelated individuals. Despite 13/14 variants being observed in two participants, none of their families was clearly seg-

Table 5. Classification of six variants by CSER sites

Site	*MSH6* c.2731C > T; p.Arg911*	*FBN1* c.4270C > G; p.Pro1424Ala	*TNNT2* c.732G > T; p.Glu244Asp	*RYR1* c.1840C > T; p.Arg614Cys	*LDLR* c.967G > A; p.Gly323Ser	*TSC2* c.736A > G; p.Thr246Ala
1[a]	Pathogenic	VUS	VUS	Likely pathogenic	VUS	VUS
2	Pathogenic	Likely pathogenic/VUS[b]	VUS	Pathogenic	VUS	VUS
3	Pathogenic	VUS	VUS	Pathogenic	VUS	VUS
4	Pathogenic	VUS	Likely pathogenic	Pathogenic	VUS	VUS
5	Pathogenic	Likely pathogenic/VUS	VUS	Likely pathogenic	VUS	Likely pathogenic
6	Pathogenic	Pathogenic/likely pathogenic	VUS	Likely pathogenic	Likely pathogenic /VUS	Likely pathogenic

[a]Classification from the EVS review of 6503 participants.
[b]Two classifications are listed when two reviewers at a site did not agree.

Table 6. GERP and CADD scores for nondisruptive variants by classification

Score (Min, Max)	Likely benign	VUS	Likely pathogenic	Pathogenic
N	136	405	32	17
GERP++	3.44 (−7.77, 6.08)	3.26 (−11.3, 6.17)	4.33 (0.633, 6.04)	4.49 (2.95, 5.67)
CADD	15.87 (0.004, 37)	15.97 (0, 37)	18.98 (10.66, 33)	20.14 (12.37, 32)

regating the phenotypes of interest. Second, although the ACMG suggests gene-disease pairs for highly penetrant actionable disorders, Lawrence et al. (2014) considered a different phenotype than the ACMG for five of their 14 variants, did not consider MAF, allowed fewer meiotic segregations to count as evidence, and relied heavily on functional assays. Interestingly, the participant they report with a putative pathogenic *APOB* variant had a normal lipid profile. As the authors note, we classified three of their putative pathogenic variants as VUSs. Of these, *SCN5A* T220I is also classified by Partners LMM as a VUS. Also, *CACNA1S* T1354S was seen 48 times in the 6503 EVS participants, yet the relevant disorder, malignant hyperthermia, would be expected in only one person in this cohort; this variant therefore was reclassified as benign for this phenotype by the NIH ClinSeq project (Gonsalves et al. 2013), despite abnormal in vitro function (Pirone et al. 2010). These lines of evidence suggest that the Lawrence et al. (2014) publication overestimates IFs. Further, it appears that the phenotypes considered and their classification criteria, not deeper exome coverage, are the critical factors contributing to their higher estimate.

The larger sample reported here confirms the deficiency of literature-derived HGMD pathogenic variants in those of African versus European ancestry that we previously reported (Dorschner et al. 2013). This deficit occurs in the portion of variants identified from the literature (summarized in HGMD) rather than in novel disruptive variants in which the proportion of variants identified in African-ancestry and European-ancestry individuals was the same. This is likely due to the underrepresentation of individuals of African ancestry in the literature or databases.

Even with clear criteria, there appears to be substantial inter-reviewer discordance and a bias toward classifying variants into higher pathogenicity categories. Discrepancies between any two reviewers of a variant were common (83/156, 53%). In the process of adjudication, the final classification of a variant generally agreed with the reviewer who initially assigned it the lower pathogenicity score. It is possible that had reviewers each considered more than 10–15 assigned variants then review would have become more consistent. High discordance among reviewers leads to some concern about plans for crowd-sourced variant classification.

Our findings suggest that discordant classification can be overcome by using multiple data sources and many experts providing input. Indeed, we had 100% concordance of our final variant classifications with the SCRP ($N = 45$) and 98% concordance with the Partners LMM ($N = 99$). Additionally, despite use of different criteria, the most common classifications made by the CSER laboratories also matched our classifications.

The criteria for pathogenicity classification should be standardized across laboratories in a way that promotes consistent determinations. A new ACMG classification proposal has been presented and is under internal review; however, this was unavailable when the University of Washington Return of Results Committee (RORC) began, and we opted for a simpler system that worked well, but that might have been improved by the consideration of in silico data. We consider six lines of evidence to be most important for variant classification:

1. Population minor allele frequency was a useful factor for variant classification, and variants observed only once in EVS were most likely to be pathogenic, supporting inclusion of MAF in classification criteria (Table 2). MAF is used by most classification systems (Duzkale et al. 2013; Eggington et al. 2013; Thompson et al. 2014) and by the ACMG draft guidelines. Highly penetrant alleles should be considerably less common than the associated dominantly inherited disorder, particularly as most disorders have high allelic heterogeneity. In general, high allele frequency in any ancestry group is evidence against pathogenicity, particularly for dominant disorders; however, founder mutations in populations with a high incidence of the associated disorder should be taken into account.

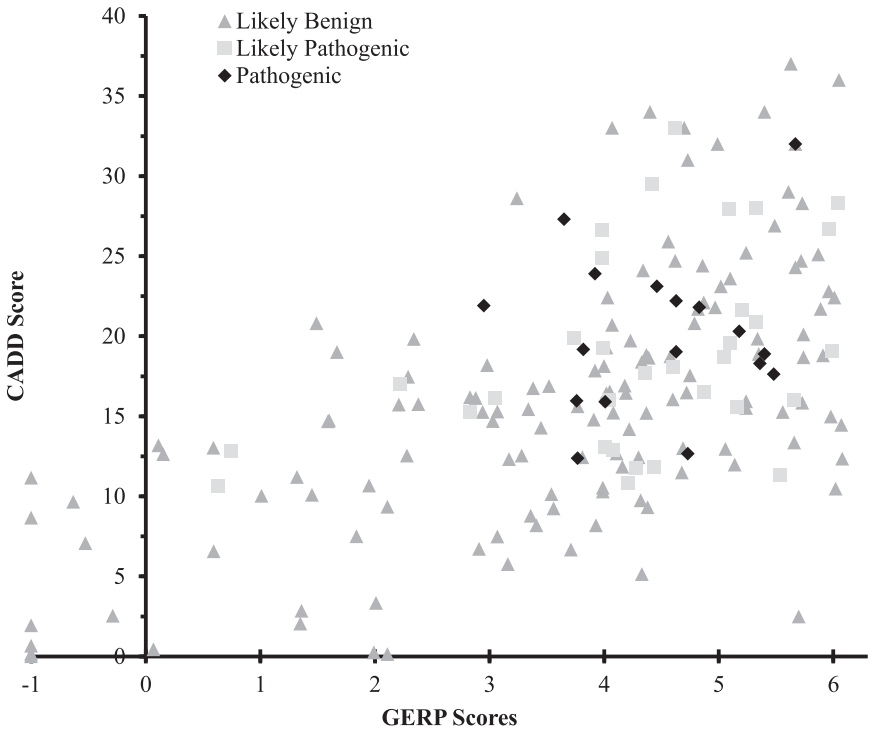

Figure 2. GERP versus CADD scores of pathogenic, likely pathogenic, and likely benign nondisruptive variants for dominant disorders. Likely benign variants with a GERP score of less than −1.0 are shown with their corresponding CADD scores along the −1 *x*-axis. Their true coordinates are (GERP, CADD): (−7.77, 0.15), (−7.34, 0.00), (−5.43, 1.93), (−4.01, 11.16), (−2.76, 8.66), (−2.25, 0.66).

2. Although we did not use in silico pathogenicity scores, we find that lower conservation scores may be of utility in categorizing variant pathogenicity. In these data, variants classified as pathogenic had GERP > 2.95 or CADD > 12.37, but high GERP and CADD scores were seen across all variant classification categories. Using a GERP < 2 as a criterion to lower the pathogenicity assignment for nondisruptive variants by one level would result in two of 32 likely pathogenic variants being reclassified as variants of uncertain significance, and 84 variants classified as uncertain significance would be reclassified as likely benign. Variants with higher in silico prediction scores would be more likely to be published and thus cited in HGMD; therefore, our analysis does not suggest that the high scores are unreliable, but that when taken in context of published literature, those with high scores may not be as helpful as low scores in identifying false positive reports of pathogenicity. In silico algorithms, particularly measures of evolutionary conservation, are considered in several other classification systems (Duzkale et al. 2013; Eggington et al. 2013; Thompson et al. 2014). However, the numerous tools can yield disparate results for any one variant.
3. Cosegregation of variant and disorder within families is useful for variant classification, but needs to be carefully defined. One reason for a discordant CSER laboratory classification was differing weighting of cosegregation data reported in the literature. To address this, the statistic for defining cosegregation in a pedigree should be established. The probability of variant sharing in the pedigree (e.g., one-half for an affected parent-child pair) is a simple number to compute and interpret. The odds ratio is simply 1 divided by that probability (2 for an affected parent-child pair), and the LOD score is the log base 10 of the odds ratio (0.3 for that pair). All of these numbers can be computed across multiple families with equal ease and, of these, the probability stated as a fraction is most intuitive. We found that computation of probability can vary if reviewers do not consider lack of the variant of interest in unaffected individuals in their cosegregation evidence and whether adjustments are made for incomplete or age-dependent penetrance. There is some disagreement regarding the level of cosegregation that should be considered evidence for pathogenicity. In this context, it is important to acknowledge that we are not mapping genes with a small prior probability of being at one specific site on the genome, for which a LOD score of 3 or 3.3 would be a usual criterion. Instead, we know the location of our genes of interest, though there may be dozens of genes of interest for a cardiomyopathy and only one for some disorders. For this reason a lower threshold is likely warranted. The Next Medicine RORC somewhat arbitrarily selected a probability of 1/16, due to its proximity to a *P*-value of 0.05. Others have suggested lower (Thompson et al. 2014) or higher thresholds (Duzkale et al. 2013; Eggington et al. 2013). Whatever threshold can be agreed on, we support the need to have a second line of evidence that supports pathogenicity in addition to cosegregation in a single family. This is because it is possible, though generally unlikely in the era of full coding sequencing, to have the correct gene segregating with disease but to have missed the true pathogenic variant and instead identified a benign variant in *cis*. Therefore, in addition to quantifying segregation, additional variant-specific evidence is needed.
4. Co-occurrence of variant with disorder is a well-accepted criterion that can be identified by careful case-control studies for more common variants. However, this evidence can be misapplied by comparing data from two studies, which should be done with great caution. Additionally, variants common enough to have been tested in a case-control study are often not highly penetrant. For very rare variants, statistically significant case-control comparisons will be unavailable. We looked for three or more affected unrelated individuals with a variant as a major component of our evidence criteria, in agreement with the criteria proposed for classifying incidental findings in 61 genes associated with cardiomyopathy and arrhythmia (Ng et al. 2013). However, we recognize that others could suggest higher or lower thresholds, depending on the desired positive predictive value and sensitivity.
5. Novel or very rare truncation variants expected to lead to nonsense-mediated decay and loss of canonical splice sites, when haploinsufficiency is known to cause the associated disorder, can be considered to be highly predictive of pathogenicity. This prediction can be confounded by a number of issues. For example, the truncation variants at the 3′ end of the gene may not be subject to nonsense-medicated decay, and thus may not cause the same phenotype as haploinsufficiency. Therefore, we excluded such variants. However, the exact location no longer susceptible to nonsense-mediated decay is likely to be gene specific and will depend on which exons are transcribed. As noted elsewhere, noncanonical splice site variants may require mRNA testing to determine if missplicing occurs and at what rate (Eggington et al. 2013).
6. A de novo mutation in an individual with a de novo disorder is evidence of pathogenicity as acknowledged by others (Duzkale et al. 2013). However, these data are rare in the literature and it is important to prove parentage when concluding that any variant is de novo (Biesecker 2012).

Other considerations have been proposed. We did not consider functional data, such as in vitro assays. We do recognize that some evidence can be very predictive. It is critical to use evidence from functional assays that are highly correlated with the associated disorder (MacArthur et al. 2014) and sufficiently validated with known variants. These assays are not widely available and we believed that their inclusion in our criteria would lead to poor and inconsistent classifications. The development and cataloging of reliable assays should be a high priority for our field. Myriad Genetics, Inc. considers "history weighting" data (Eggington et al. 2013). This considers that patients with true mutations should have the disorder in their families, even in the absence of cosegregation data. Similarly, Myriad Genetics and others (Duzkale et al. 2013) consider that, for a dominant disorder, when a variant is seen in *trans* with a known pathogenic variant in an affected person, that is evidence against pathogenicity. Finally, expert databases are considered by some. We did accept Myriad *BRCA1* and *BRCA2* variant classifications because they have a large amount of data that is not public. However, when possible, evaluation of the primary data supporting the variant classification is optimal.

An important limitation of our study is our inability to assess if the participants were ascertained based on phenotypes that enriched for any of the pathogenic or likely pathogenic variants identified. This might lead to an overestimate of the frequency of such IFs. Of most concern, several cohorts were enriched for lipid disorders, vascular disease, or chronic obstructive lung disease. This could have led to enrichment in *LDLR* and *SERPINA1* pathogenic variants. However, we do not see a marked excess in pathogenic or likely pathogenic variants in these genes, considering that the population frequencies of these disorders in participants of European ancestry are 1/500 for familial hypercholesterolemia and

between 1/500 and 1/3500 for alpha-1-antitrypsin deficiency (Rader et al. 2003; Kircher et al. 2014). Similarly, the number of variants classified as pathogenic or likely pathogenic for disorders expected to be at usual population frequencies were in the expected range, supporting the classifications for variants in genes such as *BRCA1*or *BRCA2* and those for Lynch syndrome (Janavicius 2010; Hampel and de la Chapelle 2011), expected to be found in 1/350 to 1/1000 people.

A second limitation is that some pathogenic variants may have been missed due to incomplete exome coverage or our inability to consider indels. However, as seen in Supplemental Table 5, only six genes associated with dominant disorders and two genes associated with recessive disorders had less than eightfold coverage of 90% of the coding regions. Indels and copy number variants (CNVs) may be missed in our analyses due to limitations in calling these types of variants by the exome sequencing shorter read length (50 base pairs) technology used when the ESP data was generated. However, indels and CNVs are not known to comprise a large portion of the known pathogenic variants for most disorder-gene pairs considered.

A third limitation is that this estimate of the frequency of IFs expected to be returned from exome sequencing results may not be generalizable to other ethnic groups or to children. We only considered gene-disease pairs in which the disorder could remain undiagnosed in adulthood. The addition of genes associated with disorders that would manifest before adulthood might result in more returned results.

A fourth limitation is the use of HGMD to identify potential pathogenic variants for review. It is possible that a small number of known pathogenic or likely pathogenic variants exist in the EVS that have not been published and thus would not be contained in the HGMD database. However, in the absence of a HGMD entry, it is unlikely enough data would be available for other than a VUS classification. The review of all expected disruptive variants also decreased this likelihood.

With regard to returning these results to the ESP participants, the primary authors do not have access to these cohorts. However, each cohort can address return separately. A substantial proportion of the sample contributing to the ESP included participants from one of six NHLBI cohorts in the HeartGO Consortium, including the Multi-Ethnic Study of Atherosclerosis (MESA), Framingham Heart Study, and Jackson Heart Study. Many participants in these cohorts provided consent to be recontacted for return of actionable genetic research results. Findings from the current study will inform future plans for return of results to consenting research participants.

In summary, we find that ~2.0% of adults of European ancestry and 1.1% of adults of African ancestry can be expected to have actionable highly penetrant pathogenic or likely pathogenic SNVs identified by exome sequencing at this time. These estimates are reduced to 1.6% and 1.0% for pathogenic or likely pathogenic variants in genes for which the ACMG recommends review and return of IFs to adults. Individuals of Ashkenazi ancestry are expected to have a higher rate of pathogenic variants due to founder mutations alone (Hartge et al. 1999). In addition, reviewers are likely to be inconsistent in their categorizations and biased toward more pathogenic categories. This suggests the need for simple, consistent criteria for classifying variant pathogenicity and improved variant-specific databases and knowledge bases. Finally, current literature identifies fewer pathogenic variants in those of African ancestry, likely due to the underrepresentation of these individuals in clinical and research studies.

Methods

Gene list development

The list of 112 actionable genes paired with diseases was agreed upon unanimously by the University of Washington National Human Genome Research Institute (NHGRI)-funded CSER "NEXT Medicine study" RORC; this committee, its membership, and process are detailed elsewhere (Dorschner et al. 2013). "Actionable" genes in adults were defined as having deleterious variant(s) whose penetrance would result in specific, defined medical recommendation(s) that are supported by evidence, the implementation of which would be expected to avoid significant morbidity and mortality. The benefit of intervention must be sufficient to counter any anxieties raised by the identification of an unexpected predisposition to a disorder. The University of Washington NEXT Medicine study is developing an actionable variant database for an adult population, and the EVS has ESP cohorts that were largely adults at the time of recruitment, and thus, may exclude subjects with pediatric disorders. For these reasons, only gene-disorder pairs that might remain undiagnosed in adulthood were included. The list of genes determined to date to have actionable variants has been previously published (Berg et al. 2013; Dorschner et al. 2013) and is continually updated as new and putative gene-disease associations are reviewed. Since the publication of Dorschner et al. (2013), three genes have been added to the list (*MAX*, *TGFB2*, and *TMEM127*) and five genes have been removed: (*GPD1L, HCN4, KCNE3*, *SCN1B*, and *SCN3B*). These five genes were removed based on RORC consensus that the evidence to support the gene-disease association did not reach the threshold for inclusion. The list of actionable genes is likely to grow as evidence for novel genes accumulates; however, it is likely that further genes will be rarer and rarer causes of disease and therefore a source of IFs. The full list of gene-disease pairs along with the percentage of each gene's coding region covered by the ESP sequencing technology is in Supplemental Table 5.

Criteria for classification of variants

Given that we are addressing potential IFs, our criteria for the classification of highly penetrant pathogenic variants (Table 1A,B) were stringent. Each variant from HGMD was classified as "pathogenic," "likely pathogenic," "variant of uncertain significance" (VUS), or "likely benign." Additionally, we defined "disruptive" expected pathogenic variants as truncating and missplice-causing variants not identified by HGMD as disease causing. We did not assign variants to the "benign" category, as all variants selected for review were either listed as disease-causing variants in HGMD or were disruptive SNVs (predicted to cause a premature termination or missplice). Finally, we accepted Myriad *BRCA1* and *BRCA2* variant classifications that were known to us because their classifications use data that are not available.

Multiple sources of data were evaluated to classify the pathogenicity of each variant. Ancestry-specific allele frequencies from the EVS were used to exclude variants that were too common to be highly penetrant pathogenic variants for the relevant disorder, based on the prevalence of the disorder. The references cited by HGMD Professional 2013.3 (Stenson et al. 2009), PubMed, and Google were evaluated. Additional supporting references for each variant were searched for in other databases, including the Leiden Open Variant Databases (LOVD), ClinVar, and InSiGHT, and these references were also reviewed. Variants in some of the genes of interest were also associated with disorders that were not considered highly actionable (e.g., *RYR1* may be associated with neuromuscular disease as well as the target phenotype malignant

hyperthermia), or their association with disease was not established to our required evidence level. Only variants putatively producing the phenotype of interest were considered pathogenic.

Participants and variant selection

The NHLBI ESP has 6503 participants whose variants are summarized on the EVS. We had previously evaluated variants in 1000 participants (500 European ancestry and 500 African ancestry) and now have evaluated variants in the remaining 5503 participants. These variant annotations were derived from accessing the ESP database on November 7, 2013, using the EVS version v.0.0.22. We pooled these data to improve ancestry-specific estimates. ESP participants are from 18 cohorts with heart, lung, and blood phenotypes. Further details regarding these phenotypes are available on the ESP website (http://evs.gs.washington.edu/EVS/). The sequence data from this study have been submitted to the NCBI database of Genotypes and Phenotypes (dbGaP; http://www.ncbi.nlm.nih.gov/dbgap) under accession numbers phs000254.v2.p1, phs000279.v2.p1, phs000281.v5.p3, phs000290.v1.p1, phs000291.v2.p1, phs000296.v3.p2, phs000327.v1.p1, phs000334.v1.p1, phs000347.v1.p1, phs000362.v1.p1, phs000398.v2.p1, phs000399.v1.p2, phs000400.v3.p1, phs000401.v7.p9, phs000402.v2.p1, phs000403.v3.p3, phs000422.v1.p1, phs000518.v1.p1, phs000546.v1.p1, phs000556.v1.p1, phs000581.v1.p1, phs000582.v1.p1, phs000587.v1.p1, and phs000632.v1.p1.

Ancestry was inferred from analysis of principal components (Patterson et al. 2006; Price et al. 2006). It has been previously reported that ~3.2% of the entire cohort of 6503 participants have Ashkenazi ancestry (Dorschner et al. 2013). All of these 6503 participants' exome variants were reviewed for the 112 genes paired with phenotypes of interest (so that the initial 1000 participants were investigated for variants in the three new genes) for any SNV listed as disease causing in HGMD and any disruptive expected pathogenic variants. Indels were not included due to difficulty of accurately calling these with the shorter read length used in generating the ESP sequence data. Variants with a MAF greater than 0.005 in genes associated with autosomal dominant disorders were not evaluated as they were too common to be considered a highly penetrant pathogenic variant for a dominant disorder given the frequencies of the disorders under consideration. This is the same allele frequency used as a threshold by the International Society for Gastroenterology and Hereditary Tumors (InSiGHT) (Thompson et al. 2014). We did not eliminate the possibility of low penetrance pathogenic variants with MAF > 0.005. A single reviewer classified 20 variants with MAF < 0.005, but with 10 or more occurrences in EVS (MAF ~0.0008). Variants for disorders inherited in an autosomal recessive pattern were reviewed regardless of MAF, but only when a single participant had two potentially pathogenic variants, each annotated in HGMD or considered disruptive. We assumed that the recessive variants were carried on separate alleles, in *trans*, as this is more likely than the variants being in *cis*. Carrier status was not assessed in this study.

Expert variant review of EVS variants

Each of 52 expert reviewers considered a subset of all potential pathogenic variants. All reviewers were geneticists or reviewed with a geneticist partner: 48 were clinical geneticists, genetic counselors, or molecular geneticists, and the remainder had significant relevant genomic expertise. Each reviewer was provided an Excel spreadsheet with detailed information on the 10–15 variants assigned to them for classification and links to publications cited in HGMD. They were each asked to determine whether the allele frequency was less than a disease-specific maximum frequency (DAF) and to review the primary literature and databases to document these data and to determine if the evidence met the pathogenicity criteria (Table 1A,B). Reviewers were instructed to calculate the maximum allowable allele frequencies for each disorder under a conservative model, which included the assumption that the given disorder was wholly due to that variant considering the mode of inheritance of the disorder. When disorder frequencies were unknown, reviewers were asked to conservatively overestimate. Reviewers were provided with total minor allele frequency and ancestry-specific allele frequencies from EVS and from the 1000 Genomes Project data (Brownstein et al. 2014) for each variant. Reviewers were instructed to ignore the first occurrence of the variant when considering the EVS derived MAF, because all SNVs were ascertained from the EVS, biasing the MAF upward. We did not have EVS identifiers or phenotypes, so that genotype–phenotype correlation was not possible. Reviewers were trained on all aspects of review by an in-person conference or a videoconference, and a YouTube training video was available for reference at all times (https://www.youtube.com/watch?v=fa01IZzNc20). The training video file can also be accessed in the Supplemental Material. A genetic counselor was available at all times to answer questions, and articles that reviewers could not locate were provided to them.

Each reviewer filled out a spreadsheet that summarized the findings relevant to the pathogenicity categorization; these data were reviewed by a genetic counselor to ensure that the classification matched the evidence summarized by the reviewer. Reviewers recorded the time in minutes it took to review each variant and were asked to nominate very difficult-to-categorize variants for committee review.

Classification of non-HGMD disruptive variants

Any variants in the first 90% of the predicted amino acid sequence that were not listed in HGMD as disease-causing variants but may introduce a premature termination codon directly or as a result of ±1,2 splice site variant were also evaluated. We did not include variants at the 3′ end of the gene that met the "position-of-an-exon-exon-junction" rule of being <50 nucleotides from the final exon–exon junction to be expected to escape nonsense-mediated decay of the mRNA (Maquat 2004; Conti and Izaurralde 2005) and result in functional protein products, albeit sometimes pathogenic products (Isidor et al. 2011). We reviewed whether truncating variants were reported to cause the disorder of interest. Literature, ClinVar, and other relevant databases were reviewed to search for prior reports of these variants. In rare cases, available expert knowledge of the pathogenic variant spectrum for certain genes and disorders was also taken into account.

Classification quality control

For quality control, initially 25% (156/615) of the variants were also examined for pathogenicity by a second reviewer, blinded to the first review. Discordant classifications were reanalyzed by an experienced third reviewer. Discordance between reviewers was evaluated in a number of ways, including comparison of reviewers who had participated in the prior analysis (Dorschner et al. 2013) and those who had not. Several reviewers made systematic errors, such as including EVS participants with unknown phenotype as affected with the disorder; such variants were reclassified by a second, experienced reviewer. Additionally, to minimize erroneous classifications, all variants that were initially classified as pathogenic or likely pathogenic were evaluated by a second, experienced reviewer.

Comparison with other variant classification systems

All variant classifications from the initial 1000 ESP participants and from the remaining 5503 ESP participants with a MAF <0.0008 (n = 595) were compared to classifications by the Partners LMM (http://personalizedmedicine.partners.org/Laboratory-For-Molecular-Medicine/Default.aspx) and collected through the SCRP (http://sharingclinicalreports.org/). In addition, six variants were randomly selected within groups of varying pathogenicity assignments and were classified blindly by five research and clinical laboratories within the CSER consortium (http://www.genome.gov/27546194) according to their routine laboratory procedures.

Evaluation of pathogenicity measures

We evaluated two measures of predicted pathogenicity, GERP mammalian conservation scores and the CADD summary score of conservation, substitution, and regulation (Cooper et al. 2005; Davydov et al. 2010; Kircher et al. 2014), to determine if these scores were correlated with pathogenicity classification. Scores for nondisruptive variants in genes associated with dominant and recessive disorders were compared across pathogenicity assignments. These measures were not used as part of our classification criteria, which allowed us to assess their utility in predicting classification.

Statistical analyses

One-sided binomial tests were used to evaluate whether variants seen only once, versus those seen more than once, were seen in excess among 64 pathogenic or likely pathogenic variants in genes associated with dominant disorders. Disruptive variants not listed as disease causing in HGMD were excluded from this exercise. The null hypothesis that the MAF of each variant was independent of pathogenic, likely pathogenic, and variant of uncertain significance (VUS) classifications was tested using an analysis of variance (ANOVA). We excluded the likely benign class from this test, as many variants were placed in this class due to their high MAF. This test considered only variants in genes associated with dominantly inherited disorders, given that variants in genes associated with disorders with a recessive inheritance pattern would have different MAF ranges. Further, this test considered the higher ancestry-specific MAF for each variant because a higher MAF in either population would be considered evidence of a benign variant.

Data access

All 626 variant annotations have been submitted to the NCBI ClinVar (http://www.ncbi.nlm.nih.gov/clinvar) under submitter name CSER_CC_NCGL.

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Actionable exomic incidental findings in 6503 participants: challenges of variant classification

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ORIGINAL RESEARCH

Design and Evaluation of a Decision Aid for Inviting Parents to Participate in a Fragile X Newborn Screening Pilot Study

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Abstract The major objectives of this project were to develop and evaluate a brochure to help parents make an informed decision about participation in a fragile X newborn screening study. We used an iterative development process that drew on principles of Informed Decision Making (IDM), stakeholder input, design expertise, and expert evaluation. A simulation study with 118 women examined response to the brochure. An independent review rated the brochure high on informational content, guidance, and values. Mothers took an average of 6.5 min to read it and scored an average of 91.1 % correct on a knowledge test. Most women rated the brochure as high quality and trustworthy. When asked to make a hypothetical decision about study participation, 61.9 % would agree to screening. Structural equation modeling showed that agreement to screening and decisional confidence were associated with perceived quality and trust in the brochure. Minority and white mothers did not differ in perceptions of quality or trust. We demonstrate the application of IDM in developing a study brochure. The brochure was highly rated by experts and consumers, met high standards for IDM, and achieved stated goals in a simulation study. The IDM provides a model for consent in research disclosing complicated genetic information of uncertain value.

Keywords Newborn screening · Informed decision making · Decision aids

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Introduction

Newborn screening (NBS) historically has been a mandatory public health program, on the assumption that some health conditions are so serious and require such urgent treatment as to warrant screening without consent (Grosse et al. 2006). The U.S. Department of Health and Human Services Secretary's Advisory Committee on Heritable Disorders in Newborns and Children currently recommends that states screen all newborns for 31 core conditions, such as phenylketonuria or galactosemia, and report out 26 "secondary conditions" that are also detected when screening for the core conditions. A current list of recommended conditions may be obtained at www.hrsa.gov/advisorycommittees/mchbadvisory/heritabledisorders. But many parents are not aware of or do not remember

receiving information about NBS (Davis et al. 2006; DeLuca et al. 2011; Hasegawa et al. 2011), often because information is provided in the hospital shortly before or after birth. Parents prefer to receive this information prenatally (Detmar et al. 2007), as recommended by professional associations (Faulkner et al. 2006; AAP American Academy of Pediatrics (AAP) Newborn Screening Taskforce 2000). However, in practice this rarely happens (Kim et al. 2003), especially for low-income mothers (Tluczek et al. 2009), or the information is included with other prenatal materials and is not noticed (Kemper et al. 2005). Brochures often have suboptimal readability and clarity, either about NBS (Arnold et al. 2006) or associated practices such as blood spot retention (Haga 2010). Parents need more complete, timely, and noticeable information (Cunningham-Burley 2006; Mann et al. 2006).

Experimental screening for conditions under consideration for NBS is more complicated. Although ethics guidelines argue that parental consent is necessary for pediatric research (Diekema 2006), informed consent is difficult and expensive to obtain in large pilot studies (Feuchtbaum et al. 2007). Some authors suggest that under certain circumstances (high potential benefit, minimal risk, impracticability) waiving consent may be appropriate (Tarini et al. 2008). However, this decision is likely to be nuanced and contingent on many factors, including whether the study is state authorized or the value of information disclosed.

Some studies, such as our fragile X pilot NBS study, clearly must be performed under a consent protocol. Fragile X syndrome (FXS) is the most common inherited form of intellectual disability, caused by a CGG repeat expansion within the *FMR1* gene at Xq27.3. Individuals with 200 or more CGG repeats are considered to have FXS, in which a protein necessary for normal brain development is reduced or absent. Individuals with 55–199 repeats are considered "fragile X(FX) carriers," meaning that they have a gene expansion that increases their risk of having a child with FXS. In the absence of population screening, FXS typically is not diagnosed until 36 months or later (Bailey et al. 2009). Parents are frustrated by diagnostic delays, 25 % to 30 % have a second affected child before the first is diagnosed, and despite lack of medical treatment, identified children would be eligible to participate in early intervention programs (Bailey et al. 2005). Parents of affected children strongly support NBS (Bailey et al. 2012), but lack of data on early phenotype, the fact that a DNA-based screening test detects carriers, and absence of a proven FX-specific treatment mean that the evidence base is insufficient for FXS to meet current criteria for population screening (Calonge et al. 2010).

To provide a stronger evidence base, we are conducting a pilot study, designed as the social science equivalent of a Phase I clinical trial. In this case, the "treatment" is the information families receive from screening. We are interested in whether and why parents agree to have their child screened and the "safety" of screening, as evidenced by any "adverse events" (e.g., postpartum depression, anxiety, disrupted parent-child relationships) (Bailey et al. 2008). Families are offered FXS NBS using a test that also detects carriers (Tassone et al. 2008). We recently reported a consent rate of 67.5 % for mothers and 63 % for couples (Skinner et al. 2011).

Given the genetic complexities of the *FMR1* gene (X-linked inheritance pattern with potential implications for many family members, triplet repeat expansion with "anticipation," adult-onset conditions), we needed written materials to communicate this information. At the onset of the study, we created a pink and blue trifold brochure (no photographs) providing brief answers to 13 questions: (1) what is FXS? (2) how do children get FXS? (3) what is NBS? (4) what is the FX NBS study? (5) how is the study different from state NBS? (6) how will my baby be screened? (7) what will the study do? (8) why should I consider participating? (9) what risks are involved? (10) will I be contacted after the research screening is done? (11) will some babies need more testing? (12) what happens if my baby needs more testing? and (13) what else should I know about being in this study?

However, following an initial period of study implementation, four factors led us to decide to revise the original brochure. First, less than half of the parents reported reading it. Second, because carriers are more common than affected children, we wanted to emphasize the likelihood of carrier identification. Third, African American families were less likely than other families to agree to screening (Skinner et al. 2011), and we wanted to ensure that parents knew that FX affects all ethnicities. Finally, exposure to literature on Informed Decision-Making (IDM) led us to question whether parents were making truly informed decisions. IDM in health care is generally defined as the process by which patients are supported and involved in decisions about treatments or tests, weighing various considerations, examining values and preferences, and making a decision in partnership with a health professional (Briss et al. 2004; Charles et al. 1999; Mullen et al. 2006). IDM is especially important in situations where there is no right or wrong decision, because insufficient evidence exists to advise one option over another, or because the options all have risks and benefits that an individual must consider in order to be comfortable with the final decision (Elwyn et al. 2010). Often, printed materials or "decision aids" are developed to support IDM, using words, pictures, and figures to convey information, suggest reasons to consider or reject a course of action, and emphasize making a choice consistent with individual values and preferences (Bekker et al. 2003).

Decision aids work by providing information directly relevant to decision making and placing decisions in the context of personal values (Mullen et al. 2006). A systematic review of decision aids for prostate cancer screening (Volk et al. 2007) found that they generally result in improved knowledge and greater decisional confidence. A Cochrane review of 55 randomized clinical trials (O'Connor et al. 2009) concluded that decision aids increase knowledge and reduce decisional conflict.

The literature on IDM primarily focuses on helping patients make decisions about medical tests or treatments. With a few exceptions (e.g., Sorenson et al. 2004), less attention has been given to using decision aids to help individuals decide whether or not to participate in a research study. Nonetheless, the assumptions underlying IDM, namely that people ought to be supported in making health care decisions in a way that is consistent with their values and preferences, are directly applicable to decisions about study participation. Drawing on the IDM literature, we designed a new brochure to move beyond meeting Institutional Review Board requirements for informed consent, to meeting well-accepted standards for informed decision making. We wanted printed information that would: (1) be more visually appealing, hopefully increasing the chances that parents would read it; (2) clearly convey the likelihood of carrier identification; (3) use pictures to show that FX affects all races/ethnicities; and (4) support informed decision making. As the first step in a two-stage evaluation process, this article describes the process by which the brochure was developed; summarizes findings from an independent evaluation relative to decision aid standards; and reports the results of a simulation study with pregnant women or recent mothers. A subsequent paper will examine the effect of the brochure when implemented in a hospital recruitment environment. We conclude with a discussion of the growing need for informational aids to help parents understand complicated genetic information well enough to make an informed choice about participation in genetic research.

Materials and Methods

Brochure Design

Brochure development was guided by four IDM principles. It needed to (1) promote understanding of the study, risks, and uncertainties; (2) foster consideration of preferences; (3) support participation in decision making at a level that is desirable and personally comfortable; and (4) lead to a decision consistent with personal values (Mullen et al. 2006). A draft was developed by the first author and colleagues in health communications, then shared with the FX research team, including a certified genetic counselor, a medical geneticist, an anthropologist, an attorney, an early childhood special educator, and an experienced bilingual (Spanish) research assistant. Multiple drafts were exchanged between the research team and the brochure development team. A pilot study was conducted with six pregnant women, and their suggestions were incorporated.

The text underwent several editorial reviews. We followed the tools/tips from PlainLanguage.gov, including the *Document Checklist for Plain Language* (www.plainlanguage.gov/howto/quickreference/checklist.cfm), for content and layout (e.g., useful headings, organized to serve readers' needs, active voice). Photographs, white space, and other design elements were used to enhance clarity and appeal. The SMOG readability formula (McLaughlin 1969) indicated that the brochure is written at a 9th grade level (+/- 1.5 grades), primarily due to 3-syllable words such as "family," "carrier," "development," and "genetics." Because these words were essential to understanding the study, by retaining them we were unable to further reduce reading grade level.

This iterative process resulted in a full-color, 8-page brochure with numerous photographs depicting infants and parents of multiple ethnicities. The first two pages differentiate fragile X syndrome from fragile X carrier, describe the incidence rate of each, and makes the point that carriers are much more likely to be identified than affected children. The brochure states that although there is no cure for FXS, children can receive help from early intervention programs and doctors can treat some symptoms. Following a description of what will happen in the study, two pages are devoted to "things to consider when making your decision." One page lists reasons to participate and another lists reasons not to participate. Also included are two quotes from parents who had decided to participate (e.g., "I'm the type of person who just wants to know") and two from parents who had declined (e.g., "I don't want to know if my child is a carrier; I think I would worry unnecessarily"). The final page contains five "questions to help you decide": (a) would you want to know if your infant has FXS? (b) would you want to know if your newborn is a FX carrier? (c) are you OK knowing that right now there is no cure for FXS? (d) do you have the information you need to make a decision? and (e) do you feel prepared to learn the answer of the screening test? The brochure concludes: "If you answered 'yes' to most of these questions, maybe you are ready to have your newborn screened. If you answered 'no' to most, maybe this is not the right decision for you."

IPDAS Review

A near-final version of the brochure was submitted to an independent review group (the Cardiff University Decision Laboratory) to assess adherence to standards established by the International Patient Decision Aid Standards Collaboration

(IPDAS) (http://www.ipdas.ohri.ca/IPDAS_checklist.pdf) (Elwyn et al. 2006). The Decision Laboratory provided a detailed formative and summative assessment, including recommendations for improvement. The result was near-perfect scores on *informational content* (93 %—the brochure describes the problem, the decision to be made, and the options available. Positive and negative features are presented using equal detail in a format that allows fair comparison); *guidance* (100 %—the brochure provides structured guidance toward making a decision); and *values* (95 %—the brochure facilitates the expression and clarification of user values and attitudes). The brochure received low ratings on other items because it (1) did not include data on chances of false positive or false negative results and did not present probabilities in multiple ways; (2) did not provide details about the development process; (3) did not provide evidence supporting brochure content; and (4) lacked evidence for efficacy. These lower ratings were expected, given that the brochure was still in development; the primary purpose of the review was to determine whether the content of the brochure was consistent with recommended practices for developing patient decision aid.

Participants

The study was reviewed and approved by the Institutional Review Board at RTI International. A local firm recruited 118 pregnant women (59 %) or recent (within the past 6 months) mothers (41 %) for a simulation study. The women had a mean age of 30.4 years, ranging from 18–43. The group was relatively well educated: only 13 % had a high school degree or less, 24 % had some college or technical school, and the remainder had at least a college degree. Nine (7.6 %) were Hispanic/Latino (one also self-identified as African American), 47 (39.8 %) African American, and 62 (52.5 %) white. Most (74 %) were married and 58 % were employed. Their median household income was approximately $50,000; 11 % had a household income of less than $20,000 and 16 % over 100,000. Twenty (17 %) had heard of FXS but only 3 (2.5 %) knew someone with FXS. Participants received $50 upon activity completion.

Procedures and Instrumentation

Each woman participated in one of nine 1-hour group sessions facilitated by a member of the research team. They were told that the goal was to understand their opinions and reactions to a brochure about a research study. They were not given any other information about FXS, the study, or NBS. The women needed 2–26 min to read the brochure, an average of 6.5 min. Each then responded to the following statement: "Based on the information I read in the brochure about the FX NBS study, if I was approached by someone to participate in this study, I would agree/not agree to have my baby screened" and wrote reasons for their decision. Women then took a knowledge test containing 12 true-false statements designed by the authors to assess factual recall. They completed a survey containing 31 statements rated on a 5-point scale from Strongly Agree to Strongly Disagree. The statements addressed reactions to the brochure (e.g., I like the way this brochure looks; I trust the information) and included selected items adapted from the Decisional Conflict Scale (DCS) (O'Connor 1995).

Results

Descriptive statistics and summary scores were used to characterize performance on the knowledge test, reactions to the brochure, and hypothetical screening decisions. We used structural equation modeling to examine whether selected demographic variables were associated with test performance, perceptions of the brochure, decisional uncertainty, or screening decisions.

Knowledge

Mean percentage correct on the knowledge test was 91.1, ranging from 50–100 % (Table 1). All but three items were answered correctly by >91 % of the participants. The terms "small gene change" and "large gene change" used to differentiate carriers from affected children resulted in some confusion. About 20 % of the women incorrectly thought that an extra prick of the baby's heel was needed for the study.

Perceptions of the Brochure and Decisional Support

Combining ratings of agree and strongly agree, most women reported that the brochure was easy to read (95.8 %) and understand (89.9 %); they liked the way it looked (91.6 %); and it provided helpful information (90.8 %). The majority agreed or strongly agreed that it would help them make an informed decision about participating in the study (78.2 %) and they trusted the information (69.8 %). Some (26.9 %) said that the brochure left them with many unanswered questions about FX and 21 % reported that they were still unsure about study participation. The most common suggestions for improvement were more information about FXS and the study itself.

Decision to Participate

When asked to make a hypothetical decision, 61.9 % indicated that they would agree to have their child screened. Some non-significant variation was seen across ethnic

Table 1 Percent correct on knowledge test items for participants in the simulation study (N=118)

Item	Answer	% Correct
1. Having fragile X syndrome and being a fragile X carrier are the same thing.	False	97.5
2. Children with fragile X syndrome can have delays in development, learning problems, signs of autism or anxiety.	True	98.3
3. There is a cure for fragile X syndrome.	False	98.3
4. Fragile X is only found in certain ethnic or racial groups.	False	91.5
5. Being a fragile X carrier means there is *small* change in the fragile X gene.	True	89.9
6. During the Fragile X Newborn Screening Study an extra prick of the baby's heel is needed so that a blood spot can be taken for the study.	False	79.8
7. Children who have fragile X syndrome cannot receive help from early intervention programs.	False	97.5
8. A newborn that tests positive as a fragile X carrier has a parent who also is a fragile X carrier.	True	94.2
9. Fragile X syndrome is caused by a *large* gene change.	True	63.6
10. The Fragile X Newborn Screening Study hopes to learn about the early development of children with fragile X syndrome and children who are fragile X carriers.	True	99.2
11. Most people who are carriers of fragile X already know it.	False	100
12. There are many more people who have fragile X syndrome than people who are fragile X carriers.	False	95.8

groups, with 63.9 % of non-Hispanic whites and 56.8 % of African Americans agreeing. Six (75 %) of the eight Hispanic (non-African American) women would agree to have their child screened.

An open-ended question asked women to explain their choice. Their reasons are summarized in Tables 2 and 3 and compared with the reasons reported in our larger hospital study using the original brochure (Skinner et al. 2011). Most women (91.7 %) who would agree to participate reported benefit to knowing earlier: "I would want to know if my baby had FX or was a carrier so that I could prepare for any challenges down the road"; "I would have the available resources that are out there to help my baby as well as our family to cope with this genetic disease." The next most common reasons (25 %) reflected a belief that participation posed minimal risk: "As long as the child is not undergoing any additional unnecessary pain, I only see good in testing, whether it is curable or not"; "it's a non-invasive test that can give an enormous amount of information." These two reasons were also commonly mentioned with the original brochure (Skinner et al. 2011); however, parents responding to the new brochure were less likely to mention "contribute to research" as a reason to participate.

The reasons for not participating were more diverse and exemplified a different pattern than seen with the old brochure. The most common was lack of a cure or treatment, mentioned by 51 % of the women in this study but only 5 % in the hospital study. These women made comments such as "since there is not a cure at this moment, I would prefer not to test my child"; "if there is no cure, it's just knowledge without purpose." A substantial portion (44.4 %) also indicated that they did not want to worry, compared with 21.4 % with the original brochure. These women made comments such as "I am one of those people that would worry myself about it"; "being pregnant with my first there are a lot of things that I can worry about, most of them I must choose not to." Also, 28.9 % of women who read the new brochure (compared with only 9.4 % in the hospital) reported that they would rather wait for symptoms to appear: "I will continue to monitor my child to see if any developmental issues appear over time." Other responses (28.9 %) referenced issues regarding test accuracy: "the brochure mentions that the test results could be wrong"; "if there was a wrong diagnosis, that would upset me as well." Understandably, concerns about logistics (e.g., the context or timing is not good) were more common in the hospital group (21.4 %) compared with the simulation group (4.4 %).

Decisional Uncertainty

The three items in the Decisional Uncertainty subscale of the Decisional Conflict Scale (O'Connor 1995) were adapted for this study, displayed in Table 4. About 75 % of the mothers agreed or strongly agreed that the brochure made it easier to decide about study participation; 62.2 % disagreed or strongly disagreed that they were still uncertain about study participation, and 66.4 % agreed or strongly agreed that the brochure made it clear "what the best choice is for me."

Table 2 Reasons for accepting screening: percentages across studies and ethnic groups

Reason	Skinner et al. (2011) (*n*=1288)	Simulation Study Total (*n*=72)	African American (*n*=28)	White (*n*=38)	Hispanic (*n*=6)
Knowing is good; benefit to knowing; knowing earlier is better	71.6	91.7	92.9	89.5	100
To contribute to research	32.0	6.9	3.6	7.9	16.7
Test is minimal risk; non-invasive; just an additional test	27.5	25.0	21.4	23.7	50
Participating can't hurt; nothing to lose	8.4	1.4	–	2.6	–
Family has history of problems	5.9	2.8	3.6	2.6	–
Screening is free	4.7	9.7	3.6	15.8	–
Just curious	2.1	–	–	–	–
Spouse/partner convinced me	2.1	–	–	–	–
Because the screen was offered	1.4	–	–	–	–
To provide reproductive risk information	0.6	1.4	3.6	5.3	–

Percentages sum to greater than 100 % because participants reported more than one reason

Factors Associated with Outcomes

The path diagram in Fig. 1 outlines our hypothesized model of the decision-making process. In this model, demographics and having heard of FXS were predicted to influence perceptions of brochure quality; quality, in turn, was predicted to affect the screening decision and decisional confidence, both directly and indirectly through trust in the information. We hypothesized that non-white respondents would be less likely to trust the information, given prior research showing ethnic differences in trust in research more broadly and elevated concerns about research and the consequences of research findings for members of ethnic minority groups (Bussey-Jones et al. 2010; Nwulia et al. 2011). We also hypothesized that individuals who were somewhat familiar with FXS would be more likely to trust the information in the brochure and thus more likely to accept screening, since they would be more aware of the consequences of FXS for children and families. We conducted a path analysis to test the model using the Mplus software program for structural equation modeling (Muthén and Muthén 1998–2010). Various model fit indices were used to assess goodness of fit; values of 0.95 or higher for the comparative fit index (CFI) and Tucker-Lewis Index (TLI) and values of 0.06 or less for the root mean square error of approximation (RMSEA) indicate good fit (Bentler 1990; Browne and Cudeck 1990; Hu and Bentler 1999).

The path model fit very well (Fig. 1; CFI=0.98, TLI=0.96, and RMSEA=0.05). Women with a college education rated the quality of the brochure less positively than those with less education (coefficient=-0.25, $p<0.05$); and perceived quality of the brochure was not significantly related to age, ethnicity, or familiarity with FX. Ethnicity was not associated with trust in the information; however, those who

Table 3 Reasons for declining screening: percentages across studies and ethnic groups

Reason	Skinner et al. (2011) (*n*=565)	Simulation Study Total (*n*=45)	African American (*n*=21)	White (*n*=22)	Hispanic (*n*=2)
Logistics (the context, timing is not good)	21.4	4.4	0	9.1	0
Don't want to worry	21.4	44.4	52.4	36.4	50.0
Issues regarding testing or test accuracy	19.3	28.9	38.1	22.3	0
Don't want to know	17.7	20.0	19.1	22.8	0
Don't want to be in a study, not interested	14.9	2.2	0	4.6	0
It's not necessary	13.8	2.2	4.8	0	0
Little chance of having it; no family history	12.4	11.1	19.1	0	50
Spouse/partner declined or disagreed	11.5	NA	NA	NA	NA
My baby is fine/ healthy	11.0	NA	NA	NA	NA
Rather wait for symptoms to appear	9.4	28.9	19.1	59.1	0
No cure or treatment	5.3	51.1	42.9	59.1	50

Percentages sum to greater than 100 % because participants reported more than one reason

Table 4 Percentage of respondents endorsing different levels of agreement with decision uncertainty items (N=118)

Item	Strongly agree	Agree	Neither agree nor disagree	Disagree	Strongly disagree
The brochure made it easier to make a decision about participating in the fragile X newborn screening study	26.1[a]	48.74	16.8	7.6	0.8
After reading the brochure, I'm still unsure about participating in the fragile X newborn screening study	6.8	14.3	16.8	36.13	26.1
The brochure made it clear what the best choice is for me in terms of participating in the fragile X newborn screening study	20.2	46.2	18.5	12.6	2.5

[a] Percentage of respondents

had heard of FX were significantly less likely to trust the information (coefficient=-0.22, p<0.05). Greater perceived quality was associated with greater trust in the information (coefficient=0.62, p<0.001). Women who gave high quality ratings and those who trusted the information more were significantly more likely to agree to screening and reported greater decisional confidence.

Discussion and Conclusions

Discussion

Study recruitment materials typically are designed to meet Institutional Review Board (IRB) requirements for informed consent. When research involves complicated decisions with direct ramifications for study participants, as in the case of a study disclosing genetic information of uncertain value about newborns, researchers have an obligation to provide information that supports informed decisions. Newborn screening for FXS and the disclosure of infant carrier status clearly exemplify this obligation. No urgent medical treatment is currently available for FXS, and some parents may not want to know infant carrier status. In developing new written materials about the study, our goal was not to increase study participation rates, but to develop print materials that, if read, would maximize awareness of all facets of the study and enable parents to make an important decision in a relatively short period of time.

IDM provides a theoretical framework for fulfilling this obligation, because its underlying premise is to help people make decisions consistent with their values and preferences. IDM is well established in the design and evaluation of patient decision aids, but with few exceptions (Sorenson et al. 2004), relatively little attention has been given to its application in research recruitment. This article demonstrates that using IDM as a guiding framework can result in recruitment materials that are informative, balanced, and

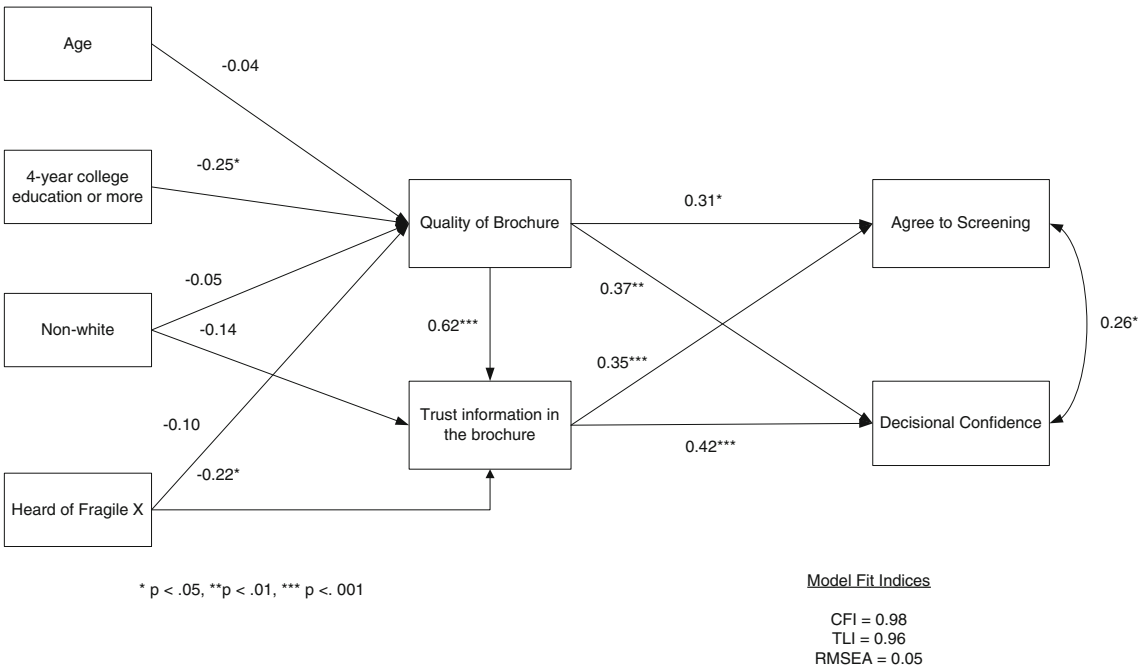

Fig. 1 Path analysis of agreement to newborn screening and decisional confidence

supportive of decision-making. Both IDM experts and women who could potentially be invited to participate in a hospital study rated the brochure high in quality and trustworthiness. The brochure required most women only about 6 min to read and resulted in high recall of facts. Perceptions of quality and trustworthiness were directly associated with the decision to participate and decisional confidence. African American and Latino mothers were no less likely to trust the brochure than white mothers and did not differ in terms of perceived quality of the brochure, suggesting that we were able to make some inroads into offsetting mistrust in research among minority families.

Interestingly, although the new brochure did not result in substantial difference in hypothetical rates of study participation, it did result in a shift in reasoning. For example, in this study few women reported "to contribute to research" as a reason for deciding to participate in the study. This difference may be due to the fact that the original report of consent rates was conducted in a teaching hospital where research is more common, or it may be due to the fact that the simulation study was focused on reactions to the brochure whereas in the hospital the focus was on making an actual decision to have your baby screened. Women who said they would decline after reading the new brochure were much more likely to mention lack of a cure and not wanting to worry as reasons for not participating in the study. The original brochure did not mention "no cure," and so it is not surprising that few parents mentioned it in our original study. The fact that more than 50 % of decliners in the simulation study mentioned lack of a cure and 44 % did not want to worry suggests that for these women, a clear treatment option is a salient factor in their decisions about whether they would want to know information about their child's fragile X status.

Study Limitations

The study has several limitations. It is possible that the study participants, by virtue of the fact that they agreed to be in the simulation study, were more inclined to be in and trust research, and thus their perspectives on research might be more favorable than the general population. We did not directly compare the old and new brochure nor did we do a knowledge pre-test, so we cannot say that the new brochure was better than the original. We were unable to reduce the reading level below 9th grade without eliminating essential 3-syllable words such as *family* or *carrier*. Most women performed well on the knowledge test, but we do not know the literacy threshold below which this brochure would not be effective, given that study participants were relatively well educated. Alternative strategies are clearly needed for low-literacy parents. The finding that mothers who had heard of FXS were less likely to trust the brochure is puzzling, and we have no data to suggest why that might be the case. The survey only asked mothers if they had heard of FXS, but we do not know how or what they knew about it. Finally, in the IDM literature, decision aids typically are used in conjunction with discussions with health care providers or family members. In our original hospital study, a research assistant was available to talk with families about the study, but the simulation study offered no such opportunity, so our findings are limited to hypothetical decisions made alone by women after a single reading.

Future Directions

Using IDM as a foundational framework, we developed a study recruitment brochure that was highly rated by experts and consumers, met high standards for IDM, and achieved some of our stated goals in a simulation study. But a brochure only has the potential to be useful if it is read. We are conducting a companion implementation study in a hospital to test its ultimate utility, assessing whether the brochure was more likely to be read and the extent to which the new brochure changes rates of study participation.

Practice Implications

With the advent of DNA-based and other next-generation sequencing technologies, research will be needed to determine how families understand and use genetic information, and, more fundamentally, whether they want that information at all. For example, if these technologies became standard for newborn screening, the public health screening program as we know it could change fundamentally (Goldenberg and Sharp 2012). State health departments will obtain information about a wide range of genetic variants and decisions will have to be made about what information to disclose, when to disclose it, and how. Systematic practice-based research will be needed and ultimately newborn screening may need to include a voluntary component for disclosing information that does not meet the "public health emergency" standard. Parents will expect to have a say in the information disclosed, but their decision must be informed and supported. IDM provides a set of relevant guiding principles, because this context mimics prior applications of IDM to health care decisions where there is no right or wrong answer. But making the information understandable and finding realistic opportunities for parents to weigh alternatives and make an informed decision will be an enormous public health challenge.

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ORIGINAL RESEARCH ARTICLE | Genetics inMedicine

Can a decision aid enable informed decisions in neonatal nursery recruitment for a fragile X newborn screening study?

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Purpose: To determine whether a brochure based on principles of informed decision making improved attention to study materials or altered decisions made by parents invited to participate in a fragile X syndrome newborn screening study.

Methods: A total of 1,323 families were invited to participate in a newborn screening study to identify infants with fragile X syndrome as well as premutation carrier infants. Of these families, 716 received the original project brochure and 607 were given a new decision aid brochure.

Results: Families were more likely to look at the new decision aid and mothers were more likely to read it completely, but the proportion of mothers who read the entire decision aid was only 14%. Families were more likely to rate the decision aid as very helpful. Consistent with informed decision making theory and research, participants receiving the decision aid brochure were less likely to agree to participate.

Conclusion: The decision aid increased attention to and perceived helpfulness of educational information about the study, but most families did not read it completely. The study suggests that even well-designed study materials are not fully reviewed in the context of in-hospital postpartum study recruitment and may need to be accompanied by a research recruiter to obtain informed consent.

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Key Words: fragile X; informed decision making; neonatal nursery; newborn screening; parent decisions

INTRODUCTION

Recruiting research participants requires careful delineation of risks and benefits and should be accompanied by a rigorous informed consent process, but recruiting families to participate in neonatal research, especially for genetic testing and the return of results, poses special challenges. Ideally, parents should be informed about research during the prenatal period, allowing time to consider options, understand ramifications of study participation, and decide whether to participate. However, logistical and financial barriers in talking with parents during this period are substantial, often leading to in-hospital recruitment immediately before or shortly after birth—clearly a suboptimal time for thoughtful decisions.

The newborn screening context

The challenges of ethical recruitment are especially salient in newborn screening. In the United States, newborn screening is usually performed without informed consent on the assumption that the urgent need to treat identified conditions outweighs the ethical stipulation of consent. Parents are typically informed about newborn screening, however, and many states allow parents to opt out of screening for religious or moral reasons, although few parents do so. State decisions about which conditions merit screening are guided by recommendations from the Secretary's Advisory Committee on Heritable Disorders in Newborns and Children.[1] Most parents accept the trade-off between the need for rapid action and the loss of parental autonomy,[2] although cross-country variations exist.[3–5] Some ethicists suggest that the possibility of whole-genome or whole-exome sequencing and the breadth of information potentially available will only heighten the debate, forcing mandatory screening to be reconsidered and strengthening the case for informed consent.[6,7]

Newborn screening pilot programs can be conducted by states without parental consent if the study meets institutional review board (IRB) criteria for minimal risk, protection of rights and welfare, and impracticability.[8] However, preliminary studies gathering data needed before state-sponsored pilots must have a robust consent process. Such was the case with our fragile X newborn screening study, the project on which this article is based.

The fragile X newborn screening pilot study

Fragile X syndrome (FXS) is the most common inherited form of intellectual disability. Because FXS lacks phenotypic specificity in the early years and developmental delays only gradually appear, most children are not identified until age 36 months or later.[9] Delayed diagnosis has significant consequences for children (e.g., inability to participate in early intervention) and families (e.g., long diagnostic odysseys, costs in finding a correct diagnosis, and/or a second affected child).[9–11]

Newborn screening for FXS could benefit affected children and families but has not been included on state screening panels because it lacks a proven medical treatment that must begin early. In addition, screening relies on a DNA-based assay that

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simultaneously identifies *FMR1* premutation carriers. Although carrier identification might be useful to some families because it leads to identifying parental carrier status, carriers are also at increased risk for adult-onset conditions such as FX-primary ovarian insufficiency, FX-tremor ataxia syndrome, or other neuropsychological or emotional problems.[12,13] Therefore, FX screening of neonates evokes difficult ethical considerations. The newborn's test results have uncertain value, suggesting an increased risk but not the certainty of disease. Most results indicate carrier status and predict the possibility of largely untreatable, adult-onset disorders for both baby and a parent.[6,14–16] These realities dictate that parents play a meaningful role in deciding whether or not their child should participate in such a study.[17–20]

The study was designed to determine parents' interest in screening, their reasons for accepting or declining, family adaptation to learning about carrier status, and the early developmental progress of identified infants. Parents were recruited in the hospital shortly after their child's birth; those interested in hearing about the study were given a brochure and consent form by a bilingual research assistant (RA). The RA returned later to answer questions, provide clarification, and obtain consent. Screening results were relayed several weeks later by phone if positive and by letter if negative. Parents of screen-positive infants were offered genetic counseling, diagnostic confirmation, and parental carrier testing, and were invited to participate in a longitudinal study of infant development and family adaptation. An initial publication reported an acceptance rate of 63%, which remained relatively constant throughout the study, with black families significantly less likely to participate as compared with other parents.[21]

Pilot study educational materials

A study brochure was developed in accordance with IRB regulations, but during the course of the study the authors became concerned that its emphasis on FXS may have prevented parents from fully understanding that the most likely screen-positive result would identify carriers. In an informal preassessment, fewer than half of the parents reported looking at it. A possible explanation for the lower participation of black families could have been that the original brochure did not state that FXS affects all ethnic/minority groups.

To address these concerns, a new brochure was designed to be more visually appealing, to include photographs of families from multiple racial groups, and to place more emphasis on carrier identification. The design incorporated well-accepted principles of informed decision making (IDM) to create a "decision aid" that supported families in weighing various factors to help them arrive at a decision consistent with their values and preferences.[22]

IDM is a method recommended in health-care situations in which patients must make a decision for which there is no objective right or wrong answer. A decision is considered effective when it is consistent with the person's own values and preferences.[23] IDM is usually supported by decision aids—print or audiovisual materials describing the decision to be made and providing strategies for weighing choices and personal values.[24] Reviews of decision aids suggest that they improve knowledge and inform decisions.[25,26] For example, a recent study of young women with early-stage breast cancer found that, as compared with usual care, women who received the decision aid knew more about their options, were more certain about their decisions, and had less decisional regret.[27] Ironically, the principles of IDM are rarely incorporated in the consent process. A review of consent documents used in 139 clinical trials concluded that most failed to meet international standards for supporting informed decisions,[28] and, with only a few exceptions,[29] the application of IDM to the consent process has not been studied.

We recently described our decision aid, provided details about how it was developed, and reported initial evidence of usefulness.[30] The decision aid, designed in a brochure format, received high scores on informational content, guidance, and values from an independent panel of experts using the International Patient Decision Aids Standards Collaboration checklist.[31] In a simulation study, pregnant women or new mothers rated the aid high in quality and trustworthiness. They scored an average of 91.1% correct on a knowledge test after reading it once (in an average of 6.4 min). When asked to make a hypothetical decision, 61.9% would choose to have their child screened; of note, minority women were not significantly less likely to trust the aid or agree to screening.

Research questions

The ultimate test of the usefulness of a decision aid can only be determined in an actual decisional context, reported here to answer five questions:

- *Was the decision aid brochure more likely to be looked at than the original brochure?*

The decision aid was more colorful than the original brochure, with numerous photographs of parents and infants. We hypothesized that it would increase the number of families in which at least one person looked at it.

- *Was the decision aid more likely to be read completely by mothers than the original brochure?*

Because of the visual appeal and revised format, we hypothesized that mothers would be more likely to read it completely than those receiving the original brochure.

- *Did parents rate the decision aid as more helpful than the original brochure in deciding whether to participate?*

Because the decision aid was developed with techniques supporting IDM, we hypothesized that it would receive higher ratings of perceived helpfulness than the original brochure.

- *Did the decision aid alter decisions about study participation?*

Two reviews of decision aids found that they often result in lower uptake rates.[25,26] Accordingly, we hypothesized that, in families in which the mother read the entire brochure, there would be a lower rate of participation in those who read the decision aid as compared with the original brochure.

- *To what extent were maternal education and race/ethnicity associated with variation in the answers to the first four questions?*

We hypothesized that the decision aid's pictorial depiction of families from multiple races would lead to an increase in minority mothers who read it and greater cross-ethnic similarity in study participation.

MATERIALS AND METHODS

We used a baseline-intervention design to evaluate the effects of the decision aid. The original brochure was used in the recruitment process for 7 months, followed by a 6-month period using the decision aid.

Subjects

A total of 1,323 families who had given birth in a university-based hospital agreed to hear about the study from an RA, giving them the opportunity to read the recruitment materials; of these, 716 received the original brochure and 607 received the decision aid. Their demographic characteristics are displayed in **Table 1**. The mothers had a mean age of 28.7 years and represented a diverse range of ethnicities: 45.7% white, 14.8% black, 33.9% Hispanic, and 5.7% other. Approximately 26% had less than a high school education, 18% had a high school or GED diploma, 15% some college or community college education, 18% a college degree, and 22% a post-baccalaureate degree.

Procedures and instruments

As detailed in the original report,[21] all mothers aged 15 years or older (excluding those with medically ill infants, infants given up for adoption, and mothers who spoke neither English nor Spanish) were approached by a bilingual RA in the postpartum unit and asked if they were interested in hearing about a research study. If interested, they were given a brochure and consent form (English and Spanish versions were available for the original brochure, the decision aid, and the consent form) and had the opportunity over the next few hours to discuss the project with the RA. For a 7-month period, families were given the original brochure, followed by a 6-month period with the decision aid. Otherwise, all recruitment procedures remained the same throughout the course of the study.

Families decided whether they wanted to have their children screened. Once they had decided, the RA asked whether they would answer a few questions about demographic characteristics and three brief questions about the brochure. Those who verbally assented (almost all parents) provided this information. The university IRB approved the survey questions and did not ask for documentation of consent. The following questions were asked:

- Have you or anyone in your family looked at this brochure about the study? Mothers indicated whether they, the father, or another family member had looked at it.
- Which of the following best describes how much you (the mother) were able to read: none; looked at the cover; quickly glanced through it but did not read it all; or read the whole thing?
- How helpful was the brochure in deciding whether the study is right for your child: not at all; somewhat; or very ?

Data analyses

Demographic characteristics of participants receiving the decision aid and the original brochure were compared using χ^2 tests. We conducted logistic regression models to examine the impact of brochure type on whether anyone looked at it, whether mothers read the whole brochure, and whether family members who looked at it considered it "very helpful." Regression models controlled for marital status, education, mother's race/ethnicity and age, and whether Spanish was her primary language. In addition, we tested for interactions between brochure type and race/ethnicity for each racial/ethnic group. Finally, we conducted similar logistic regression models to compare participation rates among parents based on brochure type and demographics, as well as whether the mother had read the whole brochure.

RESULTS

Demographic characteristics of participants by brochure type are shown in **Table 1**. Participants receiving the decision aid did not differ significantly from those receiving the original brochure on marital status, mother's age, education, race/ethnicity, or primary language.

Use and perceptions of brochures

Families receiving the decision aid were significantly more likely to report that at least one family member looked at it than those receiving the original brochure ($P = 0.02$; **Table 2**). Forty-four percent of families receiving the decision aid looked at it as compared with 39% of families receiving the original. Married parents were more likely than single parents ($P = 0.045$) and Spanish speakers were less likely than non-Spanish speakers ($P = 0.017$) to look at either brochure.

Mothers receiving the decision aid were more likely to read the whole brochure, controlling for demographics ($P = 0.043$). Fourteen percent read the new brochure completely as compared with 11% with the original brochure. Across both brochures, mothers who spoke Spanish were less likely to have read the whole brochure ($P < 0.001$). Testing interaction effects revealed that the impact of the new brochure was greater among Hispanic mothers than white mothers (odds ratio (95% confidence interval) = 1.30 (1.06, 1.61), $P = 0.014$). As shown in **Figure 1**, the percentage of Hispanic mothers reading the entire brochure increased from 6% for the original brochure to 14% for the decision aid.

Families were more likely to rate the decision aid as very helpful ($P = 0.015$; **Table 2**) than the original brochure. Although

Table 1 Demographic characteristics, use of brochure, and study participation by brochure type

	All (*N* = 1,323)	New brochure (*n* = 607)	Old brochure (*n* = 716)	Test statistic	
Variable	***n* (%)**	***n* (%)**	***n* (%)**	**χ^2 (df = 1)**	***P***
Demographics					
Married	783 (60)	350 (59)	433 (61)	0.92	0.339
Mother's age					
<25	342 (26)	161 (27)	181 (25)	0.37	0.562
25–29	347 (26)	153 (25)	194 (27)	0.51	0.473
30–34	383 (29)	172 (28)	211 (29)	0.15	0.700
≥35	229 (17)	109 (18)	120 (17)	0.39	0.533
Mother's education					
Less than high school	341 (26)	157 (26)	184 (26)	0.01	0.909
High school graduate/some college	454 (34)	209 (35)	245 (34)	0.02	0.891
4-year college or more	507 (38)	230 (39)	277 (39)	0.06	0.812
Mother's race/ethnicity					
White	595 (46)	263 (44)	332 (47)	1.09	0.296
Black	192 (15)	93 (16)	99 (14)	0.64	0.423
Hispanic	441 (34)	207 (35)	234 (33)	0.36	0.547
Other	74 (6)	33 (5)	41 (6)	0.04	0.834
Mother speaks Spanish	362 (27)	171 (28)	191 (27)	0.43	0.511
Use of brochure/study participation					
Anyone in family looked at brochure	544 (41)	268 (44)	276 (39)	4.28	0.039
Mother read the whole brochure	161 (12)	85 (14)	76 (11)	3.34	0.068
Perceived helpfulness of brochure[a]					
Very	73 (14)	46 (17)	27 (10)	5.79	0.016
Somewhat	210 (40)	82 (31)	128 (49)	17.04	<0.001
Not at all	244 (46)	136 (52)	108 (41)	5.66	0.017
Family agreed to participate in study	996 (75)	443 (73)	553 (77)	3.19	0.074

[a]Includes only those who reported looking at the brochure.

not statistically significant at $P < 0.05$, families in which the mother had less than a high school education (vs. 4-year college or more) and those who were black (vs. white) tended to be more likely to find the decision aid very helpful ($P = 0.051$ and $P = 0.056$, respectively). A significant interaction suggests that the effect of the new brochure is greater for black vs. white mothers (odds ratio (95% confidence interval) = 1.37 (1.04, 1.81), $P = 0.025$). Among black families, 77% described the decision aid format as very helpful vs. only 44% for the original brochure; in comparison, these values were 43% vs. 39% for white families (**Figure 2**).

Agreement to participate in the study

Controlling for demographics, participants receiving the decision aid were less likely to participate in the study than those receiving the original brochure ($P = 0.028$; **Table 3**). Seventy-three percent of all families who received the decision aid agreed as compared with 77% who received the original brochure. (These percentages are higher than the 63% reported in Skinner et al.[21] because our numbers are of those families who agreed to consider study participation (and thus had the opportunity to read the brochure) whereas Skinner et al.[21] reported the percentage of all families approached, some of whom were not interested in any research and so were not given a brochure.) Families in which the mother was black ($P < 0.001$) or other race (vs. white) ($P = 0.009$) were less likely to participate. Although not statistically significant at $P < 0.05$, the interaction between brochure type and reading the whole brochure suggests that the decision aid had a greater impact on study participation when the mother had read the entire brochure ($P = 0.057$). Among those receiving the original brochure, 72% of those who read all of it agreed to participate as compared with 78% of those who did not read it. However, only 54% of those who read the entire decision aid agreed to participate in the study as compared with 76% among those who did not read the entire decision aid.

DISCUSSION

Our primary goal was to test whether the use of IDM principles to design a decision aid about a study involving genetic testing and the return of results would increase parents' attention to and perceived usefulness of recruitment materials. In a simulation study[30] we had demonstrated that the decision aid (i) met established criteria for IDM, (ii) resulted

Table 2 Logistic regression models of use and perceived helpfulness of the brochure

	Anyone in family looked at brochure		Mother read whole brochure		Perceived brochure to be very helpful	
Variable	**Adjusted OR (95% CI)**	***P***	**Adjusted OR (95% CI)**	***P***	**Adjusted OR (95% CI)**	***P***
Brochure type						
New brochure	1.30 (1.04, 1.63)	0.020	1.41 (1.01, 1.98)	0.043	1.55 (1.09, 2.20)	0.015
Old brochure	REF		REF		REF	
Marital status						
Married	1.33 (1.01, 1.75)	0.045	1.29 (0.85, 1.97)	0.238	0.73 (0.47, 1.14)	0.164
Not married	REF		REF		REF	
Education						
Less than high school	1.18 (0.76, 1.83)	0.511	1.18 (0.60, 2.33)	0.928	2.20 (1.11, 4.37)	0.051
High school graduate/some college	1.09 (0.79, 1.50)	0.992	1.38 (0.87, 2.18)	0.229	1.51 (0.91, 2.51)	0.936
4-year college or more	REF		REF		REF	
Mother's race/ethnicity						
Black	0.99 (0.69, 1.42)	0.945	1.10 (0.66, 1.83)	0.717	1.76 (0.99, 3.12)	0.056
White	REF		REF		REF	
Hispanic	1.17 (0.71, 1.94)	0.539	1.77 (0.92, 3.39)	0.088	1.05 (0.50, 2.21)	0.901
Other	0.97 (0.60, 1.58)	0.908	0.63 (0.28, 1.43)	0.270	0.87 (0.40, 1.87)	0.715
Spanish speaker						
Yes	0.53 (0.32, 0.89)	0.017	0.27 (0.13, 0.57)	<0.001	0.77 (0.34, 1.73)	0.524
No	REF		REF		REF	
Mother's age	1.01 (0.99, 1.03)	0.467	1.02 (0.99, 1.06)	0.159	1.03 (0.99, 1.06)	0.163

Odds ratios are adjusted for brochure type, marital status, education, race/ethnicity, Spanish language, and age.
CI, confidence interval; OR, odds ratio; REF, reference category.

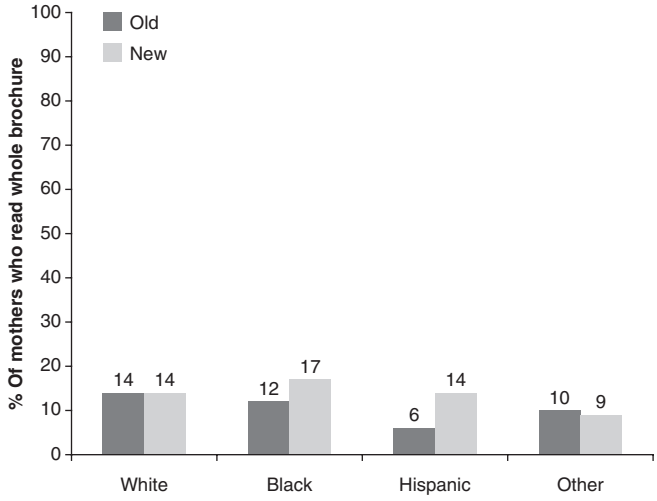

Figure 1 Percentage of mothers reading the whole brochure by race/ethnicity.

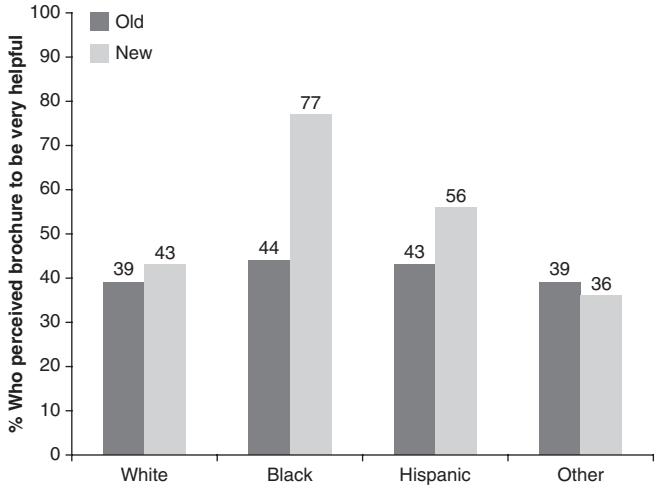

Figure 2 Percentage of families perceiving new and old brochures to be very helpful by race/ethnicity.

in a high degree of factual knowledge both about the study and about what families might learn from FX screening, and (iii) was rated highly in both quality and usefulness by a small ($N = 118$) group of pregnant women and recent mothers. The simulated population differed from the recruitment population by several key factors, including higher education level and better English fluency, not being hospitalized nor having recently given birth, and the hypothetical nature of their decisions.

The effect of the decision aid was then tested using a comparison study implemented in the FX pilot screening environment with a more diverse population. The women had just given birth and had to decide about study participation in a short period of time, before the phlebotomist obtained the baby's blood for the state screening program. Increasing the uptake of study participation was not our goal. Instead, we sought to maximize the likelihood of parents' making a fully informed decision, one that more closely aligned with their personal values and preferences.

Table 3 Logistic regression models of agreement to participate in the study

	Family agreed to participate in study		
Variable	***n* (%)**	**Adjusted OR (95% CI)**	***P***
Brochure type			
New brochure	443 (73)	0.81 (0.68, 0.98)	0.028
Old brochure	553 (77)	REF	
Marital status			
Married	614 (78)	1.04 (0.76, 1.43)	0.812
Not married	382 (74)	REF	
Education			
Less than high school	256 (75)	0.71 (0.42, 1.18)	0.345
High school graduate/some college	332 (73)	0.75 (0.51, 1.10)	0.465
4-year college or more	408 (80)	REF	
Mother's race/ethnicity			
Black	121 (63)	0.39 (0.26, 0.59)	<0.001
White	489 (82)	REF	
Hispanic	334 (76)	0.78 (0.43, 1.44)	0.427
Other	52 (70)	0.48 (0.28, 0.83)	0.009
Spanish speaker			
Yes	275 (76)	1.04 (0.56, 1.93)	0.896
No	721 (77)	REF	
Mother's age	—	0.98 (0.96, 1.01)	0.183
Read the whole brochure			
Yes	101 (63)	0.68 (0.57, 0.82)	<0.001
No	888 (77)	REF	
Period × read whole brochure		0.84 (0.70, 1.01)	0.057
New: read whole brochure	46 (54)		
New: did not read whole brochure	396 (76)		
Old: read whole brochure	55 (72)		
Old: did not read whole brochure	492 (78)		

Percentages represent the proportion of families with the characteristic who agreed to participate in the study (e.g., 78% of those who were married agreed to participate). Odds ratios are adjusted for brochure type, marital status, education, race/ethnicity, Spanish language, age, reading the brochure, and the interaction between brochure type and reading the brochure.
CI, confidence interval; OR, odds ratio; REF, reference category.

This goal was partially achieved. Consistent with our original hypotheses, when compared with the participants given the original brochure, those given the decision aid were more likely to report that someone looked at it; mothers were more likely to have read the entire brochure and they were more likely to perceive it as "very helpful." Increased attention to the brochure was likely influenced by differences in design and layout. The original brochure was a pink and blue trifold with no photographs, printed on card stock paper. The decision aid was a colorful eight-page "magazine-type" format with photographs on each page, printed on semi-gloss paper. Ratings of "helpfulness" were likely influenced by the attention given to IDM principles in the decision aid, which included two pages devoted to "things to consider when making your decision," and a set of "questions to help you decide," followed by the following concluding statement: "If you answered *Yes* to most of the questions above, maybe you are ready to have your newborn screened. If you answered *No* to most, maybe this is not the right decision for you."

Race/ethnicity played a role in these ratings. The decision aid had a greater impact on Hispanic mothers reading the whole brochure as compared with white mothers, and the increase in perceived helpfulness was greater among black mothers than among white mothers. The photographs depicting families of different ethnicities may have influenced these findings. Also, the decision aid mentioned that FXS affects all ethnic and racial groups, a fact not mentioned in the original brochure.

Of note, despite the wide range of formal education, education was not significantly associated with these or any other outcome, either as a main effect or an interaction effect. This is somewhat surprising, because a readability analysis showed that it was written at a 9th grade level, primarily due to numerous three- and four-syllable words that could not be removed (e.g., family, carrier, genetics, and development). It is possible that our multiple editorial reviews, pilot testing, attention to layout and design, and plain language reviews helped maximize readability, and the fact that many three-syllable words in the pamphlet (with the exception of "carrier" and "genetics") are common.

Enthusiasm about the statistically significant improvements seen with the decision aid, however, is tempered by the fact that fewer than half (44%) of the families reported that anyone looked at the decision aid at all. Fewer than 30% of the mothers looked at it, and of those, fewer than half read the entire brochure. In other words, of all of the mothers given the decision aid pamphlet, only ~14% read it completely. Although this is a slight improvement over the 11% who read the original brochure completely, this percentage does not come close to approaching the goal of providing informational materials that are read by most mothers. It can be argued that the timing and setting played a substantial role in creating a suboptimal environment for informed consent. Parent decisions about participation in research naturally take a back seat to other demands and priorities faced by families during this period.

Still, there is evidence that the decision aid had some influence on decisions about study participation. Across the entire sample, the decision aid resulted in a slight but statistically insignificant reduction in participation rates (77.2% with the original brochure and 73.0% with the decision aid). However, when mothers read the entire brochure, there was a substantial reduction in participation from 72% with the original brochure to 54% with the decision aid. This difference did not quite reach statistical significance, most likely because of the small sample size of women who read the whole brochure. We had hypothesized that there would be some decrease based on

literature showing that decision aids generally result in reduced acceptance of treatment or screening options.[25,26] Theoretically, placing the information about screening into a broader context of personal values enables parents to identify choices inconsistent with their values and thus reject them.[32] The magnitude of the reduction in study participation was surprising even though previous research has also reported significant drops in acceptance rates. For example, a study investigating the use of a decision aid about bowel cancer screening showed that the decision aid was associated with higher knowledge and greater confidence in decisions but reduced participation in fecal occult blood testing from 75 to 59%.[33] Our decision aid presented a balance of reasons to participate or not, giving explicit permission for parents to decline. It is also possible that the decision aid, when fully read, helped mothers realize that the information that could be learned from this screening was qualitatively different from that obtained from traditional newborn screening. The lack of specific interventions for newborns with a premutation, in combination with frank statements that FXS has "no cure," could have influenced mothers to conclude that screening was not urgent and that the value of the information gained would be uncertain and potentially worrisome. It is also possible that mothers who read the entire decision aid began to appreciate the complexities involved in making this decision and opted out because of the short time frame for deciding.

Conclusion

Many strategies could be used to improve the consent process, only one of which is improving the clarity and usefulness of informational materials.[34,35] In this study, we show that a carefully designed set of informational materials can improve parents' attention to them and, for those who do read them, the IDM format can result in lower rates of participation, an indication that the decision-making process has been influenced. The fact that only 14% of the mothers read the entire decision aid brochure is sobering but should be tempered by the recognition that parents use multiple sources of information on which to base decisions. The RAs for this project spent considerable time discussing the study with families, and we believe that before signing the consent form, most families understood the study, at least in general terms, including the broader implications of their decision to participate. We do not have independent confirmation of this, however, and were not able to study the relative impact of the RAs' interactions with families as compared with the impact of the pamphlet on families' understandings.

This study raises fundamental questions about whether written materials alone, even when well designed, will be sufficient to obtain the degree of informed consent considered acceptable for research participation during this period, especially for decisions that require comprehending complex information. Consent for research increasingly includes decisions about a wide range of genomic testing.[36] Although it could be argued that it is unrealistic to expect parents to be fully educated about genetics and the potential ramifications of genetic testing, researchers are obligated to use materials and procedures that lead to informed consent. Written materials likely will need to be viewed as a supplement to important personal interactions with a research recruiter or health-care provider, but the timing and cost of such interactions are substantial, and creative strategies are needed to minimize these costs. As one example, the educational component of a genetic counseling session has been examined to assess whether use of a "pre-visit" website to provide patients with a question prompt sheet could encourage more active and meaningful participation.[37] However, obtaining informed consent under the constraints posed by both the timing and context of newborn screening will grow even more challenging with the adoption of NextGen technologies,[38] heightening the need to develop and test effective supplemental educational methods to help individuals make decisions consistent with their values and beliefs.

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DISCLOSURE

The authors declare no conflict of interest.

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Maternal Consequences of the Detection of Fragile X Carriers in Newborn Screening

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abstract

OBJECTIVES: The possibility of newborn screening for fragile X syndrome is complicated by the potential for identifying premutation carriers. Although knowing the child's carrier status has potential benefits, the possibility of late-onset disorders in carrier children and their parents raises concerns about whether such information would be distressing to parents and potentially more harmful than helpful. This study sought to answer this question by offering voluntary fragile X screening to new parents and returning results for both the full mutation and premutation *FMR1* gene expansions. We tested the assumption that such information could lead to adverse mental health outcomes or decision regret. We also wanted to know if child age and spousal support were associated with the outcomes of interest.

METHODS: Eighteen mothers of screen-positive infants with the premutation and 15 comparison mothers completed a battery of assessments of maternal anxiety, postpartum depression, stress, family quality of life, decision regret, and spousal support. The study was longitudinal, with an average of 3 assessments per mother.

RESULTS: The premutation group was not statistically different from the comparison group on measures of anxiety, depression, stress, or quality of life. A subset of mothers experienced clinically significant anxiety and decision regret, but factors associated with these outcomes could not be identified. Greater spousal support was generally associated with more positive outcomes.

CONCLUSIONS: Although we did not find evidence of significant adverse events, disclosure of newborn carrier status remains an important consideration in newborn screening policy.

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WHAT'S KNOWN ON THIS SUBJECT: Parents generally adapt well to newborn screening results, but reactions to carrier status for X-linked conditions are unknown.

WHAT THIS STUDY ADDS: Results suggest that detection and disclosure of *FMR1* newborn carrier status may not result in significant adverse events for mothers.

Dr Bailey conceptualized and designed the study, wrote the first draft of the manuscript, integrated edits from all coauthors, and prepared the final manuscript for submission; Dr Wheeler conducted follow-up assessments at the North Carolina site, wrote part of the Results section, and reviewed and revised the manuscript; Dr Berry-Kravis conceptualized and designed the study, directed all research at the Illinois site, and reviewed and revised the manuscript; Dr Hagerman conceptualized and designed the study, directed the clinical research at the California site, and reviewed and revised the manuscript; Dr Tassone conceptualized and designed the study, developed the screening test, directed the laboratory analyses at the California site, and reviewed and revised the manuscript; Dr Powell directed the clinical follow-up at the North Carolina site and reviewed and revised the manuscript; Ms Roche provided genetic counseling at the North Carolina site and reviewed and revised the manuscript; Ms Gane provided counseling services at the California site and reviewed and revised the manuscript; Dr Sideris conducted all statistical analyses and wrote part of the Results section; and all authors approved the final manuscript as submitted.

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Fragile X syndrome (FXS) is the most common inherited form of intellectual disability. Because physical features are not evident at birth, FXS must be detected through abnormalities in development or behavior during childhood. Parents typically experience an extended "odyssey" before FXS is diagnosed.[1,2] The average age of diagnosis is 36 months for boys and later for girls, because females are usually more mildly affected.[3] As a result, many children with FXS have delayed opportunities to participate in early-intervention programs.[4] In addition, as many as 25% to 30% of families have a second child with FXS before the diagnosis of the first child.[3]

Newborn screening is the only way all children with FXS could be identified early. However, FXS does not currently meet criteria for inclusion in the Recommended Uniform Screening Panel by the Secretary's Discretionary Committee on Heritable Disorders in Newborns and Children[5] for 2 primary reasons: (1) it is not considered "medically actionable" and no studies have shown that early intervention significantly impacts development and behavior and (2) no studies have determined the costs or feasibility of conducting high-through-put screening in a state health laboratory.

Among the issues evoked by screening for FXS in newborns, one of the most concerning is the incidental detection of carriers.[6] The normal *FMR1* gene typically contains <45 repetitions of the nucleotides cytosine and guanine in CGG triplets, a number that typically remains stable across generations. Individuals with expansions of 55 to 200 repeats are premutation carriers. This repeat length is unstable and can further expand in future generations, causing female carriers to be at risk of having children of either gender with the full mutation (>200 CGG repeats), associated with *FMR1* methylation and transcriptional silencing, resulting in FXS.

A DNA-based screening test for FXS would also identify carrier infants. This information could be useful to parents, informing them of their reproductive risk of having a child with FXS and alerting them to possible future health problems. Although the American Academy of Pediatrics and the American College of Medical Genetics and Genomics[7,8] do not recommend routine carrier testing for minors, both acknowledge that if carriers are detected in newborn screening, it should be disclosed to parents. Parents of children with FXS strongly support carrier disclosure,[9,10] and a majority of parents in the general population accepted the possibility of carrier detection in 2 pilot studies.[11,12] But disclosure of *FMR1* carriers is controversial because carrier status is associated with risk of health, cognitive, and emotional problems.[13] Female carriers are at risk of primary ovarian insufficiency,[14] and both genders are at risk of fragile X–associated tremor ataxia syndrome.[15,16] Some carriers are also at risk of learning problems and brain function abnormalities,[17,18] autism spectrum features,[19] and depression or anxiety disorders.[20–22] Thus, if newborn screening for FXS were to identify carrier children, it would imply that they and the carrier parent may be at increased risk of developmental, behavioral, and medical concerns.

Learning about carrier status could increase the risk of anxiety, depression, or stress, especially for mothers. Postpartum depression and anxiety are relatively common in the general population.[23–25] Females are generally more likely to experience depression than males,[26] and mothers with the premutation are at risk of elevated depression and anxiety, risks that could be exacerbated by disclosure of their infant's carrier status.[20–22] Research on the impact of parents' learning that their healthy-appearing newborn has a disorder provides mixed evidence of increased depressive symptoms or anxiety (eg, refs 27–31). This finding may be due to evidence that other factors such as lower income, minority status, and lack of social support are also strongly associated with adverse mental health outcomes.[32]

Although much has been written about public attitudes toward the return of genomic research findings, most data come from hypothetical studies.[33] We recently completed a multisite fragile X newborn screening pilot study on the basis of the assumption that research studying the experiences of offering testing and communicating results is needed to fully understand benefits and harms. We previously reported acceptance rates and reasons for accepting or declining screening,[12] prevalence of *FMR1* premutation expansions,[34] fathers' participation in the consent process,[35] examples of how the identification of a target child can lead to identifying other family members,[36] and the development and evaluation of a brochure to support informed decision-making about study participation.[37,38]

Here we report findings from an assessment of maternal reactions to the disclosure of their child's *FMR1* carrier status after newborn screening. Our primary goal was to determine whether these mothers experienced adverse mental health outcomes (stress, anxiety, depressive symptoms, low quality of life), whether they regretted the decision to participate, and how adaptation over time varied as a function of the child's age or the availability of spousal support.

METHODS

Setting and Procedures

The study was conducted in 3 university-based hospitals in North Carolina, California, and Illinois. Study recruitment procedures[12] and laboratory methods[34] are detailed in previous reports and briefly

summarized here. Recruitment processes varied slightly at each hospital; but in general, shortly after birth, families were approached by a trained recruiter who asked if they would be willing to hear about a research project, to which most families agreed. Families were given brief written information and a short verbal overview of the study. Those who expressed interest were given much more detailed information by the recruiter, including the comprehensive consent form. Approximately halfway through the project a new brochure was developed to support informed decision-making[37,38] and was used at all 3 sites. The brochure included a section on what it means to be a "fragile X carrier," addressed the implications of carrier status for both newborns and parents, and made it clear that carrier detection was a much more likely outcome than the detection of children with FXS.

Across the 3 sites, ~20 374 families were approached, and of those, 19 951 (97.9%) agreed to hear about the study. Of those, 63.7% (12 709) agreed to have their infant screened. One infant screened positive for a full mutation (not included in this article) and 45 screened positive for a premutation allele, including 2 sets of twins.

Families of screen-positive children were called by a genetic counselor, pediatrician, or medical geneticist on the research team, notified of results, and offered a genetic counseling appointment and confirmatory testing. During this visit, families were counseled about the potential adult-onset health implications of carrier status. Thirty infants had the confirmatory testing. Of these, 2 were found not to be carriers. Sixteen families did not have confirmatory testing for the following reasons: declined genetic counseling (n = 3), failed to show for the appointment (n = 2), declined repeat testing of the infant (n = 3), or were unable to be reached via phone or mail (n = 8).

All families whose positive screening result was confirmed (n = 28) were invited to join the longitudinal component of the study. Three declined participation or were unable to be reached to schedule a visit. Twenty-three families (26 infants) participated in at least 1 longitudinal assessment, but 5 mothers did not participate in the family assessments, leaving a total of 18 mothers of premutation infants reported here. Fifteen mothers whose infants screened negative who were matched with the screen-positive group on ethnicity, language, education, and income were recruited as a comparison group.

Because a substantial number of parents did not participate in the follow-up study, we compared screen-positive participants and nonparticipants on 5 variables (maternal age, marital status, race/ethnicity, maternal education, and CGG repeat length of the identified child) using t tests for continuous variables (maternal age, CGG repeat range) and χ^2 test for categorical variables (marital status, race/ethnicity, maternal education). The results are shown in Table 1. No significant differences were detected between the groups on any of these variables.

The following 5 well-validated measures were used to determine whether mothers experienced adverse outcomes and if they were satisfied with their decision to participate: (1) the 36-item short form of the Parenting Stress Index[39]; (2) the Spielberger State-Trait Anxiety Inventory[40]; (3) the Edinburgh Postnatal Depression Scale[41]; (4) the Quality of Life Inventory (QOLI)[42]; and (5) the Decision Regret Scale.[43] The Emotional Intimacy Subscale of the Personal Assessment of Intimate Relationships Inventory[44] was used to assess spousal support.

Data Analysis

Data were collected at 1 to 7 occasions. The primary reason for this variation was length of time in the study, which lasted ~4 years. The family with 7 assessments was one of the first identified, whereas families with only 1 assessment mostly were those identified toward the end of the funding period. The mean number of assessments was 3.1 for the screen-positive group and 3.0 for the comparison group. The 3 primary research questions were as follows: (1) whether mothers of screen-positive children reported elevated stress, anxiety, depression, or low quality of life compared with mothers in the comparison group; (2) whether these mothers experienced significant regret about their decision to participate in the study; and (3) the extent to which spousal support and age of the child were related to the outcomes measured. We first tested 3-way interactions of category (premutation versus those who screened negative) × spousal support × child age. Finding no evidence for higher order effects, we retained only the 2-way interactions. The initial models also included tests of nonlinear (quadratic) change over time, but there was no evidence that such trends existed, so all models were simplified to include only linear terms. We treated the models as 2-level, random-intercept hierarchical linear models with time nested within family. Random effects are commonly used to estimate and control nonindependence in a model that arises from clustering of data[45]; in this case, data were clustered within participants, resulting from repeated measurements over time. Given our relatively small sample size, we used the Kenward-Roger[46] adjustment to the degrees of freedom to test model parameters.

RESULTS

Models were conducted testing group differences and interaction effects of child's age and spousal support on stress, depression, anxiety, quality of life, and decision regret. Parameter estimates are presented in Table 2.

TABLE 1 Comparison of Participants and Nonparticipants in the Longitudinal Study on Selected Demographic Variables

Variable	Participants	Nonparticipants	*P*
Mothers			
n	20	24	.23
Mean age (SD; range), y	30.6 (5.8; 18–44)	28.6 (4.99; 21–37)	
Marital status, %			
Married	52	48	.44
Divorced/separated	17	36	
Never married	22	12	
Unknown	9	4	
Race/ethnicity, %			
White	46	59	.97
African American	26	24	
Hispanic	9	6	
Other	13	12	
Maternal education, %			
High school or less	9	16	.61
Some college	39	40	
College degree	26	28	
Advanced degree	26	12	
Unknown	0	7	
Child			
n	23	16	.93
Mean CGG repeat range (SD; range)	68.1 (17.8; 55–129)	67.6 (19.4; 55–129)	

Parenting Stress

Across all assessments, the mean total Parenting Stress Index score was 61.8 for mothers of children with the premutation and 63.1 for mothers of comparison children. A score ≥91 is considered clinically significant and scores of 86 to 91 are considered above normal, so both groups were well within the typical range. Stress scores did not differ significantly by group (premutation versus comparison) or child age. Spousal support was strongly associated with total stress; mothers reporting high levels of spousal support reported lower stress. No interaction effects were detected, indicating that spousal support and child age were not differentially associated with stress in premutation versus comparison mothers. Across all assessments, 6% of mothers of children with the premutation and 7% of mothers of comparison children had at least 1 stress assessment in the clinically significant range.

Maternal Depression

Across all assessments, the mean Edinburgh Postnatal Depression Scale score was 4.1 for mothers of children with the premutation and 5.3 for mothers of comparison children. A score ≥10 is considered clinically significant, so both groups were well within the typical range. Depression scores did not differ significantly by group (premutation versus comparison) or child age. Spousal support was strongly associated with depression; mothers who perceived greater support reported fewer depressive symptoms. A significant interaction effect was detected; spousal support was differentially associated with stress in premutation versus comparison mothers. Across all assessments 12% of mothers of children with the premutation and 15% of mothers of comparison children had at least 1 depression score in the clinically significant range.

Maternal Anxiety

Across all assessments, the mean State-Trait Anxiety Inventory score was 34.3 for mothers of children with the premutation and 31.7 for mothers of comparison children. A score ≥45 is considered clinically significant, so the mean scores of both groups were well within the typical range. Maternal anxiety did not differ significantly by group (premutation versus comparison) or child age. Spousal support was not directly associated with anxiety, nor was a significant group × support interaction detected. However, across all assessments, 29% of mothers of children with the premutation and 7% of mothers of comparison children had at least 1 anxiety assessment in the clinically significant range.

Quality of Life

Across all assessments, the mean QOLI score was 46 for mothers of children with the premutation and 47.8 for mothers of comparison children. A score <40 is considered significantly low, so both groups were within the typical range. QOLI ratings did not differ significantly by group (premutation versus comparison). Child age was significantly associated with QOLI scores; mothers of younger children reported lower QOLI ratings than mothers of older children, but no group × age interaction was found. We did not find a main effect for spousal support but did find a significant group × support interaction. Spousal support was more important in predicting quality of life for mothers of children with the premutation than for comparison mothers. Across all assessments, 42% of mothers of children with the premutation and 38% of mothers of comparison children had at least 1 assessment with a low quality-of-life rating.

Decision Regret

The Decision Regret Scale is a 5-item measure designed to assess "remorse or distress over a decision"[43] (p 281). Here the decision for mothers was whether to have their child screened for the *FMR1* expansion. Each item (eg, "It was the right

TABLE 2 Parameter Estimates (SEs) for Total Stress (Parenting Stress Index), Depression (Edinburg Postnatal Depression Scale), Anxiety (Spielberger State-Trait Anxiety Scale), Quality of Life (QOLI), and Decision Regret (Decision Regret Scale)

Effect	Total Stress	Depression	Anxiety	Quality of Life	Decision Regret
Intercept	62.7 (2.8)	4.4 (0.9)	35.5 (1.8)	47.3 (2.2)	2.2 (0.3)
Group	−0.8 (4.2)	0.4 (1.3)	−4.4 (2.7)	1.9 (3.3)	−0.9 (0.4)*
Age	0.3 (0.2)	−0.2 (0.1)	0.03 (0.2)	−0.3 (0.1)*	−0.01 (0.01)
Spousal support	−14.4 (3.9)***	−3.7 (1.2)**	−7.1 (2.9)	10.0 (2.8)	−0.2 (0.3)
Group × age	−0.2 (0.3)	0.1 (0.2)	0.1 (0.3)	0.1 (0.3)	0.01 (0.02)
Group × support	7.6 (6.2)	5.2 (2.3)*	6.9 (4.4)	−11.0 (4.7)*	0.02 (0.5)
Age × support	0.2 (0.2)	−0.3 (0.2)	−0.2 (0.2)	0.3 (0.1)	0.00 (0.01)

*$P < .05$, **$P < .01$, ***$P < .001$.

decision") is rated on a scale from 1 (strongly agree) to 5 (strongly disagree). Because some items are stated positively (eg, "It was the right decision") and some negatively (eg, "I regret the choice that was made"), we reverse-scored the positive items so that a higher score indicated greater regret. The authors suggest converting scores to a 0 (no regret) to 100 (high regret) scale by subtracting 1 from each item, multiplying by 25, and summing the items. Across all assessments, the mean converted score was 32.3 (range: 0–100) for mothers of children with the premutation and 5.7 (range: 0–25) for mothers of comparison children. Regret scores were significantly higher for mothers of children with the premutation. The group differences were almost entirely accounted for by 2 mothers, one who reported high (90–100) regret at each assessment occasion and a second who was in the 75–80 range each time. Decision regret was not associated with child age or spousal support, and no interaction effects were found.

DISCUSSION

underThe detection of *FMR1* carriers by FXS screening in newborns and its potential for harm and benefits have been discussed extensively, but until now concerns have been speculative. Here we report findings from the first study to offer FXS newborn screening, return carrier results to parents, and follow mothers of infants to determine adaptation and reactions over time. Our primary goal was not to provide evidence that screening was beneficial but rather to attempt to detect significant potential harms.

We found no group differences in the domains assessed: depression, anxiety, stress, or quality of life. Mothers of screen-positive infants as a group were no different from a comparison group of mothers of screen-negative infants on any measure, both groups were well within the range of typical scores, and, with the exception of maternal anxiety, there were no differences in the number of mothers with clinically significant scores. Six (29%) mothers of children with the premutation had at least 1 anxiety assessment in the clinically significant range, compared with only 2 comparison mothers. An analysis of interviews and other scores with these 6 mothers reveals a complex picture, not easily leading to a generalized explanation. Three of the mothers had consistently low regret scores, 2 had high regret. Four of the 6 mothers had children with the premutation who were showing developmental or behavioral problems, and 3 of the 6 had 2 children with the premutation (2 sets of twins and 1 mother had a second child with the premutation during the study). Three mothers were premutation carriers and thus potentially at risk of elevated anxiety. These observations suggest that maternal anxiety is a complex and multifaceted construct, likely influenced by child and parent characteristics, genetic factors, family context, and spousal support.

Consistent with previous literature, we found that mothers who reported higher levels of spousal support had lower stress and lower depression scores than mothers who reported lower levels of support. We did find significant interaction effects, showing that high spousal support was more strongly associated with lower depression and higher quality of life in mothers of carrier infants than in mothers of comparison children.

We found significant group differences in decision regret. Mothers of infants with the premutation expressed greater regret about study participation than did mothers of comparison children. Comparison-group mothers had nothing to regret, and thus most of their scores were near zero. Most mothers of identified children were less likely to strongly agree with the positively worded items, but their average responses remained in the positive range; a group mean of 50 would indicate an average neutral score, and the premutation group average was 32.3. But mothers of identified children were generally more ambivalent about the study and perhaps still uncertain as to their ultimate assessment of benefit or harm. Two mothers clearly wished that they had not participated in the study. Although the written materials, including the consent form, and conversations with the recruiter clearly specified the possibility of carrier detection, the setting and timing of recruitment (a few hours after birth) may not have allowed sufficient time for these mothers to give full consideration to the study. As such, it is possible that these and other mothers had some residual regret or at least uncertainty as to whether study participation is

something they would agree to if they had the opportunity to reconsider their decision.

Our findings should be interpreted with some caution for several reasons. The first and most important limitation is potential bias in the study sample due to lack of participation in follow-up by a number of screen-positive families. To partially address this concern, we compared participants and nonparticipants on several variables (maternal age, marital status, race/ethnicity, maternal education, and CGG repeat length of the identified newborn) and found no group differences. These findings increase confidence in our conclusions, but we acknowledge that we still do not know why some families did not participate. Some may have been unconcerned about carrier status and chose not to participate because it did not seem immediately important or relevant. Others may not have participated because of adverse events or decision regret. A second limitation is the possibility that the study was not sufficiently powered to detect significant group differences. Although possible, the absolute differences between the groups were quite small, so it is unlikely that a larger sample would have affected the findings. However, the small sample size meant that we were not able to assess factors other than child age or spousal support associated with variability in the outcomes measured.

Despite these limitations, we found little evidence that the disclosure of carrier status in newborn screening for FXS, when conducted under a voluntary consent protocol with consent obtained from both parents when possible, significantly elevates the risk of stress, anxiety, depression, or low quality of life. Some mothers regretted participating in the study, suggesting that the newborn setting may hinder full understanding of the implications of consent. In addition, some mothers experienced elevated anxiety, although we cannot unequivocally demonstrate that learning their child's carrier status was the cause.

Several features of FXS currently make it unsuitable for inclusion on mandatory newborn screening panels, a situation that will remain until data show that earlier identification results in measurable benefits for children. Until then, this study suggests that the disclosure of newborn carrier status, although an important consequence to consider when making policy decisions about screening, consent, and follow-up services, may not have a significantly negative impact on mothers of identified children, especially in families where spousal support is adequate.

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ABBREVIATIONS

FXS: fragile X syndrome
QOLI: Quality of Life Inventory

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A Developmental–Contextual Model of Couples Coping With Chronic Illness Across the Adult Life Span

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A developmental–contextual model of couples coping with chronic illness is presented that views chronic illness as affecting the adjustment of both the patient and the spouse such that coping strategies enacted by the patient are examined in relation to those enacted by the spouse, and vice versa. The developmental model emphasizes that dyadic coping may be different at various phases of the life span, changing temporally at different stages of dealing with the illness as well as unfolding daily as spouses interact around dyadic stressors. In addition, couples engaged in dyadic coping are affected by broad sociocultural factors (culture and gender) as well as more proximal contextual factors (quality of the marital relationship and the specific demands of the chronic illness). The model provides a framework for understanding how couples coping with chronic illness may together appraise and cope with illness during adulthood and for determining when spousal involvement is beneficial or harmful to both patient and spousal adjustment. The developmental–contextual model to dyadic appraisal and coping has numerous research implications for the field, and the authors conclude with specific recommendations for future research.

Keywords: dyadic coping, adult development, chronic illness, marriage, coping

The diagnosis of a serious chronic illness begins a period of significant distress and adjustment for both patients and their spouses. Couples must begin to make difficult treatment decisions, redistribute household responsibilities, and adjust to the threat of a potentially life-threatening and long-term illness (Baider & De-Nour, 1999; Walsh, Blanchard, Kremer, & Blanchard, 1998). Traditionally, research has examined how patients and spouses adjust to chronic illness from an individualistic perspective to stress and coping (Carver & Scheier, 1999; Heckhausen & Schulz, 1995; Lazarus & Folkman, 1984; Maes, Leventhal, & DeRidder, 1996), measuring the adaptability of the coping strategies enacted by the patient (e.g., avoidant emotion-focused coping strategies associated with poorer adjustment, problem-focused coping associated with better outcomes). Spousal involvement is typically characterized as providing informational, tangible, and/or emotional support.

Recently, a dyadic approach to coping with chronic illness has been advanced that expands on the social support perspective by noting how spouses may frequently share stressors (appraising them as "ours" rather than "mine"), pool resources, and actively engage in joint coping efforts (Bodenmann, 2005; Lyons, Sullivan, & Ritvo, 1995; O'Brien & DeLongis, 1997; Revenson, 2003; Revenson, Kayser, & Bodenmann, 2005). According to the dyadic perspective, when couples face a stressor, such as chronic illness, the stress management resources of both partners may be activated to maintain or restore a state of homeostasis in the individual, within the marital relationship, and in relation to other social partners. As described by Bodenmann (2005), "one cannot examine one partner's stress appraisals or coping efforts without considering the effects on the other partner and the marriage" (p. 36). Consistent with Bodenmann (1997), we use the term *dyadic coping* to refer to a variety of ways that couples potentially interact as they deal with stressors (e.g., uninvolvement, support, collaboration, control, protective buffering, overprotection).

Currently in this field, two different approaches to dyadic coping have been advanced: coping congruence (see Table 1) and a more direct assessment of the patient's perceptions of the spouse's involvement (Table 2). Both approaches focus largely on the individuals composing the dyad, rather than the dyad per se. In the coping congruence approach (Revenson, 1994), dyadic coping is conceptualized as the statistical (rather than perceived) interaction between patient's and spouse's coping strategies. Coping strategies are measured individually and patterns identified through statistical analyses. Congruence in coping (e.g., both spouses using problem-focused coping or emotion-focused coping) has been posited to be associated with less distress than incongruence (e.g., coping strategies that oppose each other). However, study results (see Table 1) indicate that adjustment may depend not on congruence per se but rather on whether the dyadic unit collectively uses ineffective coping strategies (see Badr, 2004; Giunta & Compas, 1993) and is able to provide a fit between what is needed in the

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Table 1
Coping Congruence Studies

Author	Sample	Coping measure	Outcome measure	Source of perception	Significant findings
Ben-Zur et al. (2001)	73 breast cancer patients (*M* age = 52.2) and husbands (*M* age = 55.6)	COPE scale (Carver et al., 1989); problem-focused and emotion-focused scales	Brief Symptom Inventory (Derogatis & Spencer, 1982); Psychosocial Adjustment, a 15-item scale developed for this study to assess psychological adjustment	Patients and husbands	Wife-husband discrepancies in problem-focused coping were unrelated to adjustment; discrepancy in emotion-focused coping was related to greater symptom report and poorer self-reported functioning.
Giunta & Compas (1993)	153 marital dyads not experiencing chronic illness (age not given)	Ways of Coping Checklist—Revised (Vitaliano et al., 1985)	Symptom Checklist 90—Revised (Derogatis, 1983)	Husbands and wives	Cluster analysis was used to uncover different subgroups. Key to adjustment was not whether couples were congruent but whether they collectively used ineffective coping strategies. Couples who relied on escape-avoidant coping reported higher psychological symptoms.
Pakenham (1998)	101 patients with multiple sclerosis carers (*M* age = 50 for both patients and carers)	Ways of Coping Checklist—Revised (Vitaliano et al., 1985)	Brief Symptom Inventory	Patients and spouses	Differences in problem-focused coping, higher mean levels of problem-focused coping, and lower levels of emotion-focused coping were associated with better couple adjustment (less distress, lower depression). No effects were found for discrepancies in emotion-focused coping.
Revenson (1994)	103 patients with musculoskeletal or rheumatic disease and spouses (age not given)	Not given	Not given	Patients and spouses	Cluster analysis was used to uncover different subgroups of patient-spouse coping. Congruent couples did not experience lower levels of distress than did incongruent couples. Couples who were congruent and used higher amounts of problem-focused coping reported greater depression and more caregiver burden than other clusters of couples.
Upchurch et al. (2003)	45 patients with systemic sclerosis and spouses (*M* age = 49 for patients, 50 for spouses)	Ways of Coping Checklist—Revised (Vitaliano, 1991)	Psychological Adjustment to Illness Scale (Derogatis, 1986); Marital Adjustment Scale (Locke & Wallace, 1959)	Patients and spouses	Incongruent couples were marginally more distressed and reported less marital satisfaction than congruent couples.

context, for the illness, and at a particular temporal point in dealing with the illness.

Approaches to dyadic coping using a more direct assessment of the patient's perceptions of the spouse's involvement use multiple different categorizations of dyadic coping strategies: miscarried helping, protective buffering, active engagement, invisible support, overprotection, supportive coping, common dyadic coping, and hostile, ambivalent, or superficial coping (see Table 2 for an overview of the studies and the major findings in the field, organized alphabetically by investigator). As a whole, the literature suggests that the psychosocial adjustment of the patient is enhanced when patients (or spouses) perceive the spouse to be involved via support and collaboration as opposed to being involved through control (e.g., overprotection, protective buffering) or not being involved. The same general pattern of results seems to hold when one examines how the spouse perceives his or her own involvement and spousal adjustment. However, what is missing from the literature is a dyadic perspective of how the appraisal, coping, and adjustment of patient and spouse occur in relation to each other, especially over time.

A sizable literature has accumulated on a variety of forms of dyadic coping, primarily exploring their association with patient adjustment and, less frequently, spousal adjustment. The literature includes a wide array of chronic illness conditions (myocardial infarction, arthritis, cancer, diabetes, and pain), with patients of different adult ages who are at varying places in dealing with the chronic illness (e.g., diagnosis, treatment, management). Great diversity exists in the outcomes that are associated with particular types of dyadic coping, with frequent use of psychosocial outcomes (e.g., depression, self-efficacy, positive coping behaviors) and relational outcomes (e.g., marital satisfaction) and infrequent use of health outcomes (e.g., rehospitalization after surgery, pain severity). This diversity in the developmental life course, illnesses, and outcomes associated with dyadic coping makes it challenging to understand when spousal involvement is beneficial or harmful to both patient and spousal adjustment. The literature is in need of

Table 2
Coping Studies Involving Dyadic Coping

Author	Sample	Coping measure	Outcome measure	Source of perception	Significant findings
Badr (2004)	90 healthy couples and 92 couples in which one spouse was ill (multiple illnesses included) (*M* age = 42.24 for wives, 45.21 for husbands)	Brief COPE (Carver, 1997); Relationship-Focused Coping Scale (Coyne & Smith, 1991)	Dyadic Adjustment Scale (DAS; Spanier, 1976), combined husband and wife measure	Husbands and wives reported on their own coping efforts	Couple patterns were found such that couples who were congruent in their use of active engagement and complementary in their use of protective buffering and avoidance coping had better marital adjustment. Wives were less likely to engage in collaboration when ill than when well; men were more likely when ill.
Bediako & Friend (2004)	39 female patients with RA and their spouses (*M* age = 46.9 for patients, 48 for spouses)	16-item Patient Expectations Scale (developed for this study) to assess perceptions of expectations from spouses; Spouse version of the Patient Expectation Scale to assess accuracy of patient perceptions	Beck Depression Inventory (BDI; Beck et al., 1961)	Patients reported their perception of expectations from the spouse; spouses reported their own expectations	Patients' perceived inability to meet the expectation of their spouse predicted greater depressive symptoms (when disease severity and social support were controlled for).
Berg et al. (2007)	59 men with prostate cancer and their wives (*M* age = 68 for men, 65 for women)	Diary measure of daily coping and categorizations of spouse's type of involvement (uninvolved, supportive, collaborative, controlling)	Positive and Negative Affect Schedule (PANAS; Watson et al., 1988)	Patients' and wives' views of how spouse was involved in coping	Daily collaborative coping was associated with more positive mood for both men and women, with less negative mood for wives only. More negative emotional transmission occurred between husbands and wives the more frequently collaborative coping was used across a 14-day period.
Bermas et al. (2000)	79 patients with RA and 78 spouses (*M* age = 56.5 for patients, 57.6 for spouses)	Revenson's revision of the Ways of Coping Checklist	Kansas Marital Satisfaction Scale (Schumm et al., 1985)	Patients and spouses reported on their own coping efforts	Lower marital satisfaction among patients was associated with patient's use of escape into fantasy, finding blame, and spouse's use of escape into fantasy. Lower marital satisfaction among spouses was associated with spouse's use of passive acceptance and less frequent use of finding blame.
Butler et al. (1999)	125 female patients with breast cancer (*M* age = 53)	Items drawn from the Yale Social Support Index (Seeman & Berkman, 1988) to create three subscales to measure quality and quantity of emotional support	Life Events Questionnaire (Horowitz et al., 1977) intrusion and avoidance	Patients rated their own social support	Women who perceived themselves as having more aversive emotional support experienced more intrusion and avoidance symptoms related to their cancer.
Cano et al. (2000)	165 married chronic pain patients (*M* age = 48.59)	Multidimensional Pain Inventory (Kerns et al., 1985)	BDI; Marital Adjustment Test (Locke & Wallace, 1959)	Patients only	Greater perceived negative spouse responses to pain were associated with increased severity of pain and lower marital satisfaction, which resulted in increased depression.

Table 2 (*continued*)

Author	Sample	Coping measure	Outcome measure	Source of perception	Significant findings
S. L. Clark & Stephens (1996)	55 stroke patients (*M* age = 69)	Ratings of perceptions of self and spouse in the context of spouse's helpful and unhelpful actions	Center for Epidemiologic Studies Depression Scale (CES-D; Radloff, 1977)	Patients only	Spouse's unhelpful actions were associated with depression, helpful actions with positive affect.
Coyne & Anderson (1999)	211 women with history of cancer, 253 women without, undergoing genetic testing for breast cancer (*M* age = 48.5)	Inventory of emotionally supportive and unsupportive behaviors from spouse; cancer-specific social support processes	Hopkins Symptom Checklist (Hough et al., 1982)	Patients only	Frequency of unsupportive emotional behaviors from spouse was related to more distress; supportive behaviors were not related to distress.
Coyne & Smith (1991)	56 men post-MI and their wives (*M* age = 57.1 for men and 53.7 for wives)	Ratings of items reflecting active engagement and protective buffering	25-item version of the Hopkins Symptom Checklist (Derogatis et al., 1974)	Patients and wives reported on their own coping efforts	Protective buffering related to both self-distress ($r = .62$ for wives, $r = .39$ for husbands) and spousal distress ($r = .69$ for wife buffering to husband distress, $r = .44$ for husband buffering to wife distress). Only wives' use of active engagement related to both wife distress ($r = .30$) and husband distress ($r = .42$).
Coyne & Smith (1994)	Same sample as Coyne & Smith (1991)	Coyne & Smith (1991) items reflecting active engagement, protective buffering, and overprotection	Items reflecting patients' ability to deal with tasks of recovery; items reflecting wives' confidence that they could meet the personal challenges of MI	Patients and wives reported on their own coping efforts	Husbands' use of active engagement related to their higher self-efficacy ($\beta = .27$); use of protective buffering related to lower self-efficacy ($\beta = -.38$). Wives' use of protective buffering related to husbands' greater self-efficacy ($\beta = .48$); use of overprotectiveness related to husbands' lower self-efficacy ($\beta = -.25$).
Cranford (2004)	181 healthy married individuals (*M* age = 45.5)	Social Undermining Scale	BDI	One partner only	Spouse undermining at Time 1 predicted increases in depression from Time 1 to Time 2. Association between perceived stress at Time 1 and depression at Time 2 was moderated by spouse undermining at Time 1.
Dehle et al. (2001)	212 married individuals not experiencing chronic illness (*M* age = 28.4 for men, 28.3 for women)	Support in Intimate Relationships Scale (constructed for this study)	Kansas Marital Satisfaction Scale; Positive and Negative Quality in Marriage Scale (Fincham & Linfield, 1997); DAS; Perceived Stress Scale (Cohen et al., 1983); BDI	Each partner estimated the spouse's support provision	After social desirability was controlled for, perceived adequacy of social support provided by a spouse was associated with marital quality, symptoms of depression, and perceived stress.

(*table continues*)

Table 2 (*continued*)

Author	Sample	Coping measure	Outcome measure	Source of perception	Significant findings
Druley & Townsend (1998)	90 individuals with arthritis and 90 healthy controls (37 men and 53 women in each group; *M* age = 38)	Items reflective of positive interactions (e.g., willing to listen; love and affection) and negative interactions (e.g., makes too many demands, is critical of you) with spouse	Items reflecting self-esteem; CES-D	Patients only	Positive interactions with spouse related to higher self-esteem ($r = .28$) for arthritis patients only and less depressive symptoms for arthritis patients ($r = -.35$) and healthy controls ($r = -.36$). Negative interactions with spouse related to lower self-esteem (−.38) for arthritis patients only and more depressive symptoms for both arthritis patients (.33) and healthy controls ($r = .24$). For the arthritis group, self-esteem mediated the association between negative marital interactions and depression.
Druley et al. (1997)	74 women with lupus and their partners (*M* age = 43 for women, 46 for partners)	Items reflective of emotional disclosure to spouse and withholding disclosure (similar to protective buffering)	PANAS	Patients reported on their disclosure to partner	Greater reports of emotional disclosure and withholding emotional disclosure were related to more negative affect. Emotional disclosure was not associated with positive affect.
Druley et al. (2003)	Same sample as Martire et al. (2002)	Patient's pain behavior	CES-D; State-Trait Anger Expression Inventory (Forgays et al., 1997)	Patients only	When wives engaged in high levels of pain behavior, wives' depressive symptoms were positively associated with husbands' depression and anger. When wives engaged in fewer pain behaviors, their depressive symptoms were unrelated to husbands' depression and anger.
Fang et al. (2001)	197 cancer patients and their spouses (*M* age = 56 for patients, 55 for spouses)	DAS	Psychological Distress subscale of the Mental Health Inventory (MHI; Veit & Ware, 1983)	Spouses completed the DAS	Spouse perception of marital quality mediated the association between patient's physical impairment and spouse's psychological distress at all three time points. Patient distress mediated this association at only one time point.
Feldman & Broussard (2006)	71 male partners of breast cancer patients (*M* age = 51)	Dyadic Coping Scale (Bodenmann, 1997)	Quality of Life Spouses Scale (Ebbesen et al., 1990)	Partners only	Hostile dyadic coping was associated with greater illness intrusiveness.
Figueiredo et al. (2004)	66 early stage breast cancer patients (*M* age = 55.2)	Items reflective of emotional disclosure to spouse and withholding disclosure (Pistrang & Barker, 1992, 1995)	RAND 36-item Health Survey (Hays et al., 1993) to measure physical and psychological well-being; Unsupportive Social Interactions Inventory (Ingram et al., 2001); Social Support Questionnaire (Sarason et al., 1987)	Patients reported on their disclosures to partner	Failure to disclose was negatively related to emotional well-being and social support and positively related to receiving unsupportive responses from other people. Most of the unsupportive behaviors reported by patients were either minimizing or distancing.

Table 2 (*continued*)

Author	Sample	Coping measure	Outcome measure	Source of perception	Significant findings
Franks et al. (2006)	94 couples with one spouse experiencing MI (81% male patients; *M* age = 64.5 for men, 61.9 for women).	Measures of health-related support and health-related control at Time 1	Health behaviors promoting cardiac health and psychological adjustment (Mental Health scale of the Short Form-36) 6 months later	Partners reported on health-related support and control; patients reported on health outcomes	Spouses' support predicted prospective increases in psychological adjustment; spouses' control predicted prospective decreases in health behaviors and psychological adjustment.
Grant et al. (2002)	88 married women with chronic low back pain	Multidimensional Pain Inventory	Patients were asked to rate their pain intensity; State-Trait Personality Inventory (Spielberger, 1979)	Patients only	Patient's perceptions of spouse's distracting responses were associated with increases in patient anxiety; patient's perceptions of spouse's punishing responses were associated with increases in patient's pain.
Hagedoorn, Kuijer, et al. (2000)	68 cancer patients (range of cancers) and intimate partners (32 male, 36 female; *M* age = 53, diagnosed with cancer on average 2.8 years ago)	Modified items of Coyne & Smith (1991); ratings of items reflecting active engagement, protective buffering, and overprotection	Marital Quality subscale of the Maudsley Marital Questionnaire (Arrindell et al., 1983); items reflecting give-and-take in the marital relationship	Patients reported on partners' behavior; partners reported on their own behavior	Patient and partner use of active engagement related to greater patient report of marital satisfaction (*rs* = .59 and .33, respectively); protective buffering related to poorer marital satisfaction (*rs* = −.32 and −.33); overprotection related to more patient negative feelings about the relationship (*rs* = .30 and .41). Active engagement related to better marital satisfaction when patients reported high psychological distress, particularly for female patients; protective buffering was associated with lower marital quality more so for patients experiencing high levels of distress and high physical impairments.
Hagedoorn et al. (2002)	Same sample as Hagedoorn, Kuijer, et al. (2000)	Items reflecting partner's self-efficacy in providing support; items for supportive and unsupportive behavior	CES-D	Partners judged their own self-efficacy in providing support; patients rated partners' supportive/ unsupportive behavior	Feelings of insecurity and incompetence in providing support to patients were associated with their own distress in female caregivers only.
Hagedoorn et al. (2006)	67 insulin-treated patients and their partners (32 female; *M* age = 45.4)	Overprotection items used by Hagedoorn, Kuijer, et al. (2000)	Changes in internal locus of control, diabetes-related stress, and glycemic control	Patients only	Greater (compared with less) perceived overprotection was associated with less decline in diabetes-related stress, less decrease in HbA1c, and less increase in internal locus of control over a 3-month education program.
Helgeson (1991)	90 post-MI patients (70 male, 20 female; *Mdn* age = 59.5)	Disclosure to spouse	Rehospitalization and/or death, post-MI chest pain, and perceived health	Patients only	Lack of disclosure to spouse predicted worse recovery.

(*table continues*)

Table 2 (*continued*)

Author	Sample	Coping measure	Outcome measure	Source of perception	Significant findings
Helgeson & Lepore (1997)	162 male prostate cancer patients	Agency and Unmitigated Agency scales of the Extended Version of the Personal Attributes Questionnaire (Spence et al., 1979)	Scale developed for this study to measure emotional expressiveness; Cancer Rehabilitation Evaluation System (CARES; Schag & Heinrich, 1989); Health Status Questionnaire (Stewart et al., 1988)	Patients only	Unmitigated agency was related to worse functioning, more cancer-related difficulties, and difficulty expressing emotions. Agency was related to better functioning, fewer cancer-related difficulties, and ability to express emotions. The relationship between unmitigated agency and adjustment to cancer was mediated by emotional expression.
Helgeson, Novak, et al. (2004)	80 male prostate cancer patients, 52 wives	Perceived spousal control to engage in eight health behaviors	Items reflecting health behaviors; a measure of control beliefs (Lepore & Helgeson, 1998); CES-D	Patients only	Spousal control was not associated with positive changes in health behavior, and for some types of health behaviors it was associated with poorer health behaviors. Spousal control was associated with greater psychosocial distress and less personal control over time.
Kayser et al. (1999)	49 female cancer patients (*M* age = 36)	Mutual Psychological Development Questionnaire (Genero et al., 1992) to assess mutuality; Silencing the Self Scale (Jack & Dill, 1992; rating of items reflecting active engagement and protective buffering (Coyne & Smith, 1991)	Quality of life as assessed by the Functional Assessment of Cancer Therapy Scale (Cella et al., 1993); BDI; Self-Care Agency Scale (Kearney & Fleischer, 1979)	Patients only	Patients who perceived their relationship to be highly mutual reported better quality of life and self-care agency and lower depression. Patients who reported fewer self-silencing beliefs had better self-care agency. Protective buffering was related to increased depression and lower levels of self-care agency.
Kuijer et al. (2000)	106 cancer patients (range of cancers) and their partners (68% male; *M* age = 59, range = 33–83; *M* length of diagnosis = 5 years)	Modified items of Coyne & Smith (1991) reflecting active engagement, protective buffering, and overprotection	CES-D; Mastery Scale to assess patient's feelings of control (Pearlin & Schooler, 1978)	Patients reported on partners' behavior; partners reported on their own behavior	Patients and partners generally agreed in their perceptions of providing support. Protective buffering and overprotection were highly related ($r = .53$ for patient and $r = .43$ for partner perceptions). Partners reported more active engagement when patient's condition was more serious and when patients were younger and female. Female patients reported more active engagement; older patients experienced more protective buffering and overprotection. Partner self-efficacy was related to greater use of active engagement ($r = .31$) and less use of protective buffering ($r = -.47$) and overprotection ($r = -.23$). Patients reported more depression and less control when they perceived their partners as engaging in more protective buffering (rs = .22 and $-.29$) and overprotection (rs = .33 and $-.45$). Patients who reported more active engagement also reported greater relationship improvement ($r = .51$).

Table 2 (*continued*)

Author	Sample	Coping measure	Outcome measure	Source of perception	Significant findings
Manne (1999)	129 married cancer patients and their spouses (*M* age = 54 for patients and spouses)	Perceived Negative Spouse Behaviors Scale (PNSBS; Manne et al., 1997); Impact of Events Scale (IES) Intrusive Thoughts subscale	Psychological Distress subscale of the MHI	Patients reported on spouses' negative behavior	The relationship between intrusive thoughts and psychological distress was mediated by spouse criticism.
Manne, Alfieri, et al. (1999)	219 cancer patients and their spouses (*M* age = 57 for patients, 56 for spouses)	PNSBS	Negative affect (Watson et al., 1988)	Patient perceptions of spouse	The association between patient's greater functional impairment and spouse's negative behaviors was mediated by greater negative mood for spouse and more restriction in activities of the spouse.
Manne & Glassman (2000)	191 married patients with cancer (*M* age = 56, range = 29–77)	PNSBS; IES Avoidant subscale	Anxiety and Depression subscales of the MHI	Patients reported on spouses' negative behavior; patients reported on their own avoidance	The negative relationship between unsupportive behavior from spouse and patient's psychological distress was mediated by the patient's coping efficacy and the patient's engagement in avoidance coping.
Manne, Ostroff, Rini, et al. (2004)	98 women with breast cancer and their significant others (spouses or live-in partners) (*M* age = 49 for patients, 52 for partners); drawn from Manne, Ostroff, Sherman, et al. (2004)	Items reflective of emotional disclosure to spouse and perceived partner responsiveness	Items reflective of feelings of intimacy	Both patients and partners rated self-disclosure, perceived partner disclosure, and perceived partner responsiveness	Structural equation modeling revealed that for partners, the relationships between self-disclosure and intimacy were mediated by perceived partner responsiveness. For patients, the relationship between perceived partner disclosure and intimacy was partially mediated by perceived partner responsiveness. However, self-disclosure was not associated with responsiveness or intimacy.
Manne, Ostroff, Sherman, et al. (2004)	148 couples with one spouse with breast cancer (*M* age = 50 for patients, 51 for partners)	Rapid Marital Interaction Coding System (Heyman & Vivian, 1997)	Hopkins Symptom Checklist (Hesbacher et al., 1978)	Coders rated patients' and spouses' interactions	Patients were less distressed when spouses responded to self-disclosures with reciprocal self-disclosure and humor. Patients were more distressed when spouses responded to self-disclosures by posing solutions.
Manne et al. (2006)	147 breast cancer patients (*M* age = 50.6) and 127 partners (*M* age = 52.7); drawn from Manne, Ostroff, Winkel, Grana, & Fox (2005)	Communication Patterns Questionnaire (Christensen, 1988) examining mutual constructive communication, mutual avoidance, and demand-withdraw	Psychological Distress subscale of the MHI-18 (Ware et al., 1984); DAS, physical functioning on the CARES both concurrently and 9 months later	Patients and spouses rated communication and distress and completed the DAS; patients only completed the CARES	Low to moderate relations were found between patient and spouse communication patterns (*r*s ranged from .37 to .51). Mutual constructive communication reported by patient was associated with less patient and partner distress and avoidance. Demand–withdrawal communication was associated with higher distress and lower relationship satisfaction for both patient and partner. Partner reports of communication were related only to partner distress.

(*table continues*)

Table 2 (*continued*)

Author	Sample	Coping measure	Outcome measure	Source of perception	Significant findings
Manne, Ostroff, Winkel, Grana, & Fox (2005)	219 women with breast cancer and their significant others (*M* age = 49.83 for patients)	PNSBS; IES Avoidant subscale	Psychological Distress subscale of the MHI-18	Patients reported on spouses' negative behavior; spouses reported on patients' unsupportive behavior; patients reported on their own avoidance	Patient's perception of spouse's unsupportive behavior mediated the negative effect of partner's report of unsupportive behavior on distress (partner's perception of unsupportive behavior was no longer significant). Replicated Manne & Glassman (2000).
Manne, Pape, et al. (1999)	221 patients receiving treatment for advanced cancer (113 male; *M* age = 55); same sample as Manne, Alfieri, et al. (1999)	Perceived spouse support; perceived negative spouse behaviors	PANAS	Patients only	Structural equation modeling revealed that spousal support was associated with positive mood indirectly through greater use of positively focused coping. Spousal criticism was associated with negative mood indirectly through greater use of escape-avoidance coping.
Manne & Schnoll (2001)	304 married cancer patients undergoing treatment (*M* age = 57)	Partner Responses to Cancer Inventory	No outcome	Patients only	Exploratory factor analysis revealed four factors of the Partner Responses to Cancer Inventory. Emotional and Instrumental Support, Cognitive Information and Guidance, Encouraging Distancing and Self-Restraint, and Criticism and Withdrawal.
Manne & Zautra (1989)	103 women with RA and their spouses (*M* age = 55)	Rated items reflecting instrumental and appraisal support-related behaviors; spouse's critical remarks in an interview were also counted	Ways of Coping Checklist (Felton & Revenson, 1984); MHI; Activities of Daily Living (Fries et al., 1980)	Patients only reported on support behaviors	Perceptions of husband support were related to higher use of adaptive coping (r = .43 for cognitive restructuring) and less negative adjustment (r = −.25). Husband's critical remarks were related to higher use of maladaptive coping (r = .36 for wishful thinking), more negative adjustment (r = .29), and more activity limitations (r = .34).
Manne & Zautra (1990)	Same sample as Manne & Zautra (1989)	Wife's perceptions of positive and negative interaction with her husband; spouse's critical remarks in an interview were also counted	MHI	Patients only reported on marital interaction	Husband's adjustment was lower when wife perceived that their interaction was more negative and the husband was more critical in an interview; positive responses were not significantly related to husband's adjustment. Wife's adjustment was lower when husband was more critical in the interview.
Martire et al. (2003)	91 married care recipients with disabilities primarily related to arthritis, stroke, and heart disease (*M* age = 72.8)	Items reflective of the patient's perception of the quality of care received from spouse	CES-D; Pearlin & Schooler (1978) measure of global mastery	Patients only	Patient's perception of poor quality of care by spouse was associated with increased depression in patients and decreased mastery 1 year later.

Table 2 (*continued*)

Author	Sample	Coping measure	Outcome measure	Source of perception	Significant findings
Martire et al. (2002)	101 women with osteoarthritis (*M* age = 69)	Rated items indicative of two dimensions of negative reactions of spousal support (perceived incompetence and perceived powerlessness)	CES-D; items reflecting negative reactions to spousal interactions	Patients only	Greater levels of spousal support were related to fewer negative reactions for recipients who placed less importance on functional independence. Greater perceived incompetence as a result of the husband's instrumental support was related to more concurrent depression. Greater perceptions of incompetence were related to increased depression over time.
Newsom & Schulz (1998)	288 individuals with physical impairments and their spouses (52% female; *M* age = 77)	Patient-reported amount of mental or emotional strain experienced in receiving assistance from spouse in instrumental activities of daily living (IADLs)	Caregiver Health Effects Study interview; CES-D	Patients only	Helping distress was more frequent when patients reported more impairment with IADLs. The amount of received help exacerbated the detrimental effects of lower self-esteem, fatalistic beliefs, and martial conflict on negative helping. Helping distress predicted depression 1 year subsequently.
Norton et al. (2005)	143 women with ovarian cancer (*M* age = 55)	Family and friends version of the Perceived Negative Behaviors Scale (Manne & Glassman, 2000)	Psychological Distress subscale of the MHI-18	Patients only	Self-esteem mediated the relationship between perceived unsupportive behaviors from family and friends and patient's psychological distress.
Rohrbaugh et al. (2004)	191 congestive heart failure patients and their spouses (*M* age = 53 for patients, 52 for spouses)	Items to assess efficacy expectations based on respondents' rating of their confidence that the patient could meet challenges in managing illness	New York Heart Association function class (predictor of mortality)	Patient and spouse rated the patient's ability to meet challenges	Patient self-efficacy and spouse confidence predicted patient survival, but only spouse confidence remained significant when both partners' efficacy ratings were taken into account.
Schiaffino & Revenson (1995)	64 patients with RA within 2 years of diagnosis (75% female; *M* age = 53)	Participants recalled a specific pain episode and rated how often their spouse provided different types of positive (e.g., emotional, instrumental, or informational) or negative support (not perceived as helpful)	CES-D	Patients only	No simple effect of positive or negative spousal support on psychosocial outcomes was found. Moderation effects occurred such that depression increased across an 18-month period when challenge appraisals were accompanied by high amounts of positive support. Results were interpreted as evidence that some positive emotional support may be perceived by patients as miscarried helping.

(*table continues*)

Table 2 (*continued*)

Author	Sample	Coping measure	Outcome measure	Source of perception	Significant findings
U. Schulz & Schwarzer (2004)	277 patients coping with cancer surgery for a malignant tumor and their partners	Perceptions of how much instrumental, emotional, and informational support was received	Berlin Social Support Scales (Schwarzer & Schulz, 2000)	Patients reported on support received, partners reported on support provided	Significant relations were found between patient received support and partner provided support (rs = .31–.41). Social support was related to positive features of coping behavior 5 months later, with these effects much more pronounced for female than for male patients, even though male patients reported receiving greater amounts of social support than females.
Smith et al. (2004)	Patients with osteoarthritis and their spouses (M age = 62.69 for men, 59.65 for women)	Behavioral coding system based on Romano et al. (1991)	No outcome	Coding of patient and spouse behavior	Spouse facilitative behavior preceded and followed patient pain behavior more frequently than spouse solicitous behavior. Wives were more likely to show facilitative behavior than husbands.
Stephens et al. (2006)	Same sample as Martire et al. (2002)	Items reflecting pain disclosure, pain behavior, husband's emotional support (adapted from Stephens & Clark, 1996), and husband's critical attitudes	CES-D; items assessing husband's life satisfaction	Patients reported on pain disclosure and how often husbands engaged in emotional support; husbands reported on their wives' pain behavior and their own critical attitudes	Wives' expression of pain moderated the relationships between wives' pain and husbands' well-being and between wives' pain and emotional support from husbands.
Suls et al. (1997)	43 male MI survivors and their spouses (M age = 59)	Modified items by Coyne & Smith (1991) examining protective buffering	25-item version of the Hopkins Symptom Checklist	Patients and wives reported on their own coping efforts	Protective buffering was associated with greater distress in patients and spouses at both 4 weeks (r = .57 for patients; r = .57 for spouses) and 6 months (r =.69 for patients; r = .75 for spouses) postdischarge; however, use of protective buffering was not related to spouse's distress. Greater use of protective buffering by the patient at 4 weeks predicted increases in distress at 6 months (b = .78); similar effects were reported for wife distress (b = .53).
Von Dras et al. (2000)	124 male patients undergoing catheterization to detect coronary artery disease (M age = 58.94 for patients, 56.23 for spouses)	Patient's perceived social support assessed by the Interpersonal Support Evaluation List (Cohen & Hoberman, 1983); four questions reflecting spouse's perceived adequacy and desire for social support	Perceived social support	Patients and spouses reported on their own social support	Characteristics of the patient and spouse (age, mental health, social functioning, hostility, depression, perceived health) moderated their perceptions of social support.

Table 2 (*continued*)

Author	Sample	Coping measure	Outcome measure	Source of perception	Significant findings
Wright & Aquilino (1998)	129 caregiving and 119 noncaregiving wives (*M* age = 69.98 for caregiving, 68.36 for noncaregiving)	Emotional support exchange, measured with the convoy model (Antonucci & Akiyama, 1987)	Zarit Burden Scale (Zarit et al., 1980); marital satisfaction measured by a single item	Spouses only	Among caregiving wives, reciprocity of support was related to lower caregiving burden and higher marital satisfaction.

Note. RA = rheumatoid arthritis; MI = myocardial infarction.

a framework that explicates how dyadic coping may vary across the adult life span and across different contexts that couples find themselves adapting to, especially regarding the constraints of different illnesses.

In this article, we present a developmental–contextual model for studying dyadic appraisal and coping in couples that emphasizes life-span developmental and temporal processes as couples come to stressors surrounding chronic illness from different contexts (e.g., culture, gender, quality of relationship, and context of specific illnesses). This model draws on the seminal work of Reiss (1981), Revenson (1990, 1994, 2003, Bodenmann (1997, 2005), Coyne and colleagues (Coyne & Fiske, 1992; Coyne & Smith, 1991), and Lyons, Mickelson, Sullivan, and Coyne (1998), which views stress as potentially occurring in an interdependent manner in which couples may deal with stressors that arise as they deal with chronic illness. This model views dyadic coping as potentially a first line of coping for couples as they deal with stressful events, in contrast to Bodenmann (2005), who argues that individuals engage in dyadic coping when individual coping efforts have been exhausted. The developmental–contextual model pushes the dyadic coping literature beyond the individualistic constructs of coping derived from the tremendous influence of Lazarus and Folkman (1984) to a more dyadic level of analysis. In this article, we apply this model to the context of couples dealing with chronic illness; however, a dyadic approach to coping can be adopted for any stressful event (see Revenson et al., 2005, for examples) and easily extends to other social units (e.g., children, extended family members, and friends).

Before we begin, we acknowledge the scope of our review of the dyadic coping literature. First, we focus in this article on developmental and contextual factors relevant to dyadic appraisal and coping processes in chronic illness, and thus, to be included, studies must have used some measure of coping (measures of social support provided from the spouse are included here). Second, we limit the review to chronic illnesses that involve a physical disorder (rather than mental disorder, although Alzheimer's disease was included) to be consistent with the vast majority of the literature on dyadic coping in chronic illness. Although dyadic processes may be fruitfully applied to mental disorders (see Bodenmann, Widmer, Charvoz, & Bradbury, 2004), especially as these disorders may have a greater impact on marital life than physical illness (Bouras, Vanger, & Bridges, 1986), mental illness is beyond the scope of the current literature review. Third, we focus the review on psychosocial outcomes rather than physical health outcomes, as the vast majority of the dyadic coping literature does not include physical health outcomes (see Future Directions and Implications section for comments on health outcomes). Fourth, consistent with the literature on intimate relationships, we focus on heterosexual married couples (Danoff-Burg & Revenson, 2000; Revenson et al., 2005). Other couple combinations (gay and lesbian couples, cohabitating individuals) are not represented in the literature with sufficient frequency to allow firm conclusions or generalizations, and the focus of studies with gay couples is dominated by one particular illness, AIDS (Billings, Folkman, Acree, & Moskowitz, 2000; Park, Folkman, & Bostrom, 2001). Fifth, although we were interested in examining chronic illness in couples across the adult life span, the literature is heavily weighted toward examining chronic illness in middle adulthood and old age, consistent with the greater frequency of chronic illness in late life (Siegler, Bosworth, & Poon, 2003). Finally, we restrict our review to studies in which measures of social support were specific to the support received from one's spouse. Studies in which social support was assessed more broadly (e.g., total amount of support received from one's network or family support in general) were not included and constitute a large literature (e.g., Connell, Davis, Gallant, & Sharpe, 1994; Gallant, 2003; L. Hatchett, Friend, Symister, & Wadhwa, 1997; Helgeson & Cohen, 1996; Holahan, Moos, Holahan, & Brennan, 1997; King, Reis, Porter, & Norsen, 1993; Penninx et al., 1998).

To explore couples coping with chronic illness, we conducted literature searches through PsycINFO and supplemented them with the ancestry approach. We focused our search on the years between 1992 and 2006, as the early 1990s marked the appearance of several seminal articles introducing the notion of dyadic coping (Coyne & Fiske, 1992; Lyons et al., 1995; O'Brien & DeLongis, 1997; Revenson, 1994, among others). The following search terms were used in various combinations: *dyadic coping*, *chronic illness*, *spouse*, *marriage*, *social support*, *couple*, and *unsupportive behaviors*. More specific disease-related search terms were also used in combination with *marriage*, *coping*, or *spouse*, including *Alzheimer's disease*, *pain*, *fibromyalgia*, *cancer*, and so forth. Further, more specific searches were conducted to examine the effect of culture, development, temporal process, types of chronic illnesses, marital relationships, and gender on dyadic coping. Articles selected were restricted to those appearing in peer-reviewed English-language journals, using adult populations age 18 or older. When separate studies used the same sample of participants, we note this fact and include each study only if it examined a different facet of dyadic coping (e.g., self-report vs. behavioral observation, spousal reports vs. patient reports).

The Developmental–Contextual Model

We provide first an overview of the components of the developmental–contextual model (see Figure 1) and then a liter-

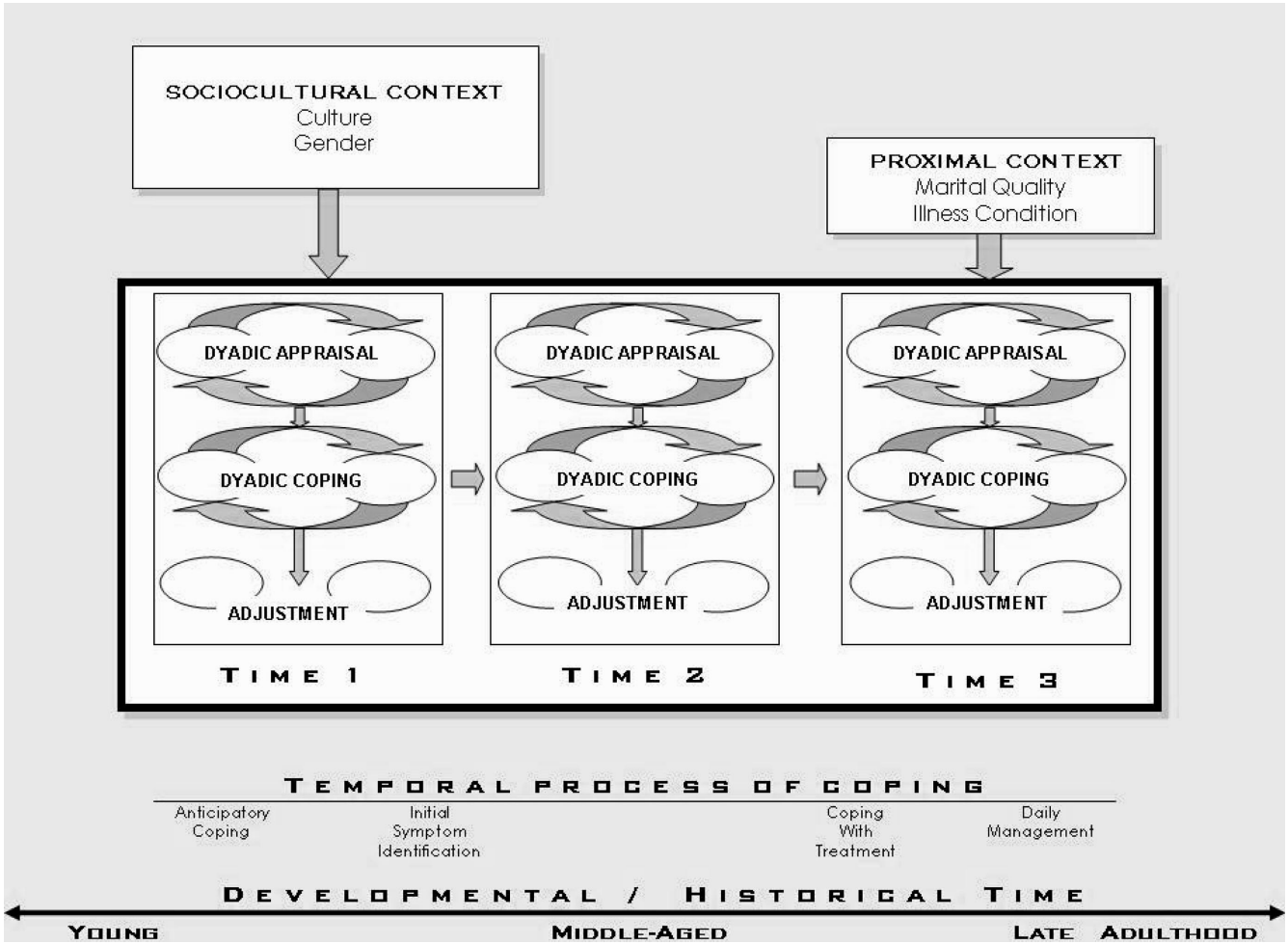

Figure 1. A developmental–contextual model of couples coping with chronic illness across the adult life span.

ature review organized by the model. The model is inherently a developmental model that emphasizes that dyadic coping may be different across the life span, during specific historical times, and during different stages of dealing with the illness (see also Revenson, 1990, 2003), as well as unfolding daily as spouses interact around dyadic stressors. The process of appraisal and adjustment may be different across adult development as couples experience normative developmental changes in self-development, emotion regulation, and marital processes that may vary with history-graded events. In addition, appraisal and coping efforts occur over time as couples move through the process of initial symptom identification, coping with treatment, and daily management of the disease.

Our model views chronic illness as affecting not only the patient but also the spouse, thereby requiring assessments of adjustment, coping, and perceptions of the spouse's involvement from both the patient and the spouse. Most of the literature thus far has been individually based, treating the patient as the focal person and examining how the spouse is involved in the patient's stressful events and how this involvement relates to the patient's adjustment (e.g., depression, marital satisfaction). (Note these relations are depicted in the links between patient coping and patient adjustment on the left side of Figure 2.) Consistent with a social contextual perspective (Rogoff, 1998; Vygotsky, 1978), our model views the dyad as the unit of examination (depicted by the circular arrows in Figure 2), such that coping strategies enacted by the patient are viewed in relation to those enacted by the spouse, and vice versa. This examination of spouses in relation to each other occurs with regard to dyadic coping, appraisal processes, and adjustment.

In our model we integrate the different categorizations currently used in the literature (e.g., supportive coping, common dyadic coping, active engagement, overprotection, protected buffering) by conceptualizing dyadic coping along a continuum of involvement ranging from uninvolvement of the spouse (patient perceives that he or she is coping individually) to overinvolvement of the spouse (e.g., patient perceives the spouse as controlling, engaging in miscarried helping). Berg, Meegan, and Deviney (1998) outlined four broad categories of ways in which individuals may perceive others to be involved in their own coping efforts: uninvolvement (person perceives that he or she is coping individually with the stressful event), support (spouse provides emotional and/or instrumental support), collaboration (spouse is more actively involved through joint problem solving), or control (spouse dominates the

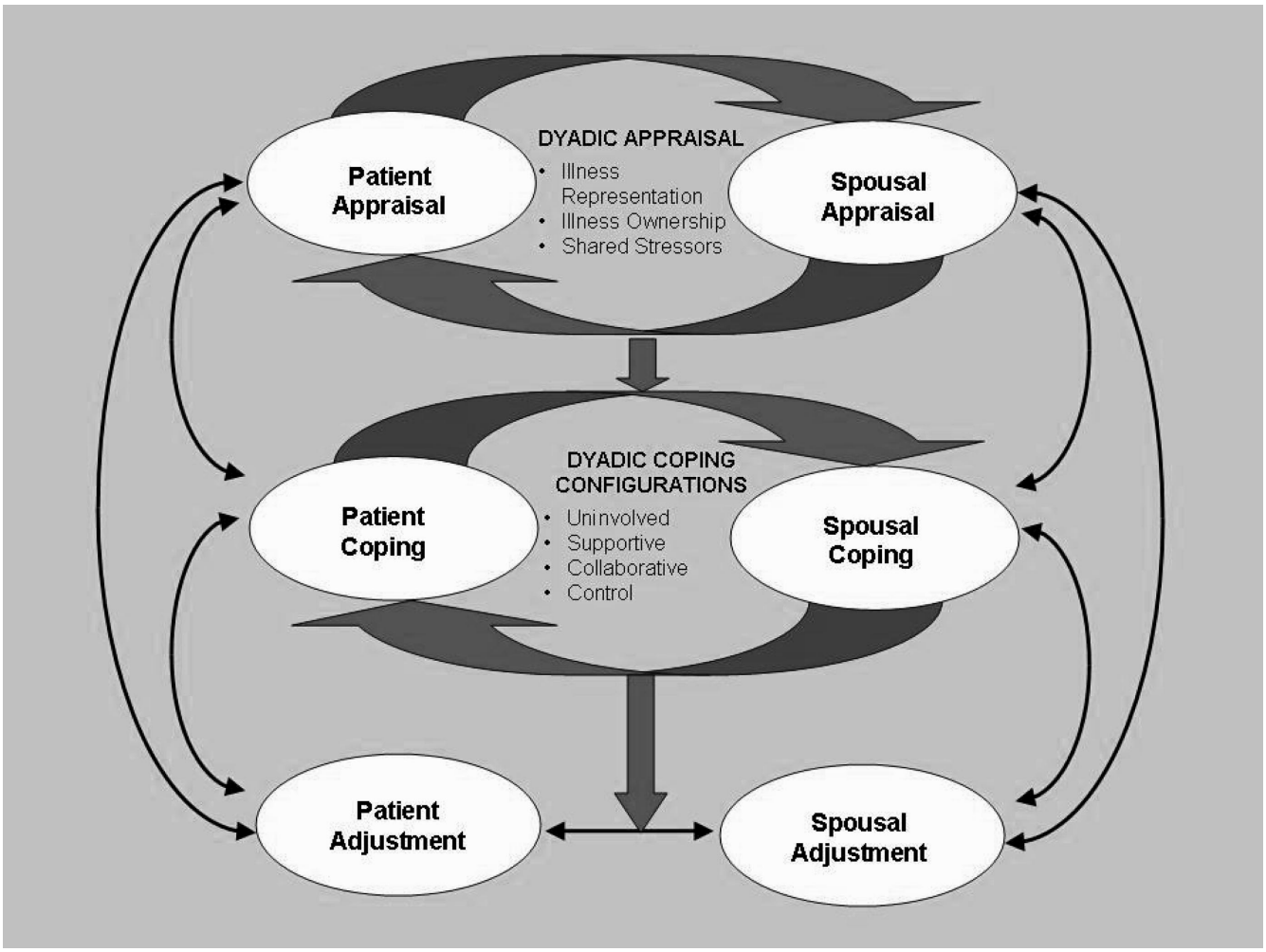

Figure 2. Dyadic appraisal, coping, and adjustment in couples.

actions of the other spouse by taking charge and telling the other person what to do).

Our approach to dyadic coping examines the dyadic coping strategies of both patient and spouse in relation, as they are mutually involved in each other's stressors. Examining the marital dyad as a unit allows for the identification of dyadic configurations of coping (e.g., invisible support, in which the patient views the spouse as uninvolved but the spouse reports providing support; see Bolger, Zuckerman, & Kessler, 2000). Dyadic coping and adjustment are part of a transactional process that unfolds over time such that multiple directions of influence are involved (not only does patient and spouse dyadic coping affect patient and spouse adjustment, but adjustment may subsequently affect future dyadic coping efforts). Further, dyadic coping is examined in the context of other appraisals, such as the similarity in spouse's illness representations (controllability, consequences), illness ownership (whose illness is it?), and shared stressor appraisals for specific stressful events (stressful event is the patient's vs. shared).

The existing literature suggests that these general patterns of relationships between dyadic coping strategies and adjustment may be moderated by a host of variables. Couples engaged in dyadic coping are affected by the larger sociocultural context (e.g., culture and gender; see also Revenson, 1990, 2003) as well as the proximal context (e.g., quality of the marital relationship and the specific chronic illness). Sociocultural factors affect the norms and expectations for the level of interdependence among spouses (Cross & Madson, 1997; Triandis, 2001), with collectivistic cultures and females more likely to represent the self in relation to others. Couples also draw on the quality of the marital relationship to engage in effective dyadic coping (Coyne & Smith, 1991; Hagedoorn, Kuijer, et al., 2000). Finally, the dyadic process of appraisal and coping may be affected by the specific chronic illness the couple faces. Chronic illnesses differ widely in their timeline, consequences, and controllability, and spouses may represent the same illness in similar or different ways (Weinman, Heijmans, & Figueiras, 2003), representations that affect forms of dyadic coping. These factors affect how couples come to appraise the stressors they face and the dyadic coping strategies they enact. Further, given the transactional nature of the model, many of these factors not only affect dyadic coping but are affected by dyadic coping. For instance, marital satisfaction can increase the likelihood of dyadic appraisal and coping and be further enhanced by such coping processes.

The model provides a framework for understanding how couples coping with chronic illness may together appraise and cope with illness during adulthood and for determining when spousal involvement is beneficial or harmful to both patient and spousal adjustment. We begin by laying out the developmental foundation of the model, followed by an examination of contextual factors, and finally we explore how these factors are reflected in dyadic appraisal and coping. Although the model advances a more dyadic understanding of coping with chronic illness, it is limited by the individualistic nature of the present literature. Therefore, the model addresses limitations in the literature and provides future directions for a more dyadic understanding of coping.

Life-Span Developmental Issues

Although numerous studies in the literature include individuals of a wide range of ages (Coyne & Smith, 1994; Kuijer et al., 2000; Manne & Zautra, 1990), only one study has explicitly examined age differences in appraisal and dyadic coping (Kuijer et al., 2000). Thus, an examination of the developmental process of dyadic coping is in its infancy, although opportunities for engaging in dyadic coping are high among older couples, as chronic illness increases with age (Siegler et al., 2003). We focus on age differences in aspects of the proximal context (quality of marital relationship, illness conditions), adjustment in relation to stressful events, and history-graded events that may affect dyadic appraisal and coping.

Life-span developmental differences occur in the marital relationship (Carstensen, Graff, Levenson, & Gottman, 1996) such that older adults experience increased marital satisfaction compared with younger couples. Long-term marriages are characterized by shared aims, goals, decision making (Lauer, Lauer, & Kerr, 1990), and intimacy (Goodman, 1999), features that may reflect shared appraisal of stressors and greater use of collaborative forms of involvement. Older marriages involve less potential for conflict and greater potential for pleasure (Levenson, Carstensen, & Gottman, 1993), less negative and more affectionate behavior during conflict discussions (Carstensen, Gottman, & Levenson, 1995), and smaller physiological responses to conflict than are evident in the marriages of middle-aged adults.

These differences in relationship quality may relate to more effective use of collaboration in older couples. As seen in the collaborative problem-solving literature, long-term married couples often demonstrate collaborative expertise (shared experiences, knowledge of each other's strengths and weaknesses), which facilitates the more active and engaged form of collaborative problem solving (Dixon & Gould, 1996). For some tasks, older adults are better able to benefit from collaborative processes than young adults (Gould, Trevithick, & Dixon, 1991), as they have greater skill at reminding and joint remembering (Wegner, Erber, & Raymond, 1991) and generating strategy discussion that facilitates problem solving (Gould et al., 1991). This collaborative skill of older couples is a valuable resource, as problems occur surrounding seeking information, making treatment decisions, and planning for long-term management of the illness. Older adults have been characterized as having a different style of decision making, one that utilizes much less information and reliance on physicians (Cassileth, Zupkis, Sutton-Smith, & March, 1980; E. A. Leventhal, Leventhal, Schaefer, & Easterling, 1993; Meyer, Russo, & Talbot, 1995). This style is thought to be adaptive in that it conserves cognitive resources, but it may be associated with more posttreatment decision regrets (J. A. Clark, Wray, & Ashton, 2001). Collaborative coping provides older adults with an additional resource in this decision process. However, this age-related decision-making style may be more indicative of a cohort effect and thus may not reflect how baby boomers will interact with health care professionals as they age, especially given their educational background (Say, Murtagh, & Thomson, 2006).

This collaborative resource of older couples is important, as chronic illness in late life is frequent (88% of all older adults have at least one chronic condition, 69% have more than one; Hoffman, Rice, & Sung, 1996), with both spouses likely to experience multiple chronic conditions. The onset of chronic illness is associated with increased dependency (Wolff, Boult, Boyd, & Anderson, 2005). Adult age is also associated with the onset of specific illness conditions, with conditions such as Alzheimer's disease, prostate cancer, rheumatoid arthritis, and osteoarthritis (Kriegsman, Penninx, & van Eijk, 1994) occurring with greater frequency in late adulthood. These illnesses are associated with specific stressors that vary in their controllability (Felton & Revenson, 1987), which affects coping strategies (Folkman, Lazarus, Pimley, & Novacek, 1987). Further, the progressive nature of many illnesses that occur in late life may increase the frequency of "downturns" (Erdal & Zautra, 1995) that affect opportunities couples have for dyadic coping (see *Types of Chronic Illness* section).

The greater debilitation associated with chronic illnesses in late life may lead to increased dependence on the spouse, challenging the need for independence. Maintaining autonomy and independence is especially important in late life (M. M. Baltes, 1996; M. M. Baltes & Silverberg, 1994), as the balance of gains and losses tips toward losses in physical functioning and social relations (P. B. Baltes & Baltes, 1990). Dependence occupies an important place in older adults' *feared selves* (i.e., selves one wishes to avoid becoming in the future, as opposed to *hoped-for selves*, which one wants to become) (Hooker, 1992; Hooker & Kaus, 1994) and is more prominent when individuals are dealing with a chronic illness (Frazier, Cotrell, & Hooker, 2003; Wiebe et al., 2003). Concerns regarding maintaining independence in the face of chronic illness mean that attempts by the spouse to assist may be interpreted as a negative reflection on one's own competencies (Martire, Stephens, Druley, & Wojno, 2002). Dyadic coping in domains that are key to defining functional independence (e.g., physical care) is particularly prone to eliciting such independence concerns (Strough, Patrick, Swenson, Cheng, & Barnes, 2003).

The greater frequency of chronic illness in late adulthood (Kriegsman et al., 1994) has been linked to the idea that chronic illness may be differentially less stressful for both patients and spouses when it comes later in adulthood rather than earlier (Coyne & Smith, 1994; Revenson, 1990; Revenson & Pranikoff, 2005; Williamson & Schulz, 1995). Better adjustment in late life to chronic illness may be a result of older adults having greater experience with chronic illness (Williamson & Schulz, 1995), as health threats are more normative in late life (Coyne & Smith, 1991) and may appear developmentally on time (Neugarten & Hagestad, 1976). Health increasingly becomes an important part of the self-system (Cross & Markus, 1991; Freund & Smith, 1999; Hooker, 1992, 1999; Hooker & Kaus, 1992, 1994) during late

adulthood in both hoped-for selves and particularly feared selves. An important area of future research will be to examine how spouses together incorporate health in their self-system and how joint health selves may affect dyadic appraisal and coping.

Developmental differences in the ability to regulate emotion and appraise stressful life events may also be important in understanding the lower distress among older adults in dealing with stressors surrounding chronic illness (see also Aldwin, 1994). Emotional understanding and regulation are oriented in late adulthood toward optimizing positive affect (Carstensen, Pasupathi, Mayr, & Nesselroade, 2000; Lawton, Kleban, Rajagopal, & Dean, 1992; Magai, 2001), with older adults experiencing difficulties in emotional regulation at primarily high levels of activation (Labouvie-Vief, 2003). Older adults appraise stressful events as more positive than young adults (Diehl, Coyle, & Labouvie-Vief, 1996) and cope via processes that focus more on accommodative changes in goals and motivations rather than persistent problem-focused coping (Brandstädter & Greve, 1994; Heckhausen & Schulz, 1995). Older adults use positive reappraisal and distancing strategies more frequently than do younger adults (Diehl et al., 1996; Folkman et al., 1987) and report being able to proactively deal with affective information (Lawton et al., 1992) via a wider variety of strategies (Blanchard-Fields & Irion, 1988). Thus, enhanced emotional regulation in late adulthood may contribute to better adjustment as couples deal with chronic illness.

The age-related differences in dyadic appraisal and coping outlined above must be placed in a broader life-span perspective (P. B. Baltes, 1987) that views development occurring through age-graded, but also history-graded, and nonnormative events. History-graded events alter the developmental context of dyadic coping and appraisal. Changes in the divorce rate (Schoen & Canudas-Romo, 2006) over the past 50 years mean that older couples less frequently find themselves in long-term marriages. Current trends for women and men to be single for longer periods of time during the life span (Roberts, 2007) suggest that friends and other family members will be important as potential dyadic partners. Further, recent advances in treating previously fatal diseases (e.g., cancer, stroke) together with greatly expanded life expectancies (U.S. Department of Health and Human Services, 2005) translate into individuals living for extended periods of the life span with chronic diseases as well as the side effects of their treatments. Thus, living with chronic illnesses such as cancer requires a long-term process of coping with the diagnosis, treatment, and potential recurrence for both patients and their spouses (Roberts, Black, & Todd, 2002). Such history-graded events create a dynamic context for the examination of dyadic coping processes across the life span.

In summary, a developmental perspective to dyadic coping poses new areas of inquiry for the field. Does the less conflictual nature of late life marriage facilitate dyadic coping and interaction such that greater mutuality and less maladaptive coping occur? Given the increasing frequency of multiple chronic illnesses with age, can we really distinguish between patient and spouse? In addition, age differences in appraisals of stressful events may activate different representations (e.g., control, dependence) that affect the enactment and interpretation of dyadic coping processes. These developmental influences may be particularly salient at different points in dealing with the chronic illness (e.g., collaborative processes may be especially beneficial as couples deal with complex treatment decisions). Further, such age-related differences in dyadic coping may be altered by changes in history-graded events that affect the experience of aging.

The Temporal Process of Coping With Chronic Illness

A second developmental aspect of the model is the temporal process of dyadic coping, which according to Revenson (2003) is "one of the most understudied in research" (p. 534). Practically speaking, research with couples experiencing chronic illness is challenging enough, but to add a longitudinal component to follow couples as they identify symptoms, seek a diagnosis and treatment, and live with the management of the illness is especially daunting. Although this process of dealing with chronic illness varies across illness conditions (Gallant, 2003), some features of illness identification and management are common across illness conditions (Maes et al., 1996; Morse & Johnson, 1991). The effectiveness of shared appraisal and dyadic coping may vary across these different phases of coping with the illness.

Maliski, Heilemann, and McCorkel (2002) followed couples as they moved from diagnosis of prostate cancer through completion of radical prostatectomy, and we detail this process as it is informative for understanding dyadic coping across time. Initially couples described dealing with the diagnosis independently. Wives described dealing with their own emotional needs somewhat separately so that they could be support providers for their husbands. Next, couples described putting themselves on a "crash course," collaboratively gathering information about prostate cancer to make an informed decision. Then couples worked toward a decision regarding treatment, both collaboratively and individually. The final treatment decision was left to the husband, although active discussions concerning the advantages and disadvantages of different treatments occurred with husbands and wives together. Searching for a specialist and preparing for surgery were described as a joint process, whereby both husband and wife together made decisions, prepared for tests, and learned what to expect postsurgery.

This research illustrates the value of tracking the temporal process from diagnosis through treatment. From this qualitative study we see that couples move in and out of different forms of dyadic coping throughout the process and need to attune their involvement to the needs and preferences of the spouse (Helgeson, 1993b). For recurring illnesses such as cancer, this process does not end when treatment is finished, as the potential for recurrence is real and often great uncertainty exists as to future health concerns (Helgeson, Snyder, & Seltman, 2004; Revenson & Pranikoff, 2005). For other chronic illnesses that involve daily management and incapacitation (e.g., osteoarthritis, rheumatoid arthritis), spousal burnout may occur that prevents the spouse's continued involvement.

Age-related differences may exist in this temporal process. Young adults who are for the first time experiencing the incidence of chronic illness may experience greater distress throughout the process, hampering their ability to engage in collaborative coping (Revenson & Pranikoff, 2005). Older adults may become quite experienced at coping collaboratively with stressful events such that coping strategies become effortless and individuals become more attuned to their spouses (Revenson, 2003). Making difficult treatment decisions as are common in illnesses such as prostate cancer, however, may be especially problematic for older adults, particularly those experiencing cognitive impairment. Several

studies have tracked couples longitudinally (Fang, Manne, & Pape, 2001; Helgeson, Snyder, & Seltman, 2004; Martire et al., 2002; Newsom & Schulz, 1998; Schiaffino & Revenson, 1995; U. Schulz & Schwarzer, 2004; Suls, Green, Rose, Lounsbury, & Gordon, 1997), establishing that different forms of dyadic coping are related to not only current but also subsequent adjustment. Extensive research is needed that follows couples across time to track changes in how couples are involved in dealing with chronic illness and how this process is similar or different for couples of different ages and across illnesses.

Contextual Characteristics

Couples across the life span who come to the experience of coping with chronic illness are affected by broad sociocultural factors (e.g., culture and gender) that make salient interdependent versus independent ways of construing relationships and chronic illness. Further, couples are affected by more proximal contextual factors present in the marital relationship and in dealing with the constraints of their specific illness. These contextual factors may be reflected in appraisal processes that affect the frequency and function of dyadic coping (see Figure 1) and also be subsequently affected by engaging in dyadic coping.

Cultural Differences

The cultural context affects interdependent appraisal and dyadic coping strategies. Although culture could shape factors such as health-related beliefs (Landrine & Klonoff, 1992), treatment seeking (Hough et al., 1987; Spactor, 1979), performance of health behaviors, adherence to treatment (Kirscht, 1983; K. A. Matthews, Kelsey, Meilahn, Kuller, & Wing, 1989), access to health care (Becker & Newsom, 2003; Hough et al., 1987), and perception of symptoms (Zborowski, 1958), we focus on how culture affects dyadic processes between patient and spouse. Culture affects how individuals view themselves in relation to others, aspects of the proximal context (most particularly the frequency of illness conditions), and coping in relation to stressful life events. To date, little empirical research has examined the effect of culture on dyadic appraisal and coping processes surrounding chronic illness either across cultures or within a diverse country such as the United States.

One dimension that has repeatedly emerged as relevant to couples coping dyadically is that of independent and interdependent self-construals (Triandis, 2001; Triandis, Bontempo, Villareal, Asai, & Lucca, 1988). Independent cultures stress becoming independent from others and expressing one's own unique attributes (e.g., Australia, most European countries, Canada, Great Britain, South Africa, and the United States; see Gudykunst, 1998). According to Markus and Kitayama (1991, 2003), independent self-construals are characterized by separateness from the social context, where individuals strive to be unique in expressing the self, realizing their self-potential, and promoting their individual goals. In contrast, individuals within interdependent cultures emphasize connectedness among themselves and the social context (e.g., cultures in Africa, South American countries, Asia continent countries, and some Middle Eastern countries such as Iran and Turkey; see Gudykunst, 1998) and are motivated to fit in, while promoting the goals of others. Cultural interdependent self-construals include the notion of simpatico (respect and sharing others' feelings) in the Hispanic culture (Triandis, Marin, Lisansky, & Betancourt, 1984) and the Hindu conception of the self as an entity that is given shape by the social environment (Marriott, 1976).

Although cultures differ in independence and interdependence, these two ways of relating to others are present in all cultures (Turiel & Wainryb, 2000) and change due to acculturation and historical influences. In a country as diverse as the United States, the interdependent cultural identity is found in many subcultures (e.g., African American: Milburn & Bowman, 1991; S. J. Hatchett & Jackson, 1993; Asian American and Pacific Islander: U.S. Department of Health and Human Services, 2001; and Hispanic) and may change due to acculturation (Gushue & Constantine, 2003; Wong, Yoo, & Stewart, 2005). In Japan the ideal of filial piety, emphasizing children's connectedness to parents, has weakened across historical time (Ogawa & Retherford, 1993), a change that has been linked to declining coresidence of older adults with adult children (Takagi & Silverstein, 2006; see also Kim, Liang, Rhee, & Kim, 1996, for a similar historical pattern occurring in Korea). These historical changes may mean that younger members of the culture evidence the interdependent versus independent construal less frequently than older members of the culture.

These culturally defined independent and interdependent construals provide a schemata of the self (Markus & Kitayama, 1991) that shapes appraisal and coping patterns valued in a particular culture (Lam & Zane, 2004). Lam and Zane (2004) found that Caucasian Americans relied more on primary control strategies as compared with Asian Americans, with independent self-construals fully accounting for the difference in primary control. Thus, Caucasian Americans' cultural emphasis on independence and autonomy may foster their use of primary control (i.e., individually mastering and controlling the environment to fit their own personal needs), as opposed to more collaborative forms of coping. In cultures that value interdependence, relatives are more likely to provide social support (Markus & Kitayama, 1991) because of their proximity, which could emphasize collective coping (Kashima & Triandis, 1986; Triandis et al., 1988). Although shared appraisals and dyadic coping may be more prevalent in cultures such as that of Asia (Lyons et al., 1998) than in Western cultures, the link between interdependent appraisals and outcomes may be similar (L. Fisher, 2005).

Different cultural groups also experience different illnesses at different rates (Aldwin & Gilmer, 2004) and have different beliefs (Bauman, 2003; Landrine & Klonoff, 1992) regarding various dimensions of illness (most particularly cause and consequence). Of particular interest for dyadic coping is the tendency for collectivistic oriented groups to view illness as somehow a "manifestation of long-term and changing relationships and dysfunctions in the family" (Landrine & Klonoff, 1992, p. 268). In this context, dyadic coping may be essential in order to "right the wrongs" of the interpersonal relationship (e.g., jealousy, violations of norms) that are the root of the problem. Currently, this aspect of illness representations is not captured in conceptualizations regarding illness and should be a focus of research examining culture and dyadic coping.

As individuals of different cultures begin the temporal process of dealing with a chronic illness by accessing the medical system, Gotay (2000) suggested, cultural differences in disclosure of medical information may also affect how couples cope with illness. In

the United States, openness and full disclosure of a patient's medical diagnosis are valued, and patients and spouses are active participants in treatment decisions (Rolland, 1998), consistent with individualistic cultures' preference for direct communication. However, in many Asian and Hispanic cultures, expectations exist that the family should protect the patient (Ballard-Reisch & Letner, 2003; Tse, Chong, & Fok, 2003), implying that the spouse, not the patient, should be informed. Culturally prescribed limitations in how medical information should be communicated between the patient and the spouse (Gotay, 2000) could affect the couple's ability to appraise and cope dyadically with an illness.

Gender Differences

The independent–interdependent distinction has also been used to describe differences between men's (independent) and women's (interdependent) self-representations (Cross & Madson, 1997). The greater interdependent self-representations (Acitelli & Antonucci, 1994; Cross & Madson, 1997) of women and larger socialization factors for women to take on the nurturant role in their relationships (Maccoby, 2002) are likely responsible for the greater importance of interdependence in appraisal and coping processes for women than for men. However, historical changes in women's status and roles may make such gender differences less apparent in younger cohorts (Twenge, 2001), creating what appear to be age-related differences in interdependence.

This interdependent self-representation may act as a lens to selectively encode and attend to information regarding relationships. An extensive literature suggests that women are more attuned to the quality of their marital relationship and the emotional experience of their spouse than are men (Kiecolt-Glaser & Newton, 2001). Women spend more time thinking about their marital relationship, have more detailed memories of specific events, are more distressed by stressors within the family (Conger, Lorenz, Elder, Simons, & Ge, 1993; Ross & Holmberg, 1992), and are more responsive to nuances of working together (Berg, Smith, et al., 2007) than men.

Individuals with a relational self-construal may also be more sensitive to the distress of their chronically ill spouse. Women typically carry a larger burden of the chronic illness of their spouse and are more affected psychosocially by the condition of their spouse than are men with a chronically ill spouse (Coyne & Fiske, 1992; Hagedoorn, Buunk et al., 2000; Lyons et al., 1995). Women's distress may be due to their perception that they are failing in the caregiver role (Hagedoorn, Sanderman, Buunk, & Wobbes, 2002). One difficulty in drawing conclusions from this literature, however, is that for many chronic illnesses (e.g., breast cancer and prostate cancer), the effect of gender and role (patient vs. caregiver) are confounded. Several studies have examined the role of gender (Hagedoorn, Buunk et al., 2000; Rohrbaugh et al., 2002; Tunistra et al., 2004) by investigating forms of cancer and congestive heart failure where women and men can be either patients or caregivers. These studies clearly show that women experience more distress than do men when either caregivers or patients are compared separately.

Women perceive greater shared appraisal and perceive collaborative and supportive forms of dyadic coping more frequently than men (Kuijer et al., 2000) and benefit more from their use (Hagedoorn, Kuijer, et al., 2000; Hovanitz & Kozora, 1989; U. Schulz & Schwarzer, 2004). Such results are consistent with research indicating women use strategies that express thoughts and feelings to others and seek emotional support (Tamres, Janicki, & Helgeson, 2002) and have goals for others rather than goals focused on the self more so than men (Strough, Berg, & Sansone, 1996). However, these gender differences may vary depending on whether the ill individual is female or male (Badr, 2004). For instance, women are less likely to engage in collaboration when they are ill than when they are well, whereas men are more likely to engage in collaboration when they are ill.

Although we have restricted our discussion of shared appraisal and dyadic coping to that involving the spouse, women may have more opportunities for dyadic coping with individuals outside of the marital relationship than men (Hess & Soldo, 1985). Across the life span women report having more close relationships (Antonucci & Akiyama, 1987; Antonucci, Akiyama, & Lansford, 1998) and more intimate friendships than men (Antonucci, 1990). In fact, during times of stress women differentially turn to same-sex persons in their network (Taylor et al., 2000), find this support more helpful than that from the spouse (Pistrang & Barker, 1998), and use this support to buffer the detrimental role of strained spousal relations (Walen & Lachman, 2000). The greater opportunities of women for dyadic coping outside of the marital relationship are especially important in the current historical context, where marriage is less frequently occurring for both men and women (Roberts, 2007). Especially during late adulthood, women may require dyadic partners other than the spouse, due to their greater life expectancy and greater years of widowhood.

In summary, when couples cope with chronic illness, they bring along broad representations of the self based on culture and gender that may affect dyadic appraisal and coping. More interdependent self-construals may set high expectations for dyadic coping, with poorer adjustment when the more interdependent and mutual forms of dyadic coping are not present. Extensive research is needed to understand how acculturation and historical changes may affect the salience of these independent and interdependent construals. Expectations for interdependence also arise as a function of more proximal contextual factors: the quality of the marital relationship and the specific demands of chronic illness conditions.

Quality of the Marital Relationship

The experience of chronic illness often brings challenges for the marital relationship, with illness associated with both reduced marital satisfaction (Hafstrom & Schram, 1984) and increased marital satisfaction (Hannah et al., 1992). Chronic illness occurs in the context of a long history of marital satisfaction (or dissatisfaction), which itself is associated with adjustment and health (Burman & Margolin, 1992; Kiecolt-Glaser & Newton, 2001). Spouses in marriages of better quality benefit in their psychosocial adjustment (Gottman & Notarius, 2000; Kiecolt-Glaser & Newton, 2001), survival from debilitating illnesses (Coyne et al., 2001), and illness management (Trief, Ploutz-Snyder, Britton, & Weinstock, 2004). In addition, marital satisfaction buffers the effects of the patient's physical impairments on spousal distress (Fang et al., 2001).

The literature supports the transactional nature of our model in that greater marital satisfaction is associated with the more beneficial forms of dyadic coping (supportive and collaborative)

(Bodenmann, 2005) and dyadic coping may lead to subsequent increases in marital satisfaction (Bodenmann, Pihet, & Kayser, 2006). Spouses whose marriage is characterized by higher marital quality more frequently perceive their spouse to be involved in effective types of dyadic coping such as active engagement and less frequently in maladaptive forms such as protective buffering (Coyne & Smith, 1991; Hagedoorn, Kuijer, et al., 2000). Highly satisfied couples also benefit more from these effective forms of dyadic coping (Hagedoorn, Kuijer, et al., 2000). In addition, spouses who experience better marital quality are buffered from more ineffective forms of dyadic coping (Cano, Weisberg, & Gallagher, 2000). Coyne and Smith (1991) found that in marriages of high quality there was no association between patients' protective buffering and wives' distress, whereas in marriages of lower quality this relationship was quite strong.

The literature on spousal involvement in chronic illness indicates that criticism and negative affect expressed during interaction are detrimental to working together, whereas warmth, love, and positive validation are positive for couple involvement (see also Cutrona, 1996). Marital interaction characterized by high negativity, low warmth, and high control may characterize critical spousal involvement and poorer coping responses (Manne, Alfieri, Taylor, & Dougherty, 1999; Manne & Zautra, 1989, 1990) and be indicative of overprotection (Hagedoorn, Kuijer, et al., 2000; Kuijer et al., 2000). Further, perceived failure to meet the expectations of the spouse is associated with depressive symptoms among rheumatoid arthritis patients, even when traditional relationship measures are controlled for (Bediako & Friend, 2004). The unsupportive behaviors exhibited by the spouse appear to be more powerful in understanding adjustment than the supportive behaviors (Cranford, 2004; Manne, Taylor, Dougherty, & Kemeny, 1997) and can exacerbate the relationship between stress and depression (Cranford, 2004).

These findings concerning positive dyadic coping are consistent with work from the marriage literature (Ball, Cowan, & Cowan, 1995; Bradbury & Fincham, 2000; Fincham & Linfield, 1997; Gottman & Notarius, 2000; Kiesler, 1996; L. S. Matthews, Wickrama, & Conger, 1996) and the collaborative coping literature (Meegan & Berg, 2002). Positive features of relationships (validation, agreement, warmth) facilitate joint problem solving and are associated with marital stability, whereas negative features (dominance, hostility) as well as the copresence of positive and negative features (Uchino, Holt-Lunstad, Uno, & Flinders, 2001) are associated with marital distress. The positive emotional support that patients need may best be accomplished when both partners reciprocate that support in a way that it is equitable (Wright & Aquilino, 1998), with reciprocal disclosure being especially beneficial for women (Manne, Ostroff, Rini, et al., 2004; Manne, Ostroff, Sherman, et al., 2004). Future research will benefit from detailed analyses of interpersonal processes (Manne, Ostroff, Sherman, et al., 2004) that draw on the marital interaction literature (Ball et al., 1995; Bradbury & Fincham, 2000; Gottman, & Notarius, 2000) to characterize how spouses interact as they deal with stressors surrounding chronic illness.

Warmth and give-and-take in interactions may facilitate the effectiveness of dyadic coping as problems are solved and decisions are made. As couples deal with chronic illness, numerous everyday problems must be approached, including treatment decisions (Davison et al., 2002; Halford, Scott, & Smythe, 2000) and redistribution of household responsibilities and financial decision making (Helgeson, 1993). Collaborative everyday problem solving, planning, and decision making are enhanced when couples engage in highly affiliative and egalitarian interactions (Berg, Johnson, Meegan, & Strough, 2003) and are worsened when couples engage in negative and controlling interactions (Miller & Bradbury, 1995). These features of positive interpersonal processes are more frequently seen in older couples (Carstensen et al., 1995), which may make collaborative coping a more effective form of dyadic coping in late life. These aspects of collaborative processes may be different depending on the specific illness that the couple must face.

Types of Chronic Illness

The literature on dyadic coping has investigated a wide array of chronic illnesses (see Tables 1 and 2). However, studies frequently have focused on a single illness (due to the practicalities of securing the sample) or included a range of illnesses with small sample sizes by condition, thereby preventing comparisons across conditions. However, the impact of chronic illness on the patient and spouse likely varies across diseases (see Kriegsman et al., 1994). Features of illnesses may be differentially salient during young or late adulthood (Kriegsman et al., 1994) or for different cultures (Aldwin & Gilmer, 2004). Further, the diagnosis and incidence of specific diseases vary across historical time with the introduction of diagnostic procedures (e.g., prostate-specific antigen test; Siegler, Bastian, & Bosworth, 2001) and health crises (e.g., obesity; Friedman, 2000).

An integration and expansion of two taxonomies (H. Leventhal, Brissette, & Leventhal, 2003; Rolland, 1984) for understanding the psychosocial impact of disease that relies on individuals' representations of illness will be used to explore differences in dyadic coping by illness (see Table 3). Consistent with Rolland (1984) and H. Leventhal et al. (2003), we distinguish illnesses in their timeline (onset and course), consequences (daily management, cognitive or communication impairments), control (how controllable the disease is), and identity (labeling symptoms). In addition, we add the consequences of the illness on the relationship, a factor not examined in conceptualizations of illness. In this section we describe how these dimensions of illness may be relevant for understanding couples' appraisal and coping.

Timeline. Illnesses differ in terms of their onset and time course (Rolland, 1984). Some illnesses have a sudden onset (e.g., myocardial infarction, forms of cancer) that does not allow for the anticipation and planning characteristic of other diseases (e.g., rheumatoid arthritis, angina pectoris). Couples are thrust into a crash course of learning about the disease and its treatment (Maliski et al., 2002), which may especially activate the collaborative resources of both spouses. Illnesses also vary in whether they involve a slow, progressive decline in functioning (e.g., Parkinson's disease, chronic obstructive pulmonary disease, Alzheimer's disease), are more constant (e.g., congenital heart arrhythmias), or are relapsing or episodic (e.g., cancers in remission, asthma). In a progressively declining illness like chronic obstructive pulmonary disease, communication needed for dyadic appraisal and collaboration is hampered due to difficulty breathing and talking (Cannon & Cavanaugh, 1998). In addition, increasing fatigue is associated with more depression and anger (Small &

Table 3
Dimensions of Chronic Illness

Dimension	Description and examples
1. Timeline	
Onset (sudden or gradual)	Illnesses characterized by a sudden onset with little forewarning (e.g., most chronic illnesses, such as myocardial infarction, multiple forms of cancer) versus gradual onset (e.g., rheumatoid arthritis, angina pectoris)
Time course	
Progressively declining	Illnesses characterized by a slowly advancing process that is increasingly debilitating to the patient (e.g., multiple sclerosis, Alzheimer's, Parkinson's, chronic obstructive pulmonary disease)
Constant	Illnesses with a largely consistent level of impairment (e.g., hypertension, diabetes)
Relapsing	Illnesses that have the potential for relapse (e.g., cancers in remission, lupus, asthma)
2. Consequences	
High daily management	Illnesses for which management requires daily tasks (e.g., asthma, diabetes)
Cognitive/communication	Illnesses that are accompanied by cognitive impairments brought on by either the illness (e.g., dementias such as Alzheimer's and Parkinson's) or the treatment of the disease (cognitive impairments associated with medications)
Relationship impact	Illnesses that have a high impact on the meaning and function of the relationship, including sexual function (e.g., prostate cancer, breast cancer)
3. Control	Illnesses for which there is the possibility for control, over the illness and its progression, through treatment and one's own efforts (e.g., Type 2 diabetes, hypertension)
4. Identity	Illnesses for which the link between symptoms and illness identity is clear (e.g., asthma) versus ill defined (e.g., fibromyalgia, chronic fatigue)

Graydon, 1992), which may compromise individuals' ability to communicate regarding stressors and cope together (Bodenman et al., 2004; Lane & Hobfoll, 1992). These more debilitating diseases occur more frequently in late life and pose challenges for spouses' typical ways of dyadic coping (Kriegsman et al., 1994).

Consequences. Chronic illnesses vary in their consequences for daily life activities, cognitive impairments, and the relationship. Illnesses that are more debilitating challenge the individual's functional independence and are sensitive to maladaptive dyadic coping processes (such as control; Martire et al., 2002). Couples coping with chronic pain struggle to find effective ways for spouses to be involved (Bush & Pargament, 1997; Newton-John, 2002; Schwartz & Ehde, 2000). Spousal involvement typically considered as supportive by the spouse (expressing empathy, assisting with daily household responsibilities) reinforces the expression of pain (Smith, Keefe, Caldwell, Romano, & Baucom, 2004). Interventions involving the spouse (see Keefe et al., 1999, 2004) suggest that spouses may need to be involved in a more collaborative manner.

Illnesses that have a high daily management component, such as diabetes, may require more frequent dyadic coping between spouses, as such illnesses require a change in lifestyle (i.e., diet and exercise) that may be best accomplished by the couple (Gallant, 2003). Directive (controlling) support from one's spouse may be detrimental to mood (E. B. Fisher, La Greca, Greco, Arfken, & Schneiderman, 1997; Hagedoorn et al., 2006). More generally, promoting health change (e.g., smoking cessation, exercise regimens) is more effective to the extent that the partner is actively engaged by enacting and modeling the desired health behavior and discussing health issues rather than exerting control (Tucker & Mueller, 2000).

When the consequences of the illness involve cognitive and communicative impairments, this may especially affect collaborative opportunities (Kriegsman et al., 1994; Rabins & Mace, 1986) and adjustment of spouses (Lieberman & Fisher, 1995). Cognitive impairments occur in illnesses involving dementia and may also result from treatments associated with illness, especially cancer treatments (for reviews, see Anderson-Hanley, Sherman, Riggs, Agocha, & Compas, 2003; Falleti, Sanfilippo, Maruff, Weih, & Phillips, 2005; Stewart, Bielajew, Collins, Parkinson, & Tomiak, 2006). The increasing dementia associated with Parkinson's disease and Alzheimer's disease may initially require that the affected person cope in an interdependent manner (Hellstrom, Nolan, & Lundh, 2005; Hirschman, Joyce, James, Xie, & Karlawish, 2005; Hodgson, Garcia, & Tyndall, 2004). With advancing disease and cognitive decline, active engagement may be impaired (R. Schulz & Martire, 2004), which becomes especially problematic for caregivers because they no longer have available the involvement of the spouse for their own coping efforts (Blieszner & Shifflett, 1990; Morrissey, Becker, & Rubert, 1990; R. Schulz & Martire, 2004). Thus, a major strain for a caregiver of someone with dementia is not having available the normal range of dyadic coping strategies.

Shared appraisal and dyadic coping may also be affected by the extent to which the illness has a relationship impact, affecting core aspects of being a couple, such as sexuality and other joint activities (e.g., leisure activities). For example, illnesses such as chronic pain, prostate cancer, and Hodgkin's disease often affect a couple's sexuality (Andersen & Lamb, 1995; Hannah et al., 1992). Decreased intimacy in sexual relations may lead to marital strain or to declines in intimacy and connectedness, which are important for shared appraisal and collaboration. Couples who maintain physical intimacy may be able to buffer the effects of disabling illness on psychological adjustment (Druley, Stephens, & Coyne, 1997).

Control. Variation also exists in the degree of control individuals have over their illness. Patients experiencing diabetes and hypertension report more control over their illness than those with cancer and rheumatoid arthritis (Felton & Revenson, 1987), and more control is associated with better illness outcomes (Kaptein et al., 2003; Keefe, 1998; Watkins et al., 2000) across diseases (diabetes, cardiovascular disease, pain). Greater perceived control

by married couples may initiate behavioral actions to address the stressful events and management of the disease, increasing the frequency of collaborative coping. Perceived control may be lower in older patients and related to important health behavior change after the illness (Gump et al., 2001), consistent with older adults' lower locus of control in general across domains (Lachman, 2006).

Identity. Finally, illnesses differ with respect to their identity (i.e., how identifiable symptoms are). Many illnesses share similar symptoms (e.g., pain, fatigue), with some illnesses poorly understood by the community and medical profession (e.g., chronic fatigue syndrome, fibromyalgia). For illnesses without a strong identity, couples will differ in their representations of the illness, with differences associated with adjustment (see Weinman et al., 2003). Older adults appeal more to age per se for cause in illnesses such as coronary artery disease (Gump et al., 2001), which could be detrimental to health-related change behaviors required to manage the illness.

In summary, although the literature examines a wide array of chronic illness conditions, we are only beginning to understand how specific chronic illnesses affect the ability of the couple to cope dyadically with the stressful events surrounding the illness at various stages of the life span. Age-related differences may exist in how adults perceive the illness, with older adults more likely to experience less control over their illness and to experience illness with progressive deterioration. The literature in general suggests that dyadic coping works rather similarly across illness conditions, with collaborative and positive supportive coping beneficial for patient adjustment and control and uninvolvement detrimental, with the primary exception the case of chronic pain. As the current literature does not compare illness conditions, strong conclusions regarding the effects of illness condition on dyadic coping are premature. Later in the article we outline studies that could begin to address this gap in the field.

Dyadic Appraisal, Coping, and Adjustment

Couples coping with chronic illness bring schemata of the self in relation to others, which reflect their place in the life span and sociocultural (e.g., culture, gender) as well as proximal contextual factors (e.g., marital quality and illness characteristics). The interdependence of couples affects appraisals of the illness, shared appraisals of specific stressors, and the ways couples cope dyadically. We now elaborate the appraisal and dyadic configurations adopted within the developmental–contextual model, illustrating the value of understanding developmental and contextual factors surrounding chronic illness.

Appraisal Configurations

Models of dyadic coping posit that spouses may view the illness or specific stressful events (Lyons et al., 1998) as shared; however, little research has linked appraisals and dyadic coping. Although we depict appraisal processes as temporally prior to coping strategies (see Figure 2), we acknowledge that coping strategies most certainly affect appraisal processes (e.g., collaborating with one's spouse leads one to think about the stressor as shared). Three aspects of dyadic appraisal are examined: (a) illness representations (Is the illness controllable? What are the consequences of the illness?), (b) illness ownership (Who owns the illness?), and (c) specific stressor appraisals (Does the spouse share the stressful event?).

Couples' illness representations. Consistent with the dimensions of illness outlined above, different facets of illness (e.g., timeline, cause, controllability) may be activated in appraisals for specific chronic conditions (Kaptein et al., 2003). Dyadic illness representations may be the starting point for forms of dyadic coping as well as be affected by dyadic coping. Couples may diverge in their representations as they cope with the illness in more separate ways, gaining differential expertise regarding the illness (Hampson & Glasgow, 1996).

The association between similarity in illness representations among spouses and adjustment is a complex one, involving the extent to which illness representations are positive as well as contextual aspects of the illness itself. Studies examining dyadic illness representations rely on a congruence approach to the examination of dyadic representations, measuring individual representations and identifying patterns of configurations among these individual representations. Figueiras and Weinman (2003) found that patient recovery from a myocardial infarction was best when couples had similar positive illness perceptions rather than similar negative or conflicting perceptions. Heijmans, de Ridder, and Bensing (1999) found that differences in the illness representations of patient and spouse (expressed as either minimization or maximization of the seriousness of the illness) may be beneficial to the patient's coping and broader psychosocial adjustment differentially by disease. Healthy spouses of chronic fatigue syndrome patients tended to minimize the seriousness of the illness (compared with the patient), whereas healthy spouses of those with Addison's disease maximized the seriousness of the illness (compared with their spouses). When these dissimilarities occurred, adjustment was better among Addison's patients, and the results were weaker for patients with chronic fatigue syndrome.

Heijmans et al. (1999) posited that maximization by spouses of Addison's patients served to balance the minimization of patients, thereby regulating patients' tendencies to overdo their activity level. Similarly, the minimization of spouses of chronic fatigue syndrome patients balanced the maximization of illness severity by patients and thereby encouraged patients to increase their physical and social activity level. An alternative possibility is that these spouses held a more medically accurate view of the illness than the patients, which served to support functioning. These results point to a more dyadic view of illness representation, taking the adaptability of the illness representations held by the dyad rather than the perspectives held by the individual. Future research needs to deterrmine whether this adaptability is enhanced in late adulthood and whether these representations vary across time (during symptom perception, diagnosis, and treatment management) and are tied to congruence in how physical symptoms of the disease are perceived (Cremeans-Smith et al., 2003).

Illness ownership. The developmental and contextual components of our model point to aspects of illness representations yet to be examined, most particularly how the illness is situated within the relationship itself, either due to its cause (as is characteristic of some collectivistic cultures) or to how the illness is shared between spouses (i.e., does the illness belong to the patient, or is it shared between patient and spouse?). Several studies (Baider & Sarell, 1984; Cannon & Cavanaugh, 1998) and clinical cases (Lyons et al., 1995; Skerrett, 2003) support the notion that chronic illness is

often identified as the property of the couple. Rolland (1994) posits that couples are often incongruent regarding illness ownership and that incongruence is most problematic for young couples, for whom chronic illness is developmentally off time.

Acitelli and Badr's (2005; Badr & Acitelli, 2005) work on relationship talk provides a promising methodology for examining illness ownership (see Hauser et al., 1993, and Beveridge, Berg, Wiebe, & Palmer, 2006, for related work in families with an ill child). Acitelli and Badr (2005) outlined how couples vary in whether they are explicit or implicit regarding illness ownership (i.e., whether it is the focus of attention or the lens through which their world is viewed). The use of personal pronouns as couples talk about the illness may be a useful way to capture this implicit representation of illness ownership (e.g., Pennebaker, Mehl, & Niederhoffer, 2003). Greater shared illness ownership may emanate from the degree to which one views the self in relation to one's spouse (Acitelli, Rogers, & Knee, 1999). Relationship talk in the context of illness is associated with marital adjustment, although the relationship is stronger for wives than for husbands (Badr & Acitelli, 2005). Furthermore, the extent to which spouses include each other's health concerns as part of their own future goals may reflect illness ownership and is associated with greater involvement in the actual care and well-being of the spouse (Pierce, Hong, Franks, & Ketterer, 2002). The greater interdependence seen in some cultural groups, women, and couples with greater marital satisfaction may facilitate viewing the illness as something that is shared within the couple.

Shared stressor appraisals. As we move toward a dyadic approach that encompasses the stressful events and coping strategies of both patient and spouse, the question arises, "Whose stressor is a particular event?" (Berg et al., 1998; Bodenmann, 1997; Lyons et al., 1998). Appraising the problem as a shared problem may be the starting point for collaborative coping with the problem or may result from such collaborative efforts (Berg, Wiebe, Bloor, et al., 2007). For instance, although a patient may initially appraise the illness as "mine," repeated daily discussions with the spouse regarding stressors and a sense of sharing these stressors may be associated with changes toward a more shared view of illness ownership and a more similar view of what the illness entails.

In our own work we have examined three distinct ways that partners may appraise stressful events (similar to categories used by Bodenmann, 2005): *individual* (an individual appraises the stressors surrounding illness as "mine"), *indirect relational* (one member of the social unit feels stress as a side effect of the other person in the dyad experiencing stress; see also Almeida, Wethington, & Chandler, 1999; Compas & Wagner, 1991), and *shared* (both patient and spouse appraise the stressor as "ours"). Further aspects of appraisal may be important for dyadic coping, as proposed by Bodenmann (2005), such as the cause and controllability of the stressor. The extent to which patient and spouse share a similar perspective of the event may contribute to positive forms of dyadic coping and mutual engagement rather than control.

The extent of these shared appraisals concerning illness representation, illness ownership, and specific events may inform different configurations of dyadic coping strategies, a link not yet explored in the literature. That is, does holding similar or shared appraisals of the illness and specific stressors increase the coupling of dyadic coping configurations? For example, shared appraisals may facilitate mutual collaboration by both patient and spouse; mismatches may increase maladaptive mutual control or control–uninvolved exchanges. Further, the mismatch between appraisal and coping strategies (e.g., a patient appraises a stressor such as dealing with finances regarding the illness as shared with the spouse yet views the spouse as uninvolved in coping efforts) will be especially problematic for adjustment (Berg, 2006).

Dyadic Coping

Our developmental–contextual approach views dyadic coping as a developmental process (see also Bodenmann, 2005; Revenson, 2003) that occurs over large-scale time across the life span, across the temporal process of coping with different aspects of illness management, and sequentially as coping unfolds in more discrete time moments across a conversation or over days (in Figure 1 from Time 1 to Time 2). The relation between dyadic coping and adjustment is a transactional one in which dyadic coping affects adjustment (the focus of most empirical studies) and is affected by adjustment. This developmental approach to dyadic coping is different from what exists in most of the empirical studies on dyadic coping, where coping is assessed at a very global level with some form of rating checklist in which participants describe how often they have used particular types of coping strategies in response to coping with the illness in general. The coping literature will benefit by examining how patients and spouses are involved in similar specific stressful encounters and how patients respond to the involvement of their spouse.

Further, our dyadic approach focuses on the stressful events experienced by both patient and spouse, examining (a) how the patient perceives the spouse's involvement and the spouse perceives his or her own involvement as well as (b) how the spouse perceives the patient's involvement and how the patient views his or her own involvement in the spouse's coping (see Figure 2). The literature thus far has largely treated the patient as the focal person, examining how the spouse is involved in the patient's stressful events and how this involvement affects the patient's adjustment (Helgeson, 1991; Martire et al., 2002; Newsom & Schulz, 1998; Schiaffino & Revenson, 1995), thereby limiting what we can derive about the dyadic nature of coping. However, a growing number of studies have examined both how the patient perceives the spouse's involvement and how the spouse perceives his or her own involvement (Bolger et al., 2000; Hagedoorn, Buunk, et al., 2000; Kuijer et al., 2000; U. Schulz & Schwarzer, 2004). Studies that have compared the involvement perceived by patients and the perceptions of that involvement as rated by the spouses have found moderate agreement (rs = .31–.50; Kuijer et al., 2000; Manne et al., 2006; Manne, Ostroff, Winkel, Grana, & Fox, 2005; U. Schulz & Schwarzer, 2004), with perceptions of controlling involvement reported higher among patients than among spouses (Hagedoorn, Kuijer, et al., 2000). Greater concordance may be enhanced in the context of relationships characterized by more intimacy and individuals with a greater interdependent orientation (Coriell & Cohen, 1995).

From the developmental–contextual framework there are numerous elements missing from the current literature. First, patient and spouse coping are rarely viewed in relation to each other (i.e., examining patterns of patient–spouse coping). Rather, patient and spousal coping are individually related to adjustment outcomes.

Second, the spouse is viewed largely as assisting the coping efforts of the patient, rather than as experiencing his or her own stressful events and benefiting from the involvement of the patient. For example, the wife of a prostate cancer patient may experience different stressful events than her husband (e.g., restriction of activities, dealing with problematic family supports), and the husband's collaborative involvement in the wife's coping efforts may be beneficial for her daily mood (Berg, Wiebe, Bloor, et al., 2007). Third, the distinction between patient and spouse becomes increasingly difficult in late adulthood, as both husband and wife are likely experiencing chronic illness (Hoffman et al., 1996). This more dyadic perspective enhances the literature and points to new methods that push the field beyond individualistic perceptions of coping with chronic illness. Dyadic coping strategies will be examined across a continuum of involvement using the strategies in our framework (e.g., uninvolved, supportive, collaborative, and control).

Patient and Spousal Dyadic Coping

The lack of spousal involvement in coping efforts from the patient's perspective (e.g., lack of social support or disclosure) is associated with poorer psychosocial adjustment outcomes for the patient (Helgeson, 1991; Helgeson & Lepore, 1997) and worse recovery for men following a myocardial infarction (Helgeson, 1991). Because such work does not include the partner's perspective, it may obscure a particular dyadic coping configuration that may be beneficial. Research by Bolger, Zuckerman, and Kessler (2000) indicates that in a number of cases when the focal person perceives the partner to be uninvolved, the partner perceives him- or herself to be supportive (invisible support). On days when invisible support occurred, the focal person's adjustment was better than on days when the focal person perceived the partner's support. Although invisible support has not been examined in the context of couples' chronic illness (see Upchurch, 2007, for the benefits of invisible support in children with Type 1 diabetes), this finding illustrates how a dyadic perspective where both patient's and spouse's views are jointly considered for specific events is necessary to understand spouses in connection to each other.

Several studies have demonstrated the effects of positive and negative social support (emotional and instrumental) for patients' adjustment and recovery (e.g., S. L. Clark & Stephens, 1996; Manne, Pape, Taylor, & Dougherty, 1999; Manne & Zautra, 1989). Unsupportive behavior relates to poorer adjustment in patients because it increases negative mood (Manne et al., 1999) and decreases coping efficacy (Manne & Glassman, 2000). However, much of this literature has not included the partner's perspective of the support that the spouse is intending to provide. Research that includes both patient's and partner's perspectives indicates that for many couples the partner's intentions of being supportive are not being received by the patient (Manne, Ostroff, Winkel, Grana, & Fox, 2005; Pistrang, Barker, & Rutter, 1997; U. Schulz & Schwarzer, 2004). In the only study comparing patient and partner unsupportive behavior, the patient's perspective alone predicted patient's subsequent maladaptive coping responses (Manne, Ostroff, Winkel, Grana, & Fox, 2005). However, mismatches in how the partner's intended support is received by the patient may be important for understanding the partner's adjustment.

Collaboration (often referred to as active engagement) involves a very active role of the partner in discussions, gathering information, brainstorming solutions, and problem solving and is associated with positive psychosocial adjustment of the patient (Coyne & Smith, 1991, 1994; Hagedoorn, Kuijer, et al., 2000; Kuijer et al., 2000) across several illness conditions (e.g., men following myocardial infarction, a range of cancers). Patient report of active engagement has been associated with higher self-efficacy (Coyne & Smith, 1994; Kuijer et al., 2000), better daily mood (Berg, Wiebe, Bloor, et al., 2007), and better relationship satisfaction both concurrently and prospectively (Bodenmann, 1997; Hagedoorn, Kuijer, et al., 2000; Kuijer et al., 2000). However, Coyne and Smith (1991) found that active engagement by the wife was related to her higher distress as the husband was recovering from a myocardial infarction. The lack of correlation between the wife's and husband's reports of active engagement in this study ($r = .09$) suggests that for many wives their active engagement was not matched by the active engagement of the husbands. At a dyadic level, collaboration may be beneficial only when both spouses engage at a high level in discussions, sharing ideas, and mutual disclosure (Beveridge & Berg, 2007). These results may also reflect that such active discussions may create a context in which the partner experiences emotional contagion from his or her ill spouse (Berg, Wiebe, Bloor, et al., 2007). Thus, in some cases, what is effective for the patient may not be so for the spouse. Active engagement may benefit those who need it the most, those who are experiencing extreme distress (Hagedoorn, Kuijer, et al., 2000) and whose cancer condition is most serious (Kuijer et al., 2000).

Spouses are also involved in coping in ways that are maladaptive in terms of patient and spousal adjustment. Overprotection and miscarried helping refer to strategies where the spouse underestimates the patient's abilities and provides an excessive amount of help (often including a restriction of the patient's activities). Protective buffering involves healthy spouses intentionally hiding their own concerns, concealing their own worries, and giving in to the ill partner to avoid conflictual interactions (Coyne & Smith, 1991, 1994; Hagedoorn, Kuijer, et al., 2000; Kuijer et al., 2000) and is generally associated with poorer marital satisfaction for the spouse. Kuijer et al. (2000) found that overprotection was commonly used together with protective buffering ($r = .53$ for patient reports). Both of these strategies are associated with lower perceived control by the receiver (Hagedoorn, Kuijer, et al., 2000; Kuijer et al., 2000) and lower self-efficacy. As Coyne, Wortman, and Lehman (1988) described, such strategies may provide a message to patients that they are incompetent and being coerced into action. Kuijer et al. (2000) found that overprotection was reported more frequently among older than among younger adult patients, potentially resulting from their greater functional need. The detrimental effects of spousal control are consistent with research indicating that the use of social control tactics by partners (e.g., telling a partner not to engage in smoking or to engage in exercise) is not effective at promoting health change and can be especially detrimental to mood (Lewis & Rook, 1999; Tucker & Mueller, 2000) and self-esteem (Tucker & Mueller, 2000), although perhaps less so among the elderly (Rook, Thuras, & Lewis, 1990).

From a dyadic perspective it is important to note that spouses who report that they engage in maladaptive strategies (e.g., over-

protection, protective buffering) are themselves experiencing greater distress (Coyne & Smith, 1991; Suls et al., 1997). Thus, spouses may engage in protective buffering because they are experiencing low self-efficacy and high distress regarding how to provide support, and/or the engagement in such strategies may result in low self-efficacy and distress (Kuijer et al., 2000). Collectively, those couples in which both members engage in protective buffering may experience the worst adjustment, with interventions needed to target both alleviating the distress (so that spouses may engage each other around the illness) and changing coping patterns. Short-term longitudinal research is needed to determine whether the negative adjustment of spouses and patients contributes to their inability to be optimally involved, consistent with the transactional perspective taken by the model.

This dyadic perspective captures the ways in which patients are in connection with spouses. In addition, we must understand how the spouse's coping efforts regarding the events experienced surrounding the illness are in relation to those of the patient. Our own research with both couples (Berg, Wiebe, Bloor, et al., 2007) and mothers and ill adolescents (Berg, Wiebe, Beveridge, et al., 2007) indicates that the patient's collaborative involvement in the healthy individual's coping efforts is associated with lower depression, negative daily mood, and more positive daily mood of the healthy individual.

The perspective of both patient and spouse in each other's coping efforts will allow for the identification of "coupled" configurations of coping, similar to the types of sequential analyses examined in marital interaction (Gottman & Notarius, 2000). Examining such coupled patterns over both short (e.g., daily) and longer time frames (e.g., from treatment decision making to daily management) would elucidate the process of dyadic coping. For instance, if at Time 1 the patient views the spouse to be controlling, the patient may subsequently withdraw from efforts to assist the spouse in his or her coping efforts (reminiscent of the demand–withdrawal pattern in the marital interaction literature; Heavy, Christensen, & Malamuth, 1995), which could lead to changes in marital satisfaction over time (Manne et al., 2006). Coupling may also occur with respect to adjustment, as patient and spousal distress are frequently related (Baider, Koch, Esacson, & De-Nour, 1998; Druley, Stephens, Martire, Ennis, & Wojno, 2003). The literature does give some hints as to coupled patterns that might be important for understanding adjustment and some indication that these patterns differ by features of the developmental–contextual model.

Coupled Patterns of Spousal Involvement

An initial approach taken to dyadic coping advanced by Revenson (1994), the congruence approach (see Table 1), revealed coupled patterns in emotion-focused and problem-focused coping at a broad level. This work revealed that it was dyadic patterns of coping (i.e., whether the dyad engaged in maladaptive or adaptive coping) rather than congruent patterns that were important for positive adjustment (i.e., at least one member of the couple used adaptive coping strategies). Similar results have been found by Badr (2004) using Kenny's (1990) actor–partner interaction model, which captures the dyad as the unit of analysis. Congruence was associated with better marital satisfaction only when couples were similar in active engagement, reflecting perhaps the essence of mutual collaboration. Revenson (1994) found developmental differences in the pattern of coupling, such that highly congruent problem-focused coping occurred in younger couples, whereas those who used complementary strategies were older. Older adults' complementarity may represent a natural division of labor among long-term couples in the tasks of daily living (Berg et al., 2003).

Similarly, research predicting spousal adjustment from both patient and spouse coping strategies reveals that coping configurations may be more adaptive for specific illness conditions. Disparities in problem-focused coping (reflecting possible division of labor) were associated with better adjustment for patients with multiple sclerosis, an illness for which couples may naturally need to divide responsibilities. Disparities in emotion-focused coping were associated with poorer adjustment for breast cancer patients (reflecting potentially a denial on the part of one of the partners of the emotional component of the illness); no associations with adjustment were found for multiple sclerosis patients (Ben-Zur, Gilbar, & Lev, 2001; Pakenham, 1998). These mixed results coming from the congruence approach regarding what coupled pattern is best point to the importance of examining the contextual constraints of specific illness conditions.

These methods provided an initial way to examine coping configurations; however, from this approach couples were assumed to interact as they individually reported coping strategies, as opposed to being assessed directly in their engagement with one another. A more complete picture regarding dyadic involvement would require both patient and spousal perceptions of how each is involved in the other's coping efforts over time. Extending the work of Bolger et al. (2000) to examine daily coping configurations together with behavioral interaction research will allow for the illumination of the developmental process of dyadic coping (see *Research Implications* section below).

Use of the Developmental–Contextual Framework to Discern When Spousal Involvement Is Beneficial or Harmful for Adjustment

The dyadic perspective advanced within our model makes predictions as to when dyadic coping will be beneficial for the adjustment of both patient and spouse. First, dyadic coping configurations will be associated with better adjustment when they match the ways in which the dyad appraises the illness and stressors. At a global level (i.e., measuring dyadic coping with the illness in general, as in much of the literature), more supportive and collaborative strategies will be associated with better adjustment when couples perceive the illness as shared by the spouse and share illness representations. Similarly, for dyadic coping with a specific stressful event, shared appraisal of that event (i.e., the stress is "ours") will be associated with better adjustment when the coping strategies involve the spouse in supportive or collaborative ways. When stressful events are appraised as the patient's own, however, uninvolvement by the spouse will be associated with better adjustment (Berg, 2006). Second, when interdependence is high (such as in Asian cultures, among women, and in those with high marital satisfaction), highly collaborative forms of coping may be expected, with poorer adjustment when those high expectations are not met with high interdependence in appraisals and strategies. During late adulthood, however, when marital satisfaction is high (Carstensen et al., 1995), the effects of detrimental

dyadic coping on adjustment may be moderated by sentiment override (T. N. Story et al., in press). Finally, the adaptability of dyadic coping configurations may depend on the consequences of the specific illness condition the couple faces, with invisible support configurations especially helpful when independence needs are high (Martire et al., 2002).

In sum, the dyadic perspective advanced within our model examines appraisal, coping, and adjustment of the patient as situated in relation to the appraisal, coping, and adjustment of the spouse. Greater interdependence by one spouse may draw more interdependence from the other spouse; similarly, greater independence may be matched by independence (Benjamin, 2003). Focusing on the dyad as the unit of analysis allows one to examine coupled patterns of interaction that may be helpful for adjustment, while at the same time examining whether that coupled pattern predicts adjustment over and above the individual's own perspective.

Future Directions and Implications

The developmental–contextual model explores how the question of whether dyadic coping is beneficial or harmful for patient and spouse adjustment depends on numerous developmental and contextual factors that affect dyadic appraisal and coping. Currently the literature is only beginning to address many developmental issues raised by our model. Numerous unanswered questions concerning the development and context of dyadic coping exist, and our developmental–contextual model is suggestive of several avenues for research that represent a new "look" to research on stress and coping.

Research Implications

The developmental–contextual model adds an important temporal component to understanding aspects of appraisal, dyadic coping, and adjustment that will require short-term and/or long-term longitudinal research (see also Helgeson, Snyder, & Seltman, 2004; Newsom & Schulz, 1998; U. Schulz & Schwarzer, 2004; Suls et al., 1997). The current longitudinal research has generally examined dyadic coping as perceived by the patient and patient and spousal adjustment at Time 1, examining the effect of dyadic coping on adjustment at a later time. Our model suggests that also examining how adjustment at Time 1 affects subsequent dyadic coping processes would reveal the transactional nature of dyadic coping and adjustment processes, both of which are likely to change over time. Our model predicts not only that dyadic coping will predict subsequent adjustment, as supported by the current literature (Helgeson, Novak, et al., 2004; Suls et al., 1997), but that poor adjustment may limit a spouse's ability to either support or collaborate with his or her spouse (Bodenmann et al., 2004).

In addition to short-term longitudinal research, the field would benefit from the use of frequent daily assessments such as are used in work on daily pain and stress (Affleck et al., 1998; Grant, Long, & Willms, 2002; Romano et al., 1992; Zautra, Smith, Affleck, & Tennen, 2001) and recent work in collaborative coping and emotion (Berg, Wiebe, Bloor, et al., 2007). Daily process research will reveal whether effective dyadic coping (e.g., collaboration) leads to more positive outcomes (lower depression, higher marital satisfaction) and/or results from a healthy working relationship in which individuals are adjusting well. Individuals with compromised mood, such as depression, may not be able to engage their resources in a high level of interpersonal involvement (Bodenmann et al., 2004) with sensitive communication. This type of work will require the use of statistical techniques that focus on the dyad as the unit of analysis, such as Kenny's (1990) actor–partner model (see also Badr, 2004; Hong et al., 2005) and multivariate hierarchical linear modeling with application to matched pairs (Raudenbush, Brennan, & Barnett, 1995).

This daily diary work will reveal coupled patterns of dyadic involvement and adjustment (see Bolger et al., 2000, for dyads coping with stressful events over time). Sequences of highly engaged coping (mutual collaboration) by couples may be in response to extremely stressful events that evoke high negative emotion (Hagedoorn, Kuijer, et al., 2000) and may be associated with decreases in negative mood over time. The combination of daily process and short-term longitudinal research will help untangle whether some dyadic coping strategies such as collaborative coping by the spouse may exert a cost on the spouse (despite the benefit for the patient) in the short term but be beneficial in the long term, especially for marital satisfaction. The existing literature suggests that when negative affect is high, collaboration with one's spouse may be detrimental for daily mood due to emotional contagion (Hatfield, Cacioppo, & Rapson, 1994); however, such collaboration may be important in the long term for marital satisfaction and adjustment (Manne et al., 2006). As the dyadic coping literature has focused heavily on negative rather than positive facets of adjustment (e.g., positive mood, meaning finding), the relations between dyadic coping processes and positive affect and meaning finding should also be examined (Folkman & Moskowitz, 2000). The effect of dyadic coping on positive and negative mood may be different as these are two separate dimensions of affective experience (Watson, Weise, Vaidya, & Tellegen, 1999; Zautra, 2003).

The developmental–contextual model suggests there are temporal points when it might be crucial to target daily process assessments in the context of specific illnesses (at diagnosis, when initiating treatment, soon after treatment ends). These time points may vary for different diseases. For illnesses that are recurrent (e.g., cancer), assessments could be timed around routine screening for recurrence (Revenson & Pranikoff, 2005). Illnesses that have a high daily management component (e.g., diabetes, pain) are easiest for determining time assessments, as couples must cope nearly daily with stressful events surrounding the disease. Following couples across longitudinal time for illnesses that involve progressive deterioration or downturns (Erdal & Zautra, 1995), especially in communication and cognitive function, will be particularly beneficial for understanding change in coupled patterns (e.g., change from mutual collaboration to spouse supporting the patient who becomes uninvolved in coping efforts). We hypothesize that spousal adjustment will be most affected when deteriorations in the patient's ability to communicate restrict the range of dyadic coping strategies. In addition, an important transition point for the couple may be when the healthy partner becomes ill and dyadic processes must be rearranged. Further, the model is suggestive of illness dimensions on which dyadic coping should be targeted (e.g., comparisons of illnesses that have a high vs. low relationship impact, comparisons of illnesses where communication is impaired vs. maintained). Such work must analyze data with an eye toward age and cultural differences, allowing for conclu-

sions as to whether the dyadic process is similar across the life span and across cultures.

The literature has relied heavily on an individual's self-report of the spouse's involvement as the primary measure of appraisal and dyadic coping. One limitation of this reliance on self-report is that measurement of dyadic coping strategies may be confounded with psychological adjustment (L. B. Story & Bradbury, 2004). Individuals who are experiencing negative mood may interpret their spouse's involvement as unsupportive and controlling. Although the social support literature shows that a person's perception of spousal involvement is key to understanding that person's adjustment (Uchino, 2004), the field would profit by incorporating a multifaceted assessment of dyadic illness appraisal and coping that includes different types of data (e.g., self-report, behavioral interactions, linguistic analyses). The extensive work of Bodenmann and colleagues (as reviewed by Bodenmann, 1997) and Manne and colleagues (Manne, Ostroff, Rini, et al., 2004; Manne, Ostroff, Sherman, et al., 2004), utilizing a wide range of methods (including interview, questionnaires, diaries, and behavioral interaction), bolsters findings for the beneficial effects of mutual support and collaboration on adjustment and marital satisfaction. Other promising approaches include Pistrang et al.'s (1997) paradigm, where the patient discloses an area of distress to the spouse and both interpret their intentions in communication, supplementing it with behavioral interactions of patient and spouse. Spouses' self-report of the coping strategies used in the interaction and their detailed analysis of the meaning ascribed to those ongoing interactions will address how the spouse's involvement is perceived as compared with how it is intended. These approaches could be supplemented by other dyadic approaches, such as analyzing behavioral interactions for "attunement in couples" (see Fogel, 1993, and Hsu & Fogel, 2001, for this approach in mother–child communication), linguistic analyses of relational language ("we" vs. "I"; Acitelli & Badr, 2005; Pennebaker et al., 2003), and coupling of spousal emotions (Butner, Diamond, & Hicks, in press) and coping strategies.

The integration of behavioral interaction research with stress and coping has the potential to provide a much needed theoretical framework to guide the various distinctions of dyadic coping and work toward common assessment measures that are theoretically derived and psychometrically sound. For instance, Trobst (2000) applied the interpersonal circumplex model (e.g., Kiesler, 1996) to social support interactions. By projecting different forms of support (e.g., emotional support, empathic concern, helpful, nonhelpful) onto a two-dimensional space (dominant to submissive and warm–agreeable to coldhearted), Trobst found that beneficial forms of support were characterized by high warmth and moderate direction. Unhelpful support was characterized by low warmth and moderate direction. Extending this interpersonal circumplex to understand forms of dyadic coping may require inclusion of not only how the interpersonal behavior is construed but also how the stressor is appraised ("mine" vs. shared). For instance, collaboration and support may both be construed as interpersonal behavior that is warm and moderately directive. However, the distinction between viewing the spousal behavior as support versus collaboration may lie in whether the spouse identifies or appraises the stressor as his or her own. By combining self-report methods with behavioral interaction, we can ascertain what factors are involved in the actual perception of and meaning ascribed to the spouse's involvement (e.g., what triggers a construal of control, support, or collaboration).

Research on dyadic appraisal and coping needs to move beyond mental health outcomes to examine the physiological concomitants associated with poorer adjustment. The role of physical symptoms of the disease and couple congruence in both psychosocial adjustment and perceptions of physical symptoms may be important in understanding the psychosocial adjustment of both patient and spouse (Cremeans-Smith et al., 2003; Druley et al., 2003). Dyadic coping may relate to many health outcomes, as marital satisfaction is associated with health (Burman & Margolin, 1992), including lower heart rate and blood pressure (Carels, Sherwood, & Blumenthal, 1998; Kiecolt-Glaser & Newton, 2001) and better immunological function (Robles & Kiecolt-Glaser, 2003). Similarly, social support can reduce the physiological effects of stress through appraisal processes (Uchino, 2004) and facilitate more positive health practices (e.g., obtaining preventive health screenings, seeking earlier treatment; Berkman, 1995; DiMatteo, 2004). To the extent that effective dyadic coping occurs in the context of better and more supportive marital relationships and may lead to improved marital satisfaction, these physiological processes are likely to be at work as couples deal with conflicts and problems associated with the chronic illness (see Zautra et al., 1998, for a demonstration of the link between spousal criticism and disease activity in arthritic patients; see Wiebe et al., 2005, for a link between collaborative coping and adherence in adolescents with Type I diabetes).

Intervention Implications

In addition to methodological implications, the work on shared appraisal and dyadic coping has important intervention implications for patients and spouses coping with chronic illness. Two meta-analyses have indicated that including the spouse in a psychosocial intervention for chronic illness is more effective than interventions focused solely on the patient (Martire, 2005) or typical medical care (Martire, Lustig, Schulz, Miller, & Helgeson, 2004). The effects of spousal interventions were more pronounced when the intervention explicitly dealt with relationship issues (e.g., communication regarding the illness and how illnesses can affect relationship quality; see Scott, Halford, & Ward, 2004, for an example). Manne, Ostroff, Winkel, Fox, et al. (2005) found that for breast cancer patients, couple-focused intervention benefited couples most for women who initially perceived their spouses to be most unsupportive. Thus, even for patients initially at greater risk for not experiencing the beneficial forms of dyadic coping (those whose spouse is perceived to be unsupportive and experiencing more physical impairment), couple-based intervention is effective. Couple interventions have also been successful in illnesses involving pain (Keefe et al., 1996, 1999), a finding that is impressive in light of the difficulties in understanding beneficial forms of dyadic coping in dealing with pain.

Interventions situated squarely within the dyadic coping perspective (Bodenmann, Charvoz, Cina, & Widmer, 2003; Bodenmann & Shantinath, 2004; Kayser, 2005; Widmer, Cina, Charvoz, Shantinath, & Bodenmann, 2005) hold great promise for strengthening the marital relationship as couples deal with chronic illness. These approaches work directly on components of effective dyadic coping such as understanding the other person's perspective re-

garding stress, couple communication, mutual problem-solving skills, and coordination and collaboration regarding daily management tasks. These interventions are demonstrating gains in adaptive forms of coping (e.g., collaboration) and reductions in maladaptive forms (e.g., hostile dyadic coping) in long-term married couples and across time. The effects are particularly strong for women. Such interventions may provide not only greater facilitation for the patient but also much needed intervention for the distressed spouse.

Summary

Dyadic coping with a chronic illness is a process in which patients and spouses are situated in a context where their adjustment, appraisal, and coping efforts exist in relation to each other. The developmental–contextual model provides a framework for understanding how this way of relating to one another is a process that may vary across the life span and at different phases of chronic illness. Couples engaged in dyadic coping are affected by broad sociocultural factors (e.g., culture, gender) and more proximal contextual factors (e.g., marital quality, specific illness conditions) that relate to the way each partner sees him- or herself in relation to the spouse. The developmental and contextual nature of this framework points to a new look for research in stress and coping that may address how spousal involvement is interpreted and how this may change across time in a way that moves beyond current individualistic approaches in the field. Future work needs to establish how this approach may be applied to couples outside the context of chronic illness (Bodenmann, 2005) and other types of partnered relationships.

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Genetics inMedicine | ORIGINAL RESEARCH ARTICLE

An informatics approach to analyzing the incidentalome

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Purpose: Next-generation sequencing has transformed genetic research and is poised to revolutionize clinical diagnosis. However, the vast amount of data and inevitable discovery of incidental findings require novel analytic approaches. We therefore implemented for the first time a strategy that utilizes an a priori structured framework and a conservative threshold for selecting clinically relevant incidental findings.

Methods: We categorized 2,016 genes linked with Mendelian diseases into "bins" based on clinical utility and validity, and used a computational algorithm to analyze 80 whole-genome sequences in order to explore the use of such an approach in a simulated real-world setting.

Results: The algorithm effectively reduced the number of variants requiring human review and identified incidental variants with likely clinical relevance. Incorporation of the Human Gene Mutation Database improved the yield for missense mutations but also revealed that a substantial proportion of purported disease-causing mutations were misleading.

Conclusion: This approach is adaptable to any clinically relevant bin structure, scalable to the demands of a clinical laboratory workflow, and flexible with respect to advances in genomics. We anticipate that application of this strategy will facilitate pretest informed consent, laboratory analysis, and posttest return of results in a clinical context.

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Key Words: clinical informatics; incidental findings; secondary findings; whole-exome sequencing; whole-genome sequencing

INTRODUCTION

The rapidly decreasing cost of whole-genome sequencing (WGS) and its ability to simultaneously analyze all human genes make it an attractive technique for genetic diagnosis. Early anecdotal reports describing the use of WGS or whole-exome sequencing (WES) have demonstrated the power of these new technologies to impact patient care.[1–3] However, there exist significant barriers to the widespread application of WGS/WES in clinical medicine. Technical hurdles are being addressed in the marketplace, where competition will lead to faster, cheaper, and more accurate sequencing.[4] Practical obstacles such as the time and effort required for analysis of clinically relevant variants, and return of complex results to patients, will require transition from traditional genetic testing approaches.

In a clinical environment, the most productive use of WGS/WES will likely be in the diagnosis of patients with distinctive features suggestive of a genetic disorder. In these individuals, there will also be genetic findings unrelated to the presenting symptoms, which are "incidental" or "secondary" findings, the aggregate of which has previously been termed the "incidentalome."[5] Arguably, the vast majority of an individual's genetic variants will be unrelated to the presenting symptoms. Therefore, the problem of how to deal with incidental findings poses a formidable problem for clinicians and laboratorians.

In the pursuit of evidence-based genomic medicine, it will be vital to avoid overwhelming patients and physicians with genomic findings of dubious clinical value. Because the use of common single-nucleotide polymorphisms for prediction of common disease risk is still of limited value clinically,[6] we have chosen to focus on monogenic disorders. Given that any individual has a very small a priori likelihood of being affected with an incidentally identified Mendelian disorder, few truly disease-causing genetic variants are expected per person. Therefore, any attempt to sift through genomic data for clinically relevant incidental findings will benefit from the recognition that the vast majority of variants bear negligible clinical significance. In other words, the identification of incidental findings should maximize specificity.

The challenge, therefore, is to synthesize collective knowledge about the genetic causation of disease and implement a practical, clinically oriented approach to the analysis of genome-scale variant data. We recently described a conceptual strategy for classifying genes into "bins" to facilitate informed consent, analysis, and return of incidental findings in a clinical setting.[7] In our proposed system, the first step is to assign genes to bins according to features such as clinical utility/actionability (Bin 1) and clinical validity (Bin 2), and the potential to cause harm (Bin 2a, 2b, 2c; see **Supplementary Materials and Methods** online for details). The second step is to select the variants in a given individual that merit detailed review. The third step involves human review of the resulting subset of variants. Because a variant of uncertain significance (VUS), by definition, has no known clinical value,

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only known mutations or likely disease-causing novel mutations would be reported as incidental findings.

Variants identified by any sequencing method can be readily sorted based on their genomic location (whether they fall within a "binned" gene) and further annotated in terms of effect on the translated protein and predicted zygosity. For recessive disorders in which a single heterozygous mutation signifies the carrier state but is not considered disease causing, heterozygous variants would be moved into a separate category, "Bin R," for reproductive implications. Our binning approach thus attempts to capture clinical differences between genes and organize them into a succinct number of categories to facilitate the pretest counseling and posttest reporting of suspected disease-causing variants when discovered incidentally during WGS/WES.

The goals of this endeavor were to evaluate the average number and type of potentially clinically relevant incidental findings and the impact of various "filters" on the output of the proposed analytic framework. We implemented a prototype of this strategy with an analysis of 80 whole genomes as a proof-of-concept, showing that multiple genomes can be efficiently analyzed to identify clinically relevant variants. This strategy can be refined with advances in our understanding of disease-causing and benign variants and offers an initial means of structured clinical assessment of WGS/WES data in a practical and efficient manner.

SUBJECTS AND METHODS

Binning of OMIM genes

OMIM files (accessed June 2011) containing entries for 12,786 genes were scrutinized using OMIM, PubMed, Gene Reviews, and other resources. Genes were placed into Bin 3 (no clinical implications) if there was no indication of association with a Mendelian disorder, if only somatic mutations were reported, or if limited evidence of pathogenicity was available. Loci mapped by linkage, for which specific genes/mutations have not been documented, were also removed from consideration. A total of 2,016 genes associated with Mendelian disorders were identified, and their respective inheritance patterns were determined.

We made two judgments about genes involved in Mendelian disorders: (i) most genes do not have clinical utility/actionability in terms of definable preventive measures or treatment and (ii) for most Mendelian disorders, the potential for psychosocial harm caused by their incidental discovery is neither trivial nor highly concerning. Therefore, all 2,016 genes were initially placed in Bin 2b. We then manually reviewed each gene and applied a first-order approximation of the previously outlined criteria to provisionally place each gene into a bin. Genes for which there existed a reasonable suggestion of beneficial interventions were provisionally assigned to Bin 1. Genes having potentially significant risk of psychosocial harm were provisionally assigned to Bin 2c.

Genome sequences

WGS was performed by Complete Genomics (Mountain View, CA).[8] Nineteen genomes were from patients enrolled in an institutional research board–approved research study for genetic evaluation of hereditary cancer susceptibility. Sixty-one genomes, representing presumably healthy individuals from diverse ethnic groups, were made publically available by Complete Genomics (http://www.completegenomics.com/sequence-data/download-data/). All genome coordinates are based on NCBI build 37.

Databases and computational analysis

Tables containing variant calls and annotations were stored in a PostgreSQL 8.4.3 database and joined with a table of allele frequencies generated from phase I consensus single-nucleotide polymorphism sites (2 May 2011) from the 1000 Genomes Project and small insertion/deletion calls from the 1000 Genomes pilot paper dataset (20 October 2010).[9] To address differences in allele frequency (AF) between different populations, we used the highest minor AF reported for a given variant in any of the phase I population groups. A local instance of the Human Gene Mutation Database (HGMD)[10] was created in another PostgreSQL database. Genomic coordinates for HGMD mutations were lifted over to NCBI Build 37 and converted to the Complete Genomics standard variant format. Variants matching with annotated disease mutations ("DM" variants) could then be readily identified in the 80 WGS samples.

A Python (2.6.5) script was written to iterate through variant files and select variants meeting the criteria outlined in the manuscript. Because Complete Genomics independently calls each allele, two separate lines in the variant file represent homozygous variants. The script collapses homozygous positions to a single line and indicates the zygosity of the variant in a separate field. For genes associated with autosomal recessive disorders, the script counts the number of variants meeting the predefined criteria and, if only one heterozygous variant exists, annotates that variant as signifying carrier status. The algorithm thus recognizes the potential for biallelic mutations (although it is important to note that further investigation is required to adjudicate whether the mutations are in *cis* or in *trans*).

RESULTS

To demonstrate the applicability of the proposed analytic framework, we provisionally binned 2,016 genes implicated in Mendelian disorders, implemented a computational analytic pipeline, and explored the output from 80 whole-genome sequences. In this first attempt at binning the genome (**Supplementary Table S1** online), 161 genes were assigned to Bin 1, 1,798 genes were assigned to Bin 2b, and 57 genes were assigned to Bin 2c. We emphasize that the binning of genes used in this study is provisional and used for illustrative purposes; the final population of bins will change over time and the choices made by our group and others may well differ.

We then explored parameters (AF cut-offs and effect of the mutation) used to select variants for further manual review (**Figure 1**). The total number of variants selected (**Figure 1a**) is decreased 10–20 fold using AF filters of <5 or <1% (**Figure 1b**). Selecting for protein-altering variants (missense, nonsense, frameshift, and splice-site) further decreases this number (**Figure 1c**). However, the resulting numbers are

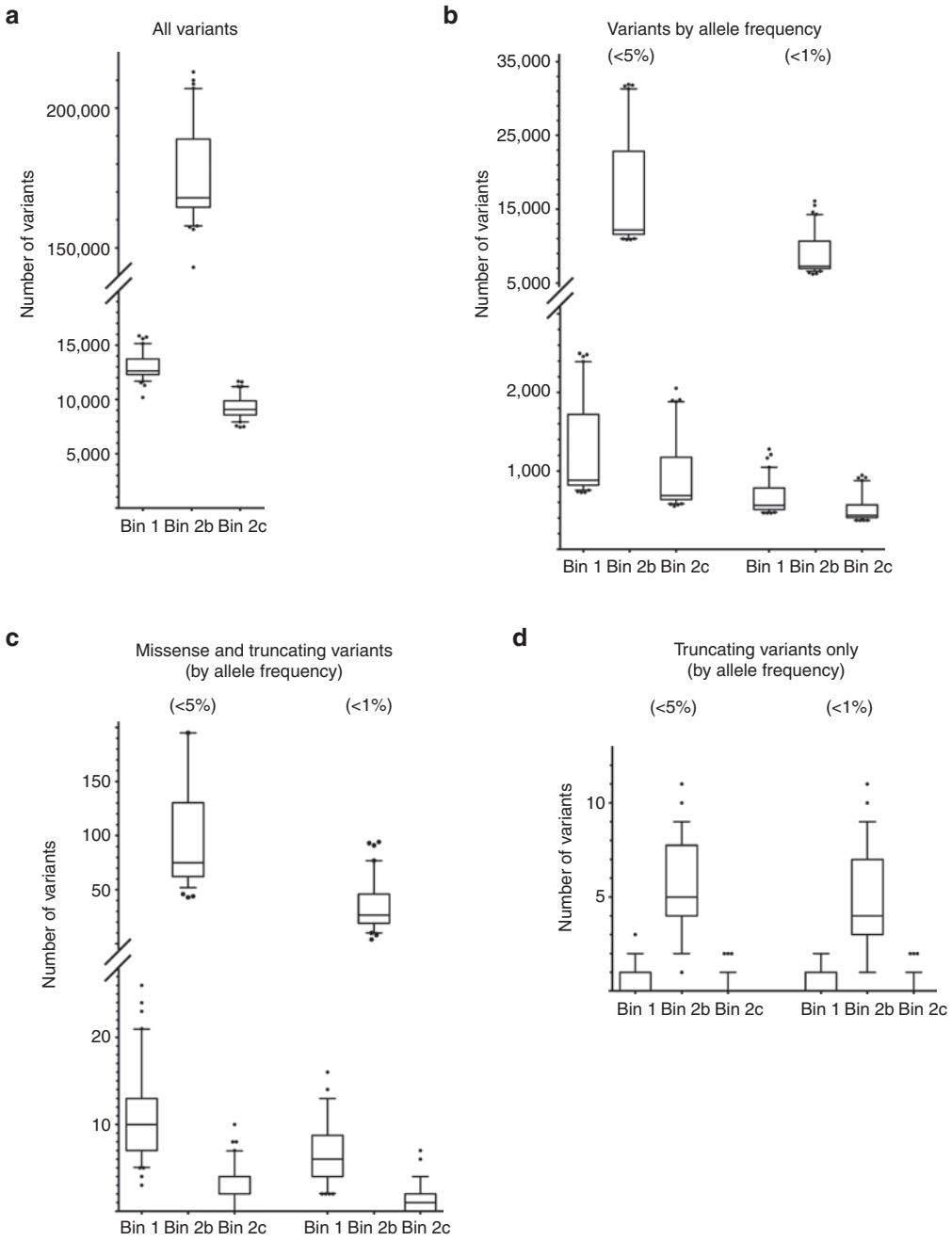

Figure 1 Selection of variants based on allele frequency and predicted effect on the translated protein. (**a**) The initial informatics analysis resulted in an average of ~13,000 variants per person in Bin 1 genes, ~175,000 variants per person in Bin 2b genes, and ~9,000 variants per person in Bin 2c genes. (**b**) Limiting these variants to <5% allele frequency (AF) or <1% AF reduces these counts ~10–15 fold. (**c**) Restricting to protein-coding variants (missense, nonsense, frameshift, and splice-site) at <5% AF results in ~10 variants per person in Bin 1 genes and 100–200 variants per person in Bin 2b genes. At <1% AF there were ~5 variants per person in Bin 1 genes and 50–100 variants per person in Bin 2b genes. (**d**) Restricting only to truncating variants (nonsense, frameshift, and splice-site) results in only a small number of variants to be analyzed by the reviewer. Of note, the AF cut-off (<5 vs. <1%) does not dramatically affect the number of truncating variants that are selected.

still incompatible with the small chance of an individual having a Mendelian disorder; thus, the vast majority of variants with <5% AF must have minimal clinical consequences. When selecting only predicted truncating (nonsense, frameshift, and splice-site) variants, the number identified per patient is more consistent with realistic expectations (**Figure 1d**).

Clearly, the sensitivity of the algorithm is decreased by the exclusion of rare missense mutations. To address this issue, we queried a local instance of HGMD for variants in these genes annotated as "DM" and identified 871 unique variants (771 missense) among the 80 whole genome sequences. On average there were 74 (range 61–106) "DM" variants per

person (Figure 2a), which is strikingly similar to the report of the 1000 Genomes Project Consortium that individuals are heterozygous for 50–100 variants classified as disease causing in HGMD.[9] Nevertheless, this large number of putatively disease-causing mutations is surprising, given the very low probability of a Mendelian disorder truly being present in any of the subjects.

Because 88% of the unique "DM" variants were missense substitutions, we hypothesized that these variants could comprise a subset of the ~150 missense variants per person identified in Bins 1, 2b, and 2c with <5% AF (Figure 2a). Surprisingly, there was minimal overlap between the less common missense variants and "DM" variants detected in the 80 genomes (Figure 2b), and upon further review, 251 of the 871 unique "DM" variants (29%) had >5% AF. As a result, 78% of "DM" variants per person were >5% AF (Figure 2c). This finding is similar to that of a previous report that 74% of HGMD variants identified in 448 genes implicated in severe recessive diseases of childhood were variants with >5% AF.[11] Although some of these variants could represent recessive alleles that are relatively frequent in certain populations, this explanation cannot account for the vast majority of these variants.

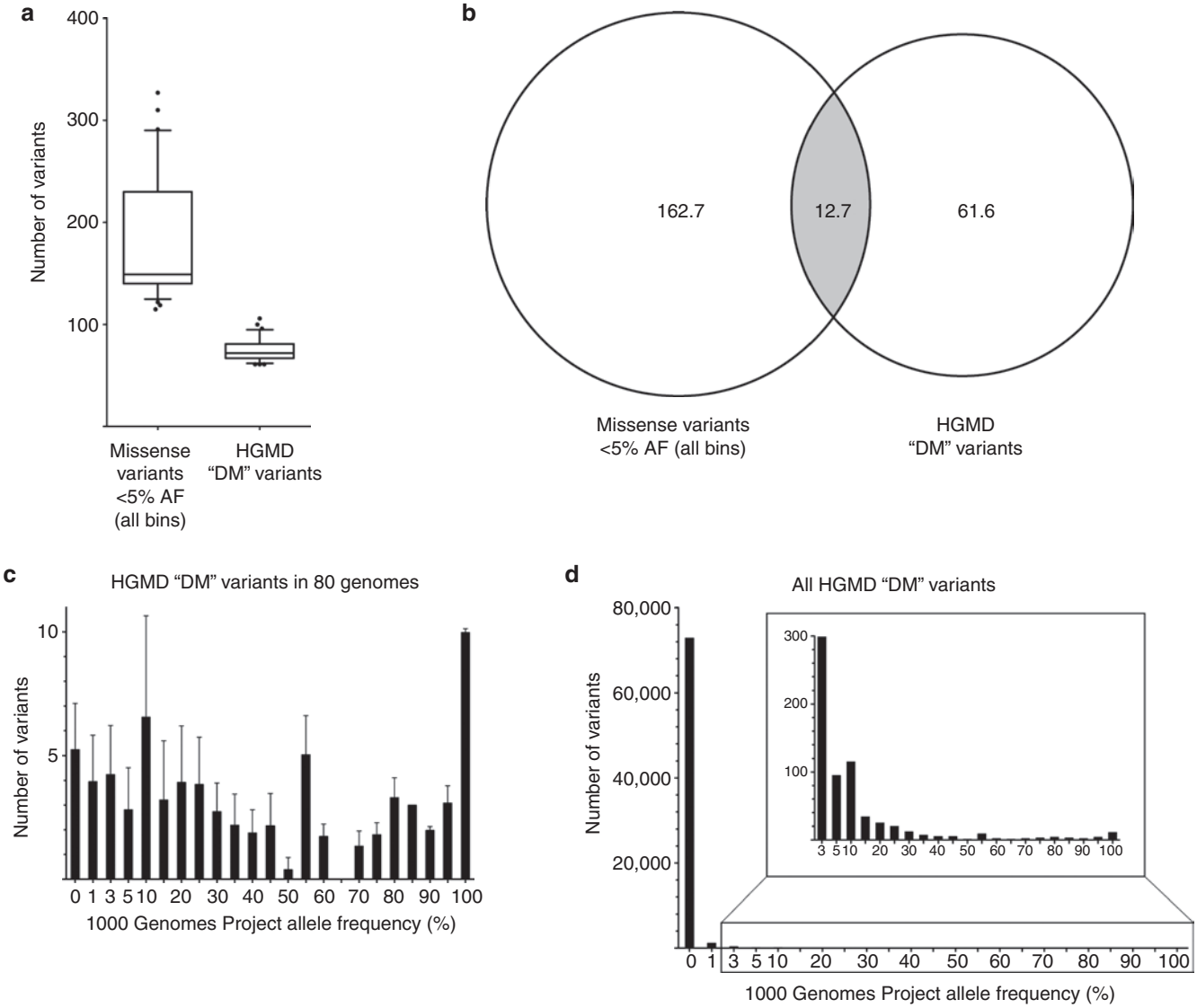

Figure 2 Analysis of mutations annotated as "DM" (disease mutation) in the Human Gene Mutation Database (HGMD). (**a**) All variants were queried against the HGMD to identify variants classified as "DM." The numbers of rare (<5% allele frequency) missense variants in all bins and the numbers of HGMD "DM" variants per person are depicted as a box-whisker plot with whiskers indicating the 5th and 95th percentiles and outliers shown as filled circles. Homozygous variants are counted twice. (**b**) The overlap between the rare missense variants and "DM" variants is depicted as a Venn diagram. (**c**) The maximum 1000 Genomes Project allele frequencies were determined for each variant identified in the 80 whole genomes, and histograms of allele frequencies were generated for each person. These histograms were then combined to depict the average number of variants per person within each range of allele frequencies (depicted as a bar plot with standard deviations). (**d**) The maximum 1000 Genomes Project allele frequencies were determined for all "DM" variants in HGMD and graphed as a histogram. The inset shows the distribution for "DM" variants with ≥3% allele frequencies.

To further assess the pervasiveness of misleading database errors, we queried the 1000 Genomes Project allele frequencies and found allele frequencies for 1,811 of 74,694 "DM" variants (mostly substitution variants). Of these, 1,152 had <1% AF, 299 had 1–3% AF, 95 had 3–5% AF, and 265 (~0.35% of all "DM" variants) had >5% AF (**Figure 2d**). The small subset of variants with >5% AF comprised the majority of "DM" variants identified in a given genome sequence, simply because of the prevalence of these variants in the general population; in subsequent analyses we restricted HGMD variants to those with <5% AF.

The final algorithm selected variants according to the following criteria: (i) presence in a binned gene, (ii) <5% AF, and either (iii) annotation as a disease-causing mutation ("DM") in HGMD or (iv) predicted to be truncating. Variants were further analyzed for zygosity to assign single heterozygous variants in recessive genes to a separate category for carrier status (Bin R). When we applied this algorithm to the 80 genomes, a total of 1,391 variants (906 unique variants) were selected. The per-person averages were 1.5 variants in Bin 1 genes, 6.4 variants in Bin 2b genes, 0.2 variants in Bin 2c genes, and 9.2 variants considered to imply carrier status for recessive disorders (**Table 1** and **Supplementary Table S2** online).

The variants selected by the algorithm were then manually reviewed using a combination of OMIM, PubMed, Google Scholar, UCSC genome browser, and locus-specific databases to assess the evidence for pathogenicity or to reclassify the variants selected from the 80 genomes. Variants were reclassified if two variants identified in an individual likely comprised a single complex substitution allele or comprised a single common haplotype. In many cases, variants annotated as "DM" in HGMD were reclassified as VUS or likely polymorphisms. In other cases, the type of variant or its location within a specific transcript was inconsistent with a pathogenic effect. Zygosity was reassessed when it was determined that two variants were likely to be in *cis* or that only one of the selected variants in a gene was likely to be pathogenic; in these cases, the remaining heterozygous variant was reassigned to Bin R. **Table 2** shows examples of binned variants, reclassified variants, and variants removed from consideration. Several detailed examples are described in the **Supplementary Materials** online. A list of binned variants from the 61 publically available genomes is available in **Supplementary Table S3** online.

After review, 705 variants were removed from consideration and 71 were reassigned to carrier status. Differing percentages of variants were reclassified or removed from consideration in each bin category (**Figure 3a**) and lower proportions of novel variants were removed (**Figure 3b**) as compared with HGMD "DM" variants (**Figure 3c**). In all, 279 of the 358 unique variants removed from consideration were HGMD "DM" variants. After the final analysis, the revised per-person averages were 0.3 variants in Bin 1 genes, 2.6 variants in Bin 2b genes, 0.06 variants in Bin 2c genes, and 5.5 variants considered to imply carrier status (**Table 1** and **Supplementary Table S2** online).

DISCUSSION

One barrier to the clinical use of WGS/WES is the legitimate concern that the burden of incidental findings will be overwhelming and lead to expensive and unnecessary follow-up despite little evidence that such variants have a strong role in causing disease.[5,12] The approach we describe here demonstrates that analysis of WGS/WES data for clinically significant incidental variants is a tractable problem and that manageable numbers of variants can be selected for manual review.

Predictive value of variants identified in an incidental context

These results indicate that a small number of potentially disease-causing variants can be readily identified using a relatively straightforward process consisting of a priori gene classification, computational filtering, and database queries. As with any medical test, the analytic parameters used in this approach represent a trade-off between sensitivity and specificity. The choices outlined in our strategy reflect the impact of sensitivity and specificity on the calculation of the negative predictive value and positive predictive value. When the prior probability of disease is very low (e.g., the chance of having a Mendelian disorder that would be discovered incidentally), a test with reduced specificity will yield results with poor positive predictive value, whereas reduced sensitivity has negligible effect on the negative predictive value. We have therefore chosen to set a threshold that emphasizes specificity, in order to enrich for incidental findings that have a high likelihood of representing truly disease-causing mutations.

Because selection of rare missense variants in known disease genes results in a large number of VUSs, which provide no

Table 1 Numbers of variants selected by the informatics algorithm

	Bin 1	Bin 2b	Bin 2c	Bin R
Total variants per person	13,129.7 (10,268–15,993)	174,576.7 (144,371–212,760)	9,251.6 (7,472–11,663)	ND
<5% AF	1,219.8 (732–2532)	16,362.1 (10,845–31,861)	915.5 (551–2,053)	ND
<5% AF and either "DM" in HGMD or predicted truncating	3.0 (0–9)	14.2 (5–26)	0.45 (0–3)	ND
<5% AF and either "DM" in HGMD or predicted truncating, analyzed for zygosity	1.5 (0–5)	6.5 (2–14)	0.2 (0–2)	9.2 (0–17)
Revised after manual review	0.3 (0–2)	2.6 (0–8)	0.06 (0–1)	5.5 (0–12)

AF, allele frequency; DM, disease mutation; HGMD, Human Gene Mutation Database; ND, not done.

Table 2 Selected examples of selected variants, reclassified variants, and variants removed from consideration after human review

Subject	Gene (OMIM no.)	NCBI 37 location	Strand	Ref.	Call	Impact	dbSNP	AF	HGMD	HGVS	Comments
Examples of predicted Bin 1 disease-causing mutations											
NA18956	*COL3A1* (120180)	chr2:189870930–189870930	+	–	insG	Splice-site, frameshifting	N/A	N/A	N/A		Type IV Ehlers-Danlos syndrome ("vascular" subtype)
NA12877	*FBN1* (134797)	chr15:48779379–48779381	–	GC	T	Frameshifting substitution	N/A	N/A	N/A		Marfan syndrome
NA18947	*KCNH2* (152427)	chr7:150645539–150645540	–	G	A	Missense	N/A	N/A	CM085481	NM_000238.2 c.2684C>T (T895M)	Long QT syndrome 2
NA12883	*MSH2* (609309)	chr2:47637458–47637458	+	–	insG	Frameshifting insertion	rs63750786	N/A	CI041960	NM_000251.1 c.592dupG	Lynch syndrome
Examples of predicted Bin 2c disease causing mutations											
NA18505	*ITPR1* (147265)	chr3:4777025–4777025	+	–	insC	Frameshifting insertion	N/A	N/A	N/A		Spinocerebellar ataxia 15; alternatively spliced exon
NA12881 NA19025	*PRKCG* (176980)	chr19:54410082–54410084	+	GA	delGA	Frameshifting deletion	N/A	N/A	N/A		Spinocerebellar ataxia 14
Examples of variants reclassified to carrier status											
NA18504	*MEFV* (608107)	chr16:3299467–3299468	–	C	T	Missense	rs11466024	0.024	CM990838	NM_000243.1 c.1223G>A (R408Q)	AR familial Mediterranean fever; R408Q and P369S reported in *cis* as single allele with highly variable clinical phenotype[11]
		chr16 3299585–3299586	–	G	A	Missense	rs11466023	0.041	CM990837	NM_000243.1 c.1105C>T (P369S)	
NA18947	*SLC34A1* (182309)	chr5:176815191–176815192	+	T	G	Splice-site disrupt	N/A	N/A	N/A		AD nephrolithiasis (heterozygous missense mutations);AR Fanconi renotubular syndrome (homozygous or compound heterozygous null mutations)
Examples of variants removed from consideration											
NA19238	*APC* (611731)	chr5:112173898–112173899	+	C	T	Missense	rs33974176	0.037	CM080070	NM_000038.3 c.2608C>T (P870S)	AD familial adenomatous polyposis; likely polymorphism
NA18505 NA19020 NA19025 NA19700	*ATM* (607585)	chr11:108121732–108121733	+	G	A	Missense	rs2235000	0.041	CM024583	NM_000051.3 c.1541G>A (G514D)	AR ataxia telangiectasia; presence of G514D and H1380Y in four unrelated individuals of African origin suggests a single allele with variants in *cis*; likely polymorphism
		chr11:108159731–108159732	+	C	T	Missense	rs3092856	0.044	CM021944	NM_000051.3 c.4138C>T (H1380Y)	
NA19025 NA19239 NA19240	*BRCA2* (600185)	chr13: 32914838–32914839	+	A	G	Missense	rs55953736	0.004	CM022331	NM_000059.3 c.6347A>G (H2116R)	AD hereditary breast and ovarian cancer susceptibility; variant of uncertain clinical significance

AD, autosomal dominant; AF, highest minor allele frequency among 1000 Genomes Project populations; AR, autosomal recessive; dbSNP, the single nucleotide polymorphism database; HGMD, Human Gene Mutation Database; HGVS, Human Gene Variation Society nomenclature; N/A, not available ; NCBI 37, National Center for Biotechnology Information human reference genome assembly 37.

Table 2 Continued on next page

Table 2 Continued.

Subject	Gene (OMIM no.)	NCBI 37 location	Strand	Ref.	Call	Impact	dbSNP	AF	HGMD	HGVS	Comments
NA18502	*LRRK2* (609007)	chr12:40704361–40704362	+	C	T	Nonsense	rs114908017	0.001	N/A		AD Parkinsonism; variant of uncertain clinical significance as disease-causing mutations are typically missense
NA12877	*HTT* (613004)	chr4:3127346–3127347	+	G	delG	Frameshifting deletion	N/A	N/A	N/A		AD Huntington; variant of uncertain clinical significance as disease-causing mutations are typically triplet repeat expansions
NA12878	*KIF1B*	chr1:10425554–10425554	+	–	insC	Frameshifting insertion	N/A	N/A	N/A		AD CMT2A1; sequential variants that likely return to correct reading frame, resulting in two amino-acid missense alterations
		chr1:10425557-10425558	+	G	delG	Frameshifting deletion	N/A	N/A	N/A		

AD, autosomal dominant; AF, highest minor allele frequency among 1000 Genomes Project populations; AR, autosomal recessive; dbSNP, the single nucleotide polymorphism database; HGMD, Human Gene Mutation Database; HGVS, Human Gene Variation Society nomenclature; N/A, not available ; NCBI 37, National Center for Biotechnology Information human reference genome assembly 37.

"actionable intelligence" for a clinician or patient, we excluded missense variants unless annotated as "DM" in HGMD. Various algorithms are used in research to predict the likely functional consequences of missense variants,[13] but these programs are not clinically validated[14] and in the absence of other supporting data they are generally insufficient to upgrade the status of a missense variant from VUS to likely pathogenic.[15] The proposed framework also excludes synonymous variants as well as variants in the untranslated portions of the transcript and introns, which are most likely benign but might alter expression of the transcript or cause splicing abnormalities. Although the exclusion of novel missense, synonymous, and noncoding variants decreases the sensitivity of the approach, the lack of any clinically validated means of selecting the true-positive mutations from among the numerous variants of unknown (or no) clinical significance requires that we sacrifice some sensitivity to maintain high specificity. Inclusion of the HGMD substantially increased the sensitivity of the algorithm, but misannotated HGMD "DM" variants (which could represent errors in the medical literature or database curation errors) still constituted a major source of false-positive results.

Because there is no gold standard against which to compare our results, we cannot definitively estimate the clinical sensitivity or specificity of this analytic framework. However, even after manual inspection, the numbers of variants selected per person (**Table 1**) indicate that a number of false positives remain. Some of the putative mutations identified in these 80 genomes could reflect sequencing artifacts, which would be revealed by follow-up Sanger sequencing. Many of the "DM" mutations remaining after manual curation may still represent VUSs or the milder end of the genotype–phenotype spectrum for a given disease. Perhaps more intriguingly, these findings could indicate a much greater degree of clinical variability and incomplete penetrance than has previously been appreciated in Mendelian disorders, which could dramatically impact the logistics of return of such information clinically. We anticipate that improvements in both clinical databases and predictive algorithms will allow us to further improve sensitivity and specificity over time.

Comparison to other reports

The average numbers of potentially clinically important variants identified in this article differ substantially from those of previous efforts to quantify the burden of clinically important incidental findings, and we feel that it represents a more realistic picture of what to expect from WGS in terms of clinical yield. These differences hinge largely on the assumptions made about disease causation and the framework we have chosen for identification of potentially clinically relevant variants. For example, whereas other groups have been inclined to report[1] and/or interpret the possible clinical significance[2] of variants that may modify risks for common diseases, we intentionally ignored common single-nucleotide polymorphisms that are weakly associated with multifactorial diseases. This decision is based on the lack of validated models for incorporating such information into medical care[6] and the inconsistent interpretive results obtained

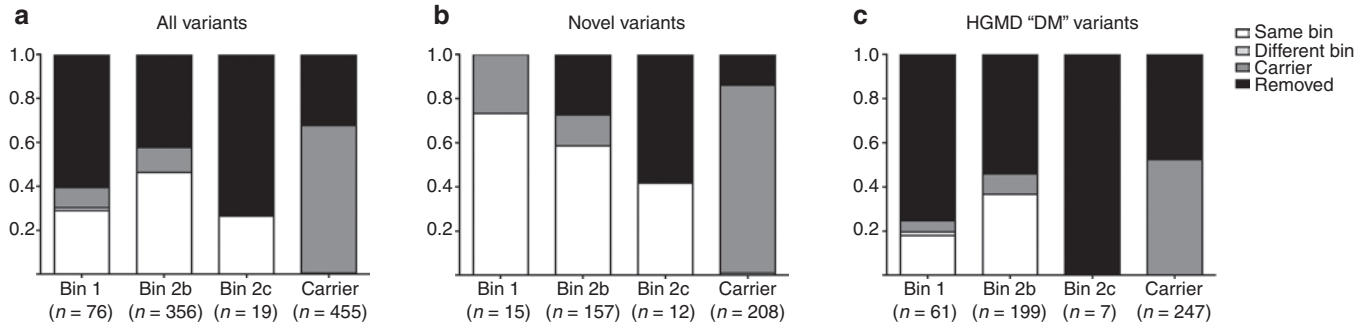

Figure 3 Results of the manual review of variants selected by the informatics algorithm. After individual review of the 906 unique variants returned by the final informatics algorithm, 45% were reassigned or removed from consideration. The graphs depict the variants initially selected within a given "bin" and the stacked segments represent the proportions of those variants that were confirmed, reassigned, or removed after review. (**a**) All 906 unique variants, (**b**) the 392 rare truncating variants identified by the algorithm, and (**c**) the 514 rare "DM" (disease mutation) variants from the Human Gene Mutation Database (HGMD). A higher proportion of "DM" variants in each bin category were removed from consideration as compared with novel truncating variants.

in different labs,[16] although the framework described here could be readily modified to include multifactorial risk calculations if warranted by advances in medical genetics and genomics. Pharmacogenomic variants can also be accommodated in the binning framework but were not considered here.

Cassa et al.[17] estimated that individuals harbor ~2,100 substitution variants that might need to be returned to research subjects, which is four orders of magnitude higher than the 0–2 likely deleterious Bin 1 variants per person identified in this study. Possible explanations for this striking difference are the stringency with which genes are categorized as having clinical utility, and the thresholds for reporting variants. We argue that a relatively high evidentiary standard should be applied in order for a gene to be placed in Bin 1, such that the expected benefits gained by improved medical management would outweigh the possible harms that could arise from the revelation of such a finding in an incidental context. Using these criteria, most known disease genes are placed in Bin 2, in which patient choice is paramount in determining whether such incidental findings should be returned. In addition, we believe that only variants that are known to be pathogenic or highly likely to be pathogenic should be returned in an incidental context. The vast majority (~96%) of variants included in the Cassa et al.[17] study originated from the HGMD, and our current data demonstrate that many of these variants are likely to represent false positives. It is difficult to discern how many of the ~2100 substitution variants per person reported by Cassa et al.[17] are actually benign common polymorphisms, although approximately one-third of these variants were homozygous (suggesting a general population AF substantially >5%), indicating that the putative "reportable" variants identified by Cassa et al.[17] include many variants that are not deleterious and should not be reported either in a research context or a clinical context.

MacArthur et al.[18] reported a survey of loss-of-function variants in the 1000 Genomes Project data and identified many challenges of interpreting WGS/WES data with respect to generating annotations and predicting the effects of possibly truncating variants. A number of known and likely disease-causing loss-of-function mutations were identified among the subjects analyzed, most of which would represent carrier status for autosomal recessive disorders. Again, however, these results point out the difficulty of predicting pathogenicity of a given variant and the importance of review by a clinical molecular diagnostician. Similar to our results, one putative disease-causing mutation listed among the loss-of-function variants by MacArthur et al.[18] was a nonsense mutation in *LRRK2*, which is of uncertain clinical significance because the reported mutations in *LRRK2*-related autosomal dominant Parkinson's disease are missense substitutions.[19]

Challenges and future directions

The bin assignments described here should be viewed as a first step in the development of the binning process. The central concept of Bin 1 is that these findings have sufficient clinical actionability that no preference would be elicited regarding their return (in effect, the "duty to warn" would supersede the patient's autonomy). This denial of the patient's "right not to know" requires us to set a very high threshold regarding the types of findings that are appropriate for this category. On the other hand, our strategy places the majority of disease genes within Bin 2, where the potential risk for harm is the organizing principle, and the concept of individual preference is paramount. Thus, we feel that our strategy strikes a balance regarding patient choice and medical paternalism. A possible future addition might be to subcategorize Bin 2b into disease groups (such as cancer, cardiovascular/sudden death, neurodegenerative, and "other" Mendelian disorders) that would allow a more refined choice in a clinical context. Of course, the disadvantage of introducing more and more categories is that the clinical decision making could devolve into a gene-by-gene menu, which would impose prohibitive demands on clinicians and laboratories with respect to informed consent and analysis.

This provisional binning of genes is not meant to represent a final or definitive list, and we expect that there will be disagreement among experts about the criteria that define Bin 1 or Bin 2 genes, or the types of incidental findings that should routinely

be returned to patients (and how they should be returned) during the course of a genome-scale diagnostic test.[20] Furthermore, there may be differences of opinion regarding the classes of variants that should be reported to patients when discovered incidentally. Our evolving understanding of the genetic underpinnings of disease will necessitate a flexible approach to the structured clinical analysis of genome sequences, and an important future direction will be to establish more granular criteria for determining the novel variants that are selected for review based on the reported spectrum of disease-causing mutations. It is likely that the large numbers of genomes currently being sequenced worldwide will greatly facilitate the clinical interpretation of variants that are found in known disease genes. Better estimates of penetrance will inform the contexts in which certain variants are reported, and many variants previously reported as disease-causing may need to be carefully scrutinized to separate those that are truly deleterious from those that simply reflect normal population variation. Therefore, the value of a centralized and rigorously maintained clinical-grade database containing known variants and their significance cannot be overstated.

Conclusion

These results represent a proof-of-concept demonstration of a structured clinical analysis of incidental findings in genome-scale sequence data that can serve as a general model for assessment of WGS/WES incidental findings. This framework makes the identification clinically relevant incidental findings much more tractable, as it reduces the number of variants requiring hand curation to a manageable number (10–20), and it should prove robust to differing bin structures or gene assignments. We expect that consensus will be possible regarding the bin assignment of many genes,[20] and we note that as of this publication there are ongoing discussions and debate among genetics professionals regarding these issues. Advances in medical genetics will also mandate a periodic re-evaluation of these bin assignments. Nevertheless, we anticipate that assignment of genes to bins based on clinical utility and stratified based on the risk of psychosocial harm will enable efficient analysis of data as well as facilitate pretest informed consent, posttest counseling, and return of results as we enter the era of clinical genomics. Further research on the implementation of this analytic framework and the responses of individuals to incidental findings is under way.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/gim

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DISCLOSURE

The authors declare no conflict of interest.

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ORIGINAL RESEARCH ARTICLE | Genetics inMedicine

Open

A semiquantitative metric for evaluating clinical actionability of incidental or secondary findings from genome-scale sequencing

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Purpose: As genome-scale sequencing is increasingly applied in clinical scenarios, a wide variety of genomic findings will be discovered as secondary or incidental findings, and there is debate about how they should be handled. The clinical actionability of such findings varies, necessitating standardized frameworks for a priori decision making about their analysis.

Methods: We established a semiquantitative metric to assess five elements of actionability: severity and likelihood of the disease outcome, efficacy and burden of intervention, and knowledge base, with a total score from 0 to 15.

Results: The semiquantitative metric was applied to a list of putative actionable conditions, the list of genes recommended by the American College of Medical Genetics and Genomics (ACMG) for return when deleterious variants are discovered as secondary/incidental findings, and a random sample of 1,000 genes. Scores from the list of putative actionable conditions (median = 12) and the ACMG list (median = 11) were both statistically different than the randomly selected genes (median = 7) ($P < 0.0001$, two-tailed Mann-Whitney test).

Conclusion: Gene–disease pairs having a score of 11 or higher represent the top quintile of actionability. The semiquantitative metric effectively assesses clinical actionability, promotes transparency, and may facilitate assessments of clinical actionability by various groups and in diverse contexts.

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Key Words: actionability; genome-scale sequencing; genomic medicine; incidental findings; secondary findings

INTRODUCTION

Genome-scale sequencing inevitably leads to the identification of many genomic variants with vastly differing clinical relevance, which requires development of innovative categorical approaches for informed consent, analysis, and return of results. Clinical genomic sequencing for suspected monogenic disorders may identify millions of genetic variants in a single patient, with only one or two "diagnostic" variants likely to explain the molecular etiology ("primary" results). Virtually all of the remaining variants are "incidental" to the original indication for analysis, although the term "secondary findings" is now the preferred term for such results when sought in a systematic fashion.[1]

We previously proposed a framework for organizing potential incidental/secondary findings into "bins" categorized by clinical validity and clinical utility[2] and developed provisional lists of binned genes.[3] Our goal is to categorize potential findings before their discovery in a patient to guide informed decision making and return of results. As part of a National Human Genome Research Institute–funded Clinical Sequencing Exploratory Research project called "North Carolina Clinical Evaluation by Next-gen Exome Sequencing (NCGENES)," we assembled a Locus-Variant Binning Committee (LVBC) to refine a category of genomic findings that we call "bin 1"—the list of clinically actionable genes to be analyzed for pathogenic variants and returned as part of the routine results.[4] Similar efforts are underway at other institutions and organizations.[5–7]

Recognizing that an expert consensus-based approach without a clear definition and framework for adjudicating actionability could lead to inconsistent and arbitrary results, the LVBC developed a semiquantitative metric for determining the clinical actionability of gene–disease pairs. This metric explicitly recognizes that actionability is a continuum, not a binary

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state.[8,9] That being said, we think that it is vital to define a core set of gene–disease pairs that reach a sufficient threshold of clinical actionability to be considered as part of the routine results of a genome-scale diagnostic test.

In parallel to the efforts of NCGENES, the Evidence-based Genomic Applications in Practice and Prevention Working Group established an evidence-based review procedure consisting of a rapid, sensitive screen for genes with possible actionability; structured data gathering organized around the elements of actionability articulated by the LVBC and detailed herein; and provided assessment by an expert deliberative committee.[10] Such a framework will be most useful, not for definitively determining actionability, but rather for identifying the minority of genes in the human genome that should undergo further scrutiny as possibly actionable in a given specific context.

The 2013 recommendations for analysis and return of certain highly actionable incidental/secondary findings by the American College of Medical Genetics and Genomics (ACMG) used a deliberative consensus method to identify gene–disease pairs within which clearly pathogenic variants should be returned as part of clinical genome-scale sequencing.[5] These recommendations were met with criticisms,[11–13] among which were concerns about the process by which the recommended gene list was developed. Also noted were concerns that some genes on the recommended list may not reach an evidentiary threshold sufficient to justify being returned as incidental/secondary findings. The development of a clear framework for the assignment of clinical actionability is therefore a necessary step toward formalizing such judgments and making assessments reproducible and updatable.

MATERIALS AND METHODS

Semiquantitative metric categories and scoring rules

The LVBC established five core characteristics of clinical actionability, with particular emphasis on the ramifications of finding a clearly pathogenic variant in a person without signs or symptoms of the disease (**Table 1**). The five characteristics are reflected by the following questions:

Table 1 Semiquantitative metric framework, questions, scores, and examples

Category	Level	Score	Notes/examples
Severity of disease: "What is the nature of the threat to health for an individual carrying a deleterious allele in this gene?"	Sudden death or inevitable death	3	Cardiac arrhythmia, vascular dissection, fatal infantile neurodegenerative conditions
	Possible death due to illness or comorbidity	2	Cancer, organ failure, moderate to severe intellectual disability
	Modest morbidity	1	Mild to moderate intellectual disability, physical limitations, early-onset neurosensory deficits
	Minimal health impact	0	Benign enzyme defects, nonmedical traits, later-onset neurosensory deficits
Likelihood of disease: "What is the chance that a serious threat will materialize?" (somewhat akin to penetrance)	>50%	3	Most individuals develop the severe outcome
	6–49%	2	Some individuals develop the severe outcome
	1–5%	1	Few individuals develop the severe outcome
	<1%	0	Outcome is very rare or cannot be reasonably estimated
Efficacy of intervention: "How effective are the interventions for preventing harm in a presymptomatic individual?"	Highly effective	3	Nearly all individuals have substantial benefit
	Moderately effective	2	The majority of individuals have some benefit
	Minimally effective	1	The majority of individuals have marginal benefit, or the minority of individuals have substantial benefit
	Ineffective/no interventions available	0	No individuals benefit; only watchful waiting recommended, or symptomatic treatments when disease manifests
Burden of intervention: "What are the burdens or potential harms of initiating interventions in a presymptomatic individual?"	Very low burden	3	Yearly screenings, routine medications, minor dietary/lifestyle modification
	Somewhat burdensome	2	Invasive screening, significant lifestyle alteration, medications with a substantial chance of side effects or more intensive delivery regimens, transplantation with rare complications
	Moderately burdensome	1	Removal of a nonvital organ, transplantation with frequent complications
	Highly burdensome	0	Removal of a vital organ
Knowledge base: "What is the evidence base for decisions about the natural history of the disease and interventions used for preventing serious outcomes?"	Substantial evidence	3	All categories scored confidently, high-quality review or practice guideline
	Moderate evidence	2	Strong primary literature, some details scored by analogy to another well-known disorder
	Minimal evidence	1	Unable to confidently score one or more categories, sparse primary literature or few reported patients
	Controversial or poor evidence	0	Uncertain natural history of disease, primary literature lacking or controversial

1. Severity: "What is the nature of the potential adverse health outcome in an individual carrying a deleterious allele in this gene?" Severity is scored from minimal health impact to modest morbidity to sudden/inevitable death.
2. Likelihood: "What is the chance that this adverse outcome will manifest?" Scoring for this category uses brackets of likelihood and is similar to penetrance.
3. Efficacy of intervention: "How effective are the established interventions for preventing the harmful outcome?" Efficacy of the intervention is scored from lack of demonstrable efficacy to highly effective intervention.
4. Burden of intervention: "How acceptable are the interventions in terms of the burdens or risks placed on the individual?" The burden or acceptability of the intervention is scored from highly consequential to minimally burdensome intervention.
5. Knowledge base: "How much is known about the gene, condition, and intervention to allow scoring in each category?" Knowledge is scored from controversial or poor evidence to substantial evidence.

All five criteria are scored on a scale of 0–3. The "outcome" and "intervention" are defined in advance and the other components of the metric are scored with respect to these parameters. It is critical to consider outcomes together with corresponding interventions to balance the clinical effects expected by natural history against the benefits and harms of these interventions in individuals who have not manifested symptoms of disease.

Gene sets scored

To judge the ability of the metric to distinguish between conditions that vary widely in terms of clinical actionability, three lists of genes were scored: (i) a list of 161 provisionally actionable genes[3] (hereafter referred to as "bin 1 genes"); (ii) a list of 57 genes originally recommended by the ACMG[4] (hereafter referred to as "ACMG genes"); and (iii) a list of 1,000 genes randomly selected from the National Center for Biotechnology Information RefSeq database (hereafter referred to as "random genes"). The random genes were selected from a 7 October 2013 RefSeq download using an in-house python script utilizing the "random" module. They were cross-referenced against Online Mendelian Inheritance in Man (http://www.omim.org) and OrphaNet (http://www.orphadata.org/cgi-bin/inc/ordo_orphanet.inc.php) and manually curated to identify those with disease associations as of that time. The majority of the random genes (889/1,000) had no documented disease association, were associated only with somatic mutations, or had a modest influence on disease risk based on association study data. These conditions scored 0 by default and were excluded from further analysis.

Although the ACMG's recommended list has subsequently been reduced to 56, all 57 original genes were analyzed with the expectation that the removed gene (*NTRK1*) would prove to be an outlier with regard to clinical actionability.

In addition, a list of "other" gene–disease pairs were scored, including conditions with phenotypes overlapping those of genes considered to be potentially actionable, disorders that are allelic to others that were scored, or conditions that were selected to evaluate the range of scores obtained for conditions considered not to be actionable. Scores for these "other genes" are included in the overall analysis but were not subject to statistical comparisons between lists because of their heterogeneity.

Assessment and consensus scoring

The multidisciplinary LVBC included clinical geneticists, genetic counselors, physicians from other specialties such as cardiology and neurology, a primary care physician, clinical laboratorians, and ethicists. Information about gene-disease relationships was obtained from OMIM, GeneReviews,[14]

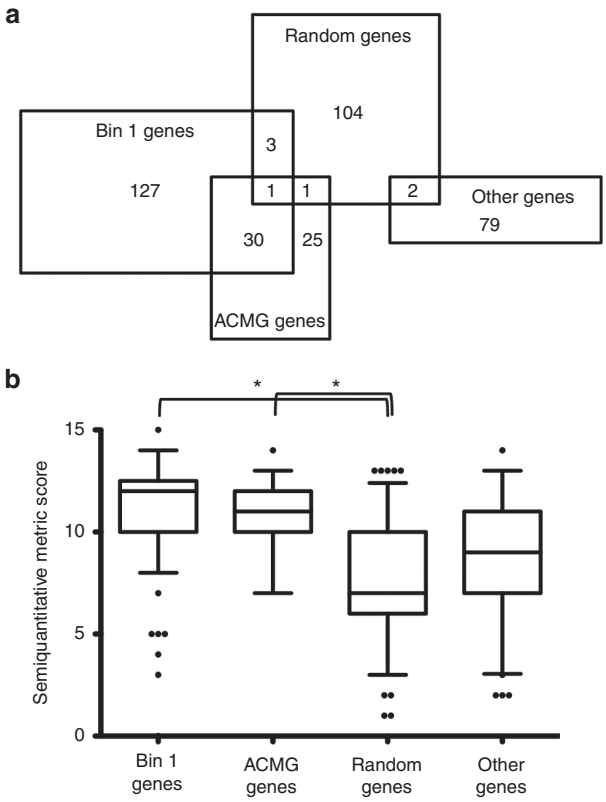

Figure 1 Semiquantitative metric scores. (**a**) Summary of overlap between the gene lists analyzed. The American College of Medical Genetics and Genomics (ACMG) genes included 26 gene–disease pairs (25 not including *NTRK*) that were not among the bin 1 genes. Conversely, 130 bin 1 genes were not among the ACMG genes. Of the 111 random genes with a defined disease association, 4 overlapped with the bin 1 genes (*ANK2*, *BRIP1*, *COL1A2*, *PROC*), 1 overlapped with the ACMG genes (*NTRK1*), and 1 gene overlapped both lists (*PTEN*). The Locus-Variant Binning Committee also evaluated 80 other gene–disease pairs, including alternative phenotypes for some genes, or different genes with similar disease phenotypes. One of these genes was on the ACMG list (*NF2*) and two were on the random list (*CASQ2* and *MAX*). (**b**) Distribution of semiquantitative metric scores. Box-whisker plots showing the median, 25th–75th percentiles (box), and 5th–95th percentiles (whiskers) of the scores for the bin 1 gene list, ACMG gene list, random gene list, and other gene list. Asterisks indicate statistically significant differences ($P < 0.0001$, two-tailed Mann-Whitney test).

Table 2 Examples of semiquantitative metric scores for selected genes

Gene	OMIM phenotype	List	Severity	Likelihood	Efficacy	Burden	Knowledge base	Total
ACADM	201450	Bin 1	3	3	3	3	3	15
Notes: Medium-chain acyl-CoA dehydrogenase deficiency is associated with a high lifetime risk of hypoglycemic episodes provoked by fasting or illness. These episodes can be fatal but are highly preventable through avoidance of fasting or provision of intravenous fluids with dextrose during illnesses.[18]								
MLH1	609310	Bin 1, ACMG	2	3	3	2	3	13
Notes: Lynch syndrome is a highly penetrant cancer predisposition syndrome in which individuals are at risk for colorectal cancer as well as other cancers. Increased colonoscopic screening is highly effective at preventing deaths due to colon cancer.[19]								
FBN1	154700	Bin 1, ACMG	3	2	2	3	3	13
Notes: Marfan syndrome is characterized by skeletal, eye, and cardiovascular involvement; the most severe aspect is increased risk for aortic aneurysm and dissection. Screening of the aortic root and arch for evidence of dilation can effectively identify individuals at high risk for dissection and allow initiation of preemptive definitive surgical management.[20]								
LDLR (Heterozygous)	143890	Bin 1, ACMG	2	3	2	3	3	13
Notes: Familial hypercholesterolemia results in elevated risk for death due to coronary artery disease. Treatment with lipid-lowering medications can somewhat mitigate the risk in affected individuals.[21]								
F8	306700	Bin 1	2	3	3	2	3	13
Notes: Hemophilia presents a risk for severe bleeding that can result in severe chronic morbidity or fatality. Recombinant clotting factor is an effective preventive measure.[22]								
HFE (C282Y homozygous)	235200	Bin 1	2	1	3	3	3	12
Notes: Hemochromatosis leads to iron overload that can cause cirrhosis, cardiomyopathy, and endocrine dysfunction. Although biochemical evidence of abnormal iron homeostasis is seen in the majority of patients, less than 10% develop severe end-organ manifestations. Biochemical screening followed by therapeutic phlebotomy is highly effective for reducing morbidity.[23]								
PTEN	153480	Bin 1, ACMG, random	2	3	2	3	2	12
Notes: PTEN hamartoma syndrome is associated with an increased risk for several malignancies, including breast cancer. Penetrance is high, and it is expected that increased screening will benefit at-risk individuals, by analogy to other cancer predisposition syndromes. However, specific data regarding screening protocols are lacking.[24]								
CASQ2	611938	Random, other	3	3	2	3	1	12
Notes: Catecholaminergic polymorphic ventricular tachycardia is characterized by episodes of arrhythmia induced by adrenergic stress. Patients can present with syncope or sudden cardiac death. Limited data suggest that intervention with β-blockers and flecainide can be effective, and in some cases implantable cardioverter-defibrillator placement is required.[25,26]								
MYH7	115196	ACMG	3	1	3	3	2	12
Notes: Hypertrophic cardiomyopathy can lead to sudden cardiac death in a small proportion of affected individuals. Management includes cardiology surveillance and risk stratification, with more aggressive interventions in those who develop symptoms.[27,28]								
SLC2A1	606777	Random	2	3	2	2	2	11
Notes: Glucose transporter type 1 deficiency syndrome has a broad phenotypic spectrum including individuals with seizures and/or complex movement disorder. Symptoms show substantial improvement with a ketogenic diet.[29,30]								
F5 Leiden (homozygous)	188055	Other	2	3	2	2	2	11
Notes: This specific mutation in the *F5* gene causes resistance to cleavage and inactivation by protein C. In the homozygous state, the result is a substantially elevated risk for venous thrombosis, which in some cases can lead to mortality due to pulmonary embolism. Awareness of this tendency allows measures to be taken to prevent immobility, reducing the chance of a clot developing.[31,32]								
SDHB	115310	Bin 1, ACMG	2	2	1	3	2	10
Notes: Heterozygous pathogenic variants in *SDHB* cause a syndrome of predisposition to paragangliomas, which can become malignant. Biochemical screening and imaging are recommended in asymptomatic individuals, with the expectation that this protocol would be somewhat effective for detecting tumors at smaller size and earlier stage.[33,34]								
ALB	615999	Random	1	1	3	3	2	10
Notes: Individuals with familial dysalbuminemic hyperthyroxinemia are clinically euthyroid, although a variant in the albumin gene leads to preferential affinity for thyroxine (T4). Some patients have been mistakenly treated for hyperthyroidism, leading to modest morbidity, which could be avoided simply by foreknowledge about this relatively benign phenotype.[35]								

ACMG, American College of Medical Genetics and Genomics; PTEN, phosphatase and tensin homolog.

[a]*NTRK1* was included in an original version of the ACMG publication but removed from the final published list.

Table 2 Continued on next page

Table 2 Continued

Gene	OMIM phenotype	List	Severity	Likelihood	Efficacy	Burden	Knowledge base	Total
FLCN	135150	Bin 1	2	1	2	3	1	9
Notes: Birt-Hogg-Dubé syndrome is a hereditary cancer predisposition syndrome associated with benign hamartomatous skin lesions, benign and malignant kidney neoplasms, and lung cysts leading to spontaneous pneumothorax. Screening protocols are recommended and assumed to be somewhat effective at detecting kidney tumors, but the evidence base for screening is limited.[36]								
TNNI3	613690	ACMG	3	1	1	3	1	9
Notes: As with *MYH7*, the *TNNI3* gene is associated with cardiomyopathy and risk for sudden death.[27] However, the degree to which screening can be an effective strategy for preventing this outcome in individuals with *TNNI3* pathogenic variants is less well known.								
MYLK	603776	ACMG	3	0	1	3	1	8
Notes: As with *FBN1*, pathogenic variants in *MYLK* are reported to be associated with increased risk of aortic dissection. However, the penetrance is essentially unknown and the pathophysiology seems to involve dissections and not aneurysms, limiting the efficacy of screening.[37] The overall knowledge base about *MYLK*-associated disease is somewhat limited.								
GCK	125851	Bin 1	1	1	1	3	2	8
Notes: Maturity-onset diabetes of the young results in a rare form of insulin-dependent diabetes. However, these individuals rarely exhibit diabetic ketoacidosis, and overall complications of diabetes are low in this disorder.[38]								
NAGLU	252920	Random	3	3	0	0	2	8
Notes: Pathogenic variants in *NAGLU* cause mucopolysaccharidosis type IIIB (Sanfilippo B), a lysosomal storage disease that leads to significant morbidity and mortality but has no effective preventive measures at this time. Supportive treatments for symptomatic manifestations are the mainstay of care but would not be expected to substantially alter outcomes.[39]								
NTRK1	155240	ACMG[a]	2	0	3	2	0	7
Notes: The *NTRK1* gene has been implicated in predisposition to medullary thyroid carcinoma, which in principle could be effectively prevented through prophylactic thyroidectomy. However, the evidence supporting causality of germ-line variants in cancer predisposition is weak, and there is insufficient data with which to estimate penetrance.[40]								

ACMG, American College of Medical Genetics and Genomics; PTEN, phosphatase and tensin homolog.

[a]*NTRK1* was included in an original version of the ACMG publication but removed from the final published list.

PubMed searches, and clinical guidelines, when available. Members of the LVBC prepared the evidence review, typically with a single member assigned primary responsibility for each gene–disease phenotype pair. The committee met regularly to review the evidence and to agree on a score for each element of the semiquantitative metric or direct additional review.

To mitigate the subjective nature of assessing certain categories and to enhance consistency between scores, the LVBC arrived at a series of scoring conventions (examples in **Table 1**). Scores for categories 1 (severity) and 2 (likelihood) are linked to the same specific outcome, either the most severe potential outcome or what is generally considered the primary outcome for the disease. However, scores for a given gene–disease pair can be calculated for more than one outcome of interest to account for disease pleiotropy. For example, different scores can be calculated for *BRCA1* depending on whether the outcome of interest is breast cancer or ovarian cancer. In effect, categories 1 and 2 reflect the medical implications of disease faced by an individual with a pathogenic finding. Scores for categories 3 (efficacy) and 4 (burden) reflect specific presymptomatic interventions targeted to the outcome described in categories 1 and 2 (e.g., bilateral risk-reducing mastectomy or bilateral salpingo-oophorectomy per the example of *BRCA1*). The semiquantitative metric thus approximates the concept of clinical utility by balancing the potential benefits and harms of intervention when an incidental/secondary finding is discovered in a presymptomatic individual.

RESULTS

A total of 1,213 unique genes were evaluated using the semiquantitative metric (**Supplementary Table S1** online). After removing genes not implicated in a single-gene disorder, 324 unique genes representing 372 gene–disease pairs were scored. These genes included 161 bin 1 genes, 57 ACMG genes, and 111 random genes associated with defined monogenic disorders. There was some degree of overlap between these lists, as depicted in **Figure 1a**. In cases where the random genes were associated with more than one condition, the highest of the scores was chosen to represent the random gene–disease pair; scores for additional gene–disease pairs were tallied in the "other" category.

The median score of the bin 1 genes was 12 (range 0–15); 84/161 gene–disease pairs scored ≥12, while 29/161 pairs scored <10. The median score for the ACMG genes was 11 (range 7–14); 25/57 gene–disease pairs scored ≥12, while 11/57 pairs scored <10. The *NTRK1* gene, originally included on ACMG's preliminary recommended list and subsequently dropped, scored 7. In comparison, the median score of the 111 random genes was 7 (range 1–13); only 14/111 gene–disease pairs scored ≥12, while 81/111 pairs scored <10. **Figure 1b** shows the distribution of scores for all of the pairs. The distributions of scores for the bin 1 genes and the ACMG genes are not significantly different from each other, but both lists are significantly different than the random genes ($P < 0.0001$, two-tailed Mann-Whitney test), indicating that the semiquantitative

metric effectively distinguishes between gene–disease pairs that were qualitatively deemed to be actionable in earlier efforts from those that would not be enriched for actionability. **Table 2** presents several scoring examples, and all scores are included in **Supplementary Table S1** online.

Among the gene–disease pairs scoring highest using this metric were *MLH1* (associated with Lynch syndrome) at 13 and *RYR1* (malignant hyperthermia) at 12. Despite its low penetrance, the *HFE* gene (implicated in hereditary hemochromatosis) scored 11 because of the availability of highly effective and noninvasive preventive measures. By contrast, some genes that were considered actionable by the ACMG, such as *SDHB*, *SDHC*, and *SDHD* (associated with hereditary pheochromocytoma/paraganglioma susceptibility) received scores below 11 because of limited evidence that biochemical screening in an otherwise asymptomatic individual would produce better long-term outcomes than treatment upon onset of symptoms. Other genes, such as *MYH11* and *MYLK* (which have recently been implicated in familial thoracic aortic aneurysm and dissection), could be considered to have effective interventions by analogy to other well-known conditions, but they scored lower because of a limited knowledge base, which precludes accurate assessment of penetrance.

As demonstrated by the range of scores observed for the selected gene–disease pairs, actionability is a continuum rather than a binary state. Using the random genes as a benchmark, 21/111 (19%) scored ≥11, while 30/111 (27%) scored ≥10. The LVBC chose to consider genes with a score ≥11, essentially the top quintile, as meeting the threshold of actionability for inclusion in the revised bin 1 list. This yields a list of 168 genes representing 176 gene–disease pairs from the total 372 pairs scored. The fact that 19% of random genes associated with single-gene disorders scored ≥11 suggests that as many as 500 genes of the ≥3,000 single-gene disorders might rise to this threshold of actionability. Thus, we have not yet identified all of the "actionable" gene–disease pairs, and a systematic screen of single-gene disorders is needed. Furthermore, scores are subject to change depending on advances in medical genetics, which will likely increase some scores over time.

DISCUSSION

Management of the vast range of heterogeneous information generated when genomic analysis is undertaken remains one of the most challenging aspects of applying genomics in the clinical realm. Patient preferences must be taken into account, especially with regard to genomic findings that have limited clinical actionability. Individuals may make greatly varying choices regarding whether they want to learn about different types of genomic findings; we are studying these preferences and the parameters that influence them as part of the NCGENES study. However, just as there are incidentally discovered laboratory values that are flagged as "critical" levels, or radiographic findings that require clinical action, it follows that when certain genomic findings exceed a threshold of actionability, the default procedure should be to provide those results as part of the routine protocol when performing clinical genome-scale sequencing tests.

It is thus critical to define a subset of clinically actionable genomic findings that are likely to be accepted by most individuals and allow a standardized and streamlined process for informed decision making in clinical genome-scale diagnostic testing. Otherwise, the decision about returning genomic findings could (at the reductio ad absurdum extremes) be relegated to an all-or-none choice irrespective of the actionability of the information, or a nearly infinite menu of potential findings organized at the level of certain genes or even specific variants. Neither of these options seems tenable in the current clinical setting.

It should be stressed that a policy of routine return of a small subset of genomic findings does not preclude patient choice by means of an informed "opt-out" at the initiation of testing, as now endorsed by the ACMG. In addition, this policy does not prohibit laboratories from offering additional categories of non–medically actionable genomic information (what we refer to as "bin 2") as an "opt-in" to those who desire such information, with appropriate education and decision making. Nevertheless, any such menu of options needs to be articulated before consent and analysis, which calls for an a priori process to define which gene–disease pairs fall into any given category.

This article describes the delineation of a novel semiquantitative metric providing a transparent definition of clinical actionability and a framework for evaluating criteria in a streamlined fashion. We outline criteria for actionability generally similar to other expert deliberative processes.[5,15] However, this framework is unique in that the dimensions of actionability can be assessed consistently across different types of disorders. The results indicate that, as expected, the ACMG list is enriched for genes that achieve high scores for actionability, both supporting their inclusion in a recommended list and generally reinforcing the parameters used in the current assessment. Future versions of the ACMG list could be informed by this scoring metric, or one similar to it, in order to remove genes that fall below a stringent threshold and to include additional genes with scores equivalent to those on the current recommended list. The percentage of individuals who will have such findings is predictable and depends on the list of gene–disease pairs being evaluated and the stringency with which variants are selected for return.[3,15–17]

Nuances in application of the semiquantitative metric

The subcategories of the metric reflect the clinical impact of a condition (severity and likelihood of a given outcome) while balancing the potential benefits (effectiveness of interventions) and harms (burden of intervention), thus approximating the clinical utility of revealing incidental/secondary findings in a presymptomatic individual. Each of these facets is necessary to include, despite the subjectivity inherent in scoring some of them. For example, both periodic phlebotomy (as in the case of hemochromatosis) and surgical removal of the stomach to prevent diffuse gastric cancer (in the case of *CDH1* mutations) are highly effective measures to prevent morbidity and mortality,

yet the burdens of these interventions are dramatically different and therefore greatly affect the concept of actionability when considering the return of genetic information in a setting in which the individual is not likely to have overt symptoms.

Scoring each category on a 0–3 scale allows for a limited degree of granularity that captures the qualitative nature of certain categories (e.g., effectiveness and burdens of intervention). It would be difficult, and potentially problematic, to spread the range of scores into finer subdivisions or to develop a more complex nonlinear scoring system. That said, different scoring systems could be explored should there be a compelling rationale to do so. In addition, customized gene lists could be generated for other contexts by applying weighting schemes or selecting a different threshold. For example, one might envision that selection of genes for primary screening of the general population should demand extremely high knowledge and efficacy scores, and those components of the metric could be weighted accordingly. In general, differential weighting of criteria will change the rank order of scores that are close to one another, and will primarily affect gene–disease pairs near the threshold used to define actionability. However, those with scores farther away from the chosen threshold would be much less likely to have their position above or below the threshold affected by changes in weighting. Finally, the evidence used in establishing the scores can be explicitly defined, and the scores can be updated to incorporate new evidence. Thus, the semiquantitative metric provides a structured framework and a more nuanced and transparent method for defining a list of clinically actionable genes than would be possible with expert consensus approaches.

In practice, the LVBC established a set of conventions for scoring different categories (see **Table 1** for examples). It is challenging to directly compare the severity of conditions that lead to bodily harm, such as death or organ failure, with conditions that lead to physical or cognitive impairment. The severity score is thus intended to judge the relative severity between disparate conditions. The likelihood of a given outcome is the most quantitatively definable component of the metric, although data are lacking for many conditions, requiring either an estimate with some uncertainty (reflected in a lower score for knowledge base) or a score of 0 when the available data are simply too limited to make a reasonable assessment, as in the case of autosomal-dominant conditions with only a few patients reported in the medical literature.

In the absence of definitive end points, the effectiveness of interventions for different clinical outcomes often relies on expert opinion. The LVBC generally considered the screening or preventive measures that all individuals with a positive finding would be expected to undergo, rather than more definitive treatments that would be required only in those who manifest symptoms. By any measure, the burden of intervention is the most subjective and personally nuanced aspect of the metric. It is likely that different individuals hold different views on what is acceptable and what constitutes an unreasonable burden in the context of their own life experiences. Thus, while we fully recognize that it can be difficult to assess the burden of a particular intervention for an individual, we attempted to define a scoring rubric that could roughly define the relative burdens of interventions across the population. This score could be replaced in the future by a more quantitative measure derived from discrete choice experiments or other means of assessing relative values, such as the methods used to measure quality-adjusted life-years.

Finally, the knowledge base score was applied as a single measure reflecting the degree to which each component of the score could be confidently defined. Alternatively, a knowledge score could be assigned to each component separately based on the knowledge base for that element. While more complicated, such an approach would provide greater granularity. The strength of the gene–disease association itself is embedded in the knowledge base score, since less well-described conditions rarely have sufficient knowledge to accurately score certain elements of the metric. However, we do not intend for this metric to be used as a stand-alone measure of the clinical validity of a gene–disease association.

Disorders that predispose to thoracic aortic aneurysm and dissection illustrate certain nuances of scoring. These conditions convey an increased risk of sudden death due to dissection, and the typical intervention is to implement a vascular imaging screening program. This intervention is a highly effective and noninvasive means to detect and monitor the size of an aneurysm before it poses significant danger of acute dissection. More invasive intervention (i.e., vascular surgery) is required only if the individual develops a clinically significant aneurysm. Overall, this combination of interventions would be a highly effective and generally acceptable way to manage the risk of sudden death due to an aortic dissection, although in some conditions the risk of dissection is not directly related to aneurysm size, in which case screening would be less effective. In addition, the effectiveness of an intervention for more rare conditions, such as *MYLK*-associated thoracic aortic aneurysm and dissection, must be extrapolated based on analogy to related conditions because of the lack of information available regarding the effectiveness of interventions specific to that condition.

Potential limitations

The current metric does not account for certain contextual factors, such as the age of the individual, the typical age at onset of disease or the age at which clinical actions would be implemented in a presymptomatic individual, the sex of the individual, the general availability and cost of recommended preventive strategies, or the ability of relevant genetic lesions to be detected. Given these limitations and the necessarily subjective nature of any assessment of actionability, the scores and evidence base generated by the application of the semiquantitative metric are best considered an initial starting point for more nuanced discussions about individual conditions or particular clinical applications.

Certain features of the metric could lead to minor irregularities in scoring. For example, if the LVBC decided that a proposed intervention for a given condition was considered ineffective (score = 0), then no score could be assigned to reflect the "burden" of that intervention because the additional points would inflate the final score. However, If the proposed intervention was considered even "minimally" effective, the total score could swing by as much as four points (effectiveness = 1 and burden = 3 points for a minimally invasive intervention). This impact could be partially mitigated by weighting schemes that emphasize the contribution of the effectiveness of the intervention over the burden score to the overall total.

The final scores for each gene–disease pair were determined through consensus of the LVBC. This process was not amenable to evaluation of interrater variability in scoring, but in practice we found that the semiquantitative metric facilitated more efficient discussions and greater consistency than earlier attempts to arrive at consensus without a structured framework. It could be argued that this process simply replaces a single idiosyncratic expert consensus about "actionability" (the current state of other deliberative processes) with several different potentially idiosyncratic decisions. However, assessment of scores for each criterion permits more systematic evidence curation and updating, as well as a more flexible approach to weighting the importance of different criteria, than would be possible otherwise.

Finally, in some cases review of the scores by domain experts may prompt revisions based on deeper understanding of the clinical scenario or greater awareness of literature that was not captured in our review process. In other cases medical advances may increase the overall scores by improving the knowledge base and perhaps the efficacy of interventions for many conditions. It is expected that there will be a need for ongoing assessment of clinical actionability and updating of results, which again is streamlined by the existence of a structured framework.

Conclusions and future questions

We have presented a framework that defines five aspects of clinical actionability, evaluates them qualitatively, and effectively distinguishes between lists of genes deemed to be potentially actionable by expert opinion versus randomly selected genes. This framework is flexible and can be adapted to different contexts. It is too early to know whether it is more efficient or more reliable than other expert consensus approaches, or whether it ultimately leads to better clinical outcomes. In addition, it remains to be determined whether other groups would arrive at the same scores. However, the inherent transparency of the framework facilitates comparison between different efforts, critical evaluation, and updating of the scores as new knowledge accrues as a result of the constantly evolving medical literature. We anticipate using this or a similar metric to evaluate all human disease genes to guide the application of genomic medicine.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/gim

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DISCLOSURE

The authors declare no conflict of interest.

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Potential Uses and Inherent Challenges of Using Genome-Scale Sequencing to Augment Current Newborn Screening

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Since newborn screening (NBS) began in the 1960s, technological advances have enabled its expansion to include an increasing number of disorders. Recent developments now make it possible to sequence an infant's genome relatively quickly and economically. Clinical application of whole-exome and whole-genome sequencing is expanding at a rapid pace but presents many challenges. Its utility in NBS has yet to be demonstrated and its application in the pediatric population requires examination, not only for potential clinical benefits, but also for the unique ethical challenges it presents.

Newborn screening (NBS) in the United States began in the 1960s, shortly after the publication of Robert Guthrie's paper describing a method for detecting phenylketonuria (PKU) in dried blood spots through a bacterial inhibition assay (Guthrie and Susi 1963). It has been defined as a public health activity aimed at the early identification of infants who are affected by certain genetic, metabolic, or infectious conditions (AAP Committee on Bioethics 2001) for which treatment can prevent unfavorable health outcomes. Millions of lives have been saved and significant morbidities prevented through universal NBS in the United States and other countries. Although a public health success, expansion of NBS has often been driven by technological advances as well as by pressure from the public and special interest groups. Rapid increases in the number of known genetic and metabolic conditions and improved methodologies have led to expansion of candidate disorders for screening. Technological advances have been closely intertwined with the ability to screen for conditions, for example, the adaptation of tandem mass spectrometry (MS/MS) for detection and quantification of multiple analytes in newborns' blood spot samples (Millington et al. 1989).

Massively parallel sequencing (MPS, also referred to as "next-generation sequencing" or NGS) uses high-throughput sequencers that are able to analyze DNA much more efficiently than

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previous methods such as Sanger sequencing. This technology provides a new methodology for screening and overcomes one of the major barriers to adding conditions to recommended newborn screening panels. Any number of conditions for which there is a known genetic basis can theoretically be screened for in an individual, potentially including the entire genome. However, screening a newborn with genome-scale sequencing raises significant complexities, including the extent to which parents should be able to learn about genetic predispositions in a newborn, particularly conditions that may not manifest clinically until adulthood, and in many cases that may not have effective preventive strategies. Children lack the autonomy of adults, who can decide for themselves whether to engage in genomic sequencing to determine whether they have a gene mutation that predisposes them to a condition such as cancer. In NBS, these decisions are made by and can directly impact the parents. This represents an additional complication that requires special consideration.

To ultimately determine the clinical utility of genome-scale sequencing in NBS and to evaluate whether such an approach offers added value, it will be imperative to assess the sensitivity and specificity of MPS for currently screened conditions and whether sequencing can provide diagnostic data as accurately as currently used screening methods such as MS/MS. Will these data significantly augment our ability to predict disease prognosis and enable more targeted management? What conditions would then meet the criteria to be added to routine NBS use? To answer these questions, it will be necessary to study the yield of sequencing for common conditions detected by current NBS (e.g., PKU, medium-chain acyl-CoA dehydrogenase deficiency [MCADD], cystic fibrosis [CF] and hearing loss), as well as conditions that meet criteria for NBS but were not possible to detect owing to lack of an adequate screening method (e.g., certain lysosomal storage disorders and primary ciliary dyskinesia). The application of sequencing in NBS will allow not only the delineation of the causative mutation in the proximally causative gene, thus augmenting studies of phenotype-genotype relationships, but it could also create a valuable long-term resource for researchers to investigate how currently unknown loci contribute to clinical heterogeneity.

APPLICATION OF DNA SEQUENCING IN NEWBORN SCREENING

Selection of Genes to Include in Sequencing-Based Newborn Screening

As articulated by Wilson and Jungner: "The central idea of early disease detection and treatment is essentially simple. However, the path to its successful achievement (on the one hand, bringing to treatment those with previously undetected disease, and, on the other, avoiding harm to those persons not in need of treatment) is far from simple though sometimes it may appear deceptively easy" (Wilson and Jungner 1968; Andermann et al. 2008). Introducing genetic testing into screening programs in the past was a relatively slow multistep process with pilot screening programs undertaken after a disease gene or method of identification for a disorder was discovered, and experts agreed was reasonable and efficacious to add to a panel (ACMG Newborn Screening Expert Group 2006; Andermann et al. 2008). Even with multiplex screening methodologies such as MS/MS that can identify analytes associated with multiple inborn errors of metabolism, pilot programs were initiated by states before widespread validation (Frazier et al. 2006).

Currently, the Discretionary Advisory Committee on Heritable Disorders in Newborns and Children evaluates conditions nominated for inclusion in NBS programs through a comprehensive systematic evidenced-based review process (see http://www.hrsa.gov/advisorycommittees/mchbadvisory/heritabledisorders/index.html; http://www.hrsa.gov/advisorycommittees/mchbadvisory/heritabledisorders/nominatecondition/reviews/pompereport2013.pdf) (Kemper et al. 2014). This review process considers not only the magnitude and certainty of net benefit, but also the capability of states to implement comprehensive screening. Twenty-three conditions were considered but

not included in the original recommended uniform screening panel (RUSP) because they lack an accurate screening method (ACMG Newborn Screening Expert Group 2006); 21 of these are detectable in some or all cases with molecular genetic analysis and, therefore, have the potential to be added to NBS panels. However, the rate at which new disease genes are being identified outpaces the ability of professionals and policy makers to assess the potential benefits and pitfalls of introducing or expanding genetic screening programs (Andermann et al. 2008), and formal review of the estimated 3260 genes with a human phenotype-causing mutation (see http://omim.org/statistics/geneMap) would be a daunting task. Ultimately, it will be necessary to construct a list of genes associated with conditions that are part of the current RUSP as well as those that are deemed to fulfill criteria for NBS and are detectable by sequencing. This panel would include conditions with onset in childhood in which early identification could allow prevention or amelioration of symptoms. The process will also need to include a mechanism for updating the list to reflect advances in medical genetics. Figure 1 depicts a timeline of advances in technology and newborn screening.

Incorporating Sequencing into Routine NBS Practice

Several challenges must be met to effectively incorporate sequencing into NBS. First, the technical capabilities of MPS need to be evaluated in

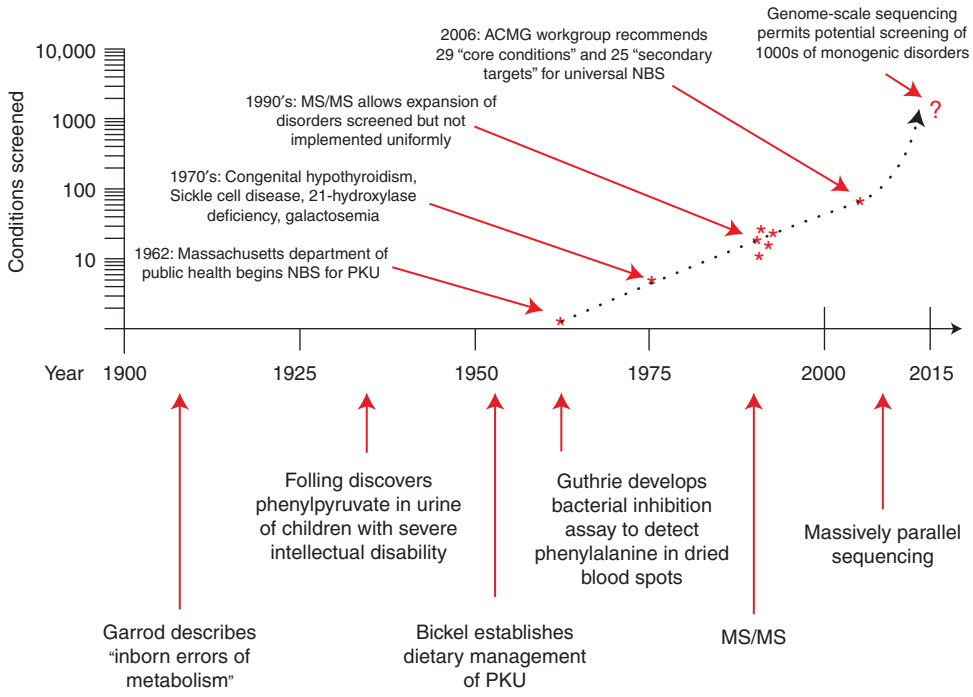

Figure 1. Milestones in newborn screening are depicted in the lower portion of the figure, with approximate dates on the horizontal axis. The number of conditions screened for (or potentially screened for) is depicted chronologically with asterisks on the vertical axis, plotted on a logarithmic scale. Screening programs have historically differed between states, most dramatically observed after development of tandem mass-spectrometry technology in the 1990s. Adoption of a recommended uniform screening panel in 2005–2006 has gradually led to greater consistency. Development of next-generation sequencing technology in the early 2000s, with subsequent reduction in the cost of genome-scale sequencing, makes it possible to analyze thousands of disease genes. The number of conditions potentially screened for is indicated with a question mark to emphasize the substantial concerns regarding the application of such technology in healthy newborns.

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comparison to standard NBS methods. Next, thoughtful choices must be made about whether to include conditions that are not amenable to current screening methods, yet, would otherwise meet criteria for screening in a public health setting versus others that would be reasonable candidates if accompanied by more rigorous informed parental consent. Finally, any serious proposal to supplement traditional NBS by genome-scale sequencing demands careful consideration of the optimal clinical setting in which parents learn about the series of complex decisions they would need to make to provide informed consent and the methods by which they are guided in this decision making process.

All 50 states in the United States currently screen for a panel of conditions that include hemoglobinopathies, disorders of amino acid, fatty acid and organic acid metabolism, congenital hypothyroidism, congenital adrenal hyperplasia, galactosemia, biotinidase deficiency, and cystic fibrosis. Thirty-four states also mandate newborn hearing screening (see http://genes-r-us.uthscsa.edu/sites/genes-r-us/files/nbsdisorders.pdf). In most states, NBS is mandated by state laws; only a few states consider it voluntary and require specific parental consent (Seashore and Seashore 2005). The public health benefits and importance of NBS must be weighed against the rights of parents to make decisions about their child. Some have argued that preventive programs conducted under public health auspices should be held to the same ethical standards as medical innovations introduced into the private sector (Skrabanek 1990). Some have proposed screening for all but a selected group of conditions, whereas others point out the potential for a "treatment odyssey" undertaken by families whose child is identified with a serious condition through screening for which no effective treatment exists (Baily and Murray 2008; Bailey et al. 2008). Although there is strong support for universal screening for disorders in which early diagnosis and treatment is lifesaving or produces great medical benefit, justifying the omission of explicit informed consent, this will not be the case for many new conditions that will be identifiable through sequencing. Available interventions for these disorders may have varying efficacy, and many disorders could have pleiotropic effects. Incorporation of genetic sequencing panels or even genome-scale sequencing into the NBS paradigm raises significant concerns about the management of such information (Bailey et al. 2008). Conditions that are clinically significant and may benefit patients greatly by surveillance and early diagnosis of complications, such as familial adenomatous polyposis, could have an equivalent impact to conditions that are currently screened. Conditions associated with developmental disability for which there may be no "cure" but for which early intervention and therapy services may be valuable are clearly different than those that have traditionally been included in NBS programs; it would be difficult to justify such screening being "mandatory," and thus parental informed decision-making will be needed. As public health proponents anticipate the use of NGS to improve health outcomes, care must be taken not to undertake a slippery slope of utilizing this technology without rigorous scientific and ethical examination of its utility, acceptance, and consequences.

Secondary Findings

If genome-scale sequencing ultimately becomes the most cost-effective means of generating sequence data for NBS, the analysis could be focused on a subset of genes through the use of informatics filters. However, whenever genome-scale sequencing is performed, there will inevitably be additional clinically significant variants in genes that may not have been the original intent of the screening. These additional findings could be considered "incidental" or "secondary" findings; whether they should be part of the routine analysis is a subject of intense debate. The American College of Medical Genetics and Genomics (ACMG) recommended that when genome-scale sequencing is performed in a diagnostic setting, known pathogenic or expected pathogenic variants in 56 genes should be reported back to patients unless they opt out of receiving such findings (Green et al. 2013). In general, the ACMG relied on the guiding principle of clinical actionability to generate this list

of genes, but the process did not use a systematic approach and the resulting list has been criticized (Burke et al. 2013; Ross et al. 2013a). The list was also not developed specifically for children, and indeed NBS conditions were excluded from consideration. As a result, the list recommended by the ACMG includes some conditions that have onset in adulthood, such as hereditary breast and ovarian cancer susceptibility caused by mutations in *BRCA1* and *BRCA2*. Should these conditions be included as part of NBS when a genome-scale sequencing method is used? Previous recommendations have argued against testing children for adult-onset disorders, preferring to defer such testing until the individual can decide whether he or she wants to have that information (see section on Ethical Considerations).

EXAMPLES OF CONDITIONS AMENABLE TO AUGMENTED NEWBORN SCREENING

Phenylketonuria (PKU)

PKU is one of the most common inborn errors of metabolism detected by NBS. It is a well-characterized amino acid disorder caused by deficiency of the liver enzyme, phenylalanine hydroxylase (PAH), leading to elevated levels of the amino acid, phenylalanine (Phe) in blood and other tissues. It is inherited in an autosomal recessive pattern. Left untreated, PKU causes severe to profound intellectual disability, microcephaly, seizures, and behavior problems. It was the first condition to be screened for in newborns and one in 15,000 infants is born in the United States with classical PKU. Milder variants, known collectively as the hyperphenylalaninemias, result from partial deficiency of the enzyme and occur in approximately one out of 48,000 births. PKU and hyperphenylalanenemia are currently detected through MS/MS of dried blood spots and screen positive cases are confirmed by measuring Phe levels in blood samples. After confirmation, patients are immediately placed on a diet that strictly controls their intake of Phe and their Phe levels are closely monitored. Dietary treatment must begin within the first weeks of life and continue throughout the patient's lifetime. The amount of protein restriction required to maintain normal blood Phe levels varies among patients. Use of the cofactor tetrahydrobiopterin (BH4; Kuvan) allows ~50% of patients to increase their protein intake thus approximating a more normal diet. The variable clinical course of PKU is, in part, based on the specific mutations in the gene encoding the L-phenylalanine hydroxylase enzyme and the amount of Phe in the diet. More than 400 mutations have been identified in the PAH gene and show a broad spectrum of types including deletions, insertions, missense, splicing, and nonsense. Although there is a modest correlation between genotype and the distinct phenotypes of classical PKU and hyperphenylalanenemia (Utz et al. 2012), there are other contributing factors as evidenced by sibling discordance, especially in the response to treatment, that is Phe restriction alone, with BH4 or with large neutral amino acids. A "genotype severity" tool has been developed to study the correlation of PAH mutation(s) with responsiveness to BH4 and its use has been recommended to help define which patients will show the best response. However, there is significant overlap between patients of different genotypes and precise predictions cannot be made before a therapeutic trial with the cofactor (Quirk et al. 2012). Genes at other loci may influence Phe transport within the brain as well as play a role in other features of the clinical phenotype; these modifier genes have been hypothesized to exist but have not yet been identified. It has been recommended that all patients with confirmed PKU have mutation analysis for genotype determination (National Institutes of Health Consensus Development Panel 2001). Information about specific PAH mutations as well as variation in other relevant genes, obtained through MPS, may help explain this phenotypic variability. It may also improve treatment outcomes by more targeted intervention and dietary regulation of Phe levels.

Hearing Loss

Hearing loss is the most common birth defect and the most prevalent sensorineural disorder

in developed countries (Hilgert et al. 2009). One of every 500 newborns has bilateral permanent sensorineural hearing loss that is greater or equal to a 40 decibel loss. Early detection and appropriate intervention results in improved development of language, cognitive, and social skills (White 2004). In 1993, a National Institutes of Health (NIH) consensus statement recommended that all infants have hearing screening shortly after birth (NIH 1993) and guidelines were established in 2000 (Joint Committee on Infant Hearing 2000). Screening is now performed in all states through Early Hearing Detection and Intervention (EHDI) programs with either otoacoustic emission (OAE) or automated auditory brainstem response (AABR) methodology. In 2009, it was estimated that 96.6% of newborns in the United States had hearing screening (see www.cdc.gov/ncbddd/hearingloss/ehdi-data2012.html).

Hearing loss represents a particularly promising avenue for realizing advances in NBS by using genomic sequencing approaches. Approximately 50% of prelingual deafness has a genetic etiology, with 70% categorized as nonsyndromic of which 75%–85% is autosomal recessive, 15%–24% autosomal dominant, and 1%–2% is X-linked or mitochondrial. Although ~50% of autosomal recessive nonsyndromic hearing loss is caused by mutations in genes encoding the proteins connexin 26 or 30, 50% is caused by mutations in at least 40 other genes.

Thirty percent of genetic prelingual hearing loss is associated with syndromes (Smith et al. 1993). Syndromic forms can have comorbid conditions including blindness, cardiac arrhythmias, kidney disease, endocrine disorders, and intellectual disability. In newborns, phenotypic features of syndromic hearing loss are usually not yet apparent, making targeted gene testing impossible. Even many of the common syndromic forms of hearing loss show considerable locus heterogeneity. One striking example is Usher syndrome, a condition with early onset deafness and retinitis pigmentosa with progressive loss of vision in later childhood, for which 18 genes or loci have been identified to date (see http://www.omim.org/phenotypicSeries/276900). Testing for mutations in these genes through standard sequencing is prohibitive because of cost and time. Not knowing the etiology may contribute to the stress reported in families after confirmation of hearing loss in their child (Vohr et al. 2008). Additionally, not all infants with significant early-onset hearing loss will have positive newborn screens (Young et al. 2011), thus preventing early identification. Sequencing could potentially detect mutations in all known syndromic and nonsyndromic hearing loss genes, thus providing a specific diagnosis not only in infants with a genetic cause of hearing loss detected through NBS but also in those who would otherwise not be identified through NBHS and thus provides a method to expand the scope of NBS. In addition, its application would increase our knowledge regarding genetic etiologies of hearing loss that are currently poorly understood.

Additional Conditions

Of the 31 conditions in the current RUSP, 27 have identified genetic etiologies, whereas the remaining four (congenital hypothyroidism, hearing loss, critical congenital heart disease, and severe combined immunodeficiencies) are frequently because of identifiable genetic causes (see Table 1). Although other screening methods remain more sensitive and economical, molecular analysis is being increasingly used for confirmatory testing and to determine prognosis and appropriate treatment for many of these conditions as well as for the 26 secondary disorders detected in the differential diagnosis of the core disorders in the RUSP (Carrillo-Carrasco and Venditti 1993; Manoli and Venditti 1993; Bhardwaj et al. 2005; Collins et al. 2010; Bhattacharjee et al. 2014; Kwan et al. 2014).

There are a number of serious and treatable childhood conditions that are not currently screened for lack of an effective test. MPS has the potential to identify the molecular basis for disorders currently included on NBS panels, but, more importantly, it could significantly expand our ability to detect a much broader range of genetic conditions. Some conditions were considered as candidates for screening by the expert panel convened by the ACMG in 2002

Table 1. Recommended uniform screening panel core conditions

	Gene(s)	Metabolic disorder			Endocrine disorder	Hemoglobin disorder	Other disorder
		Organic acid disorder	Fatty acid oxidation disorder	Amino acid disorder			
Propionic acidemia	*PCCB, PCCA*	X					
Methylmalonic academia (methylmalonyl-CoA mutase)	*MUT*	X					
Methylmalonic academia (cobalamin disorders)	*MMAA, MMAB*	X					
Isovaleric acidemia	*IVD*	X					
3-Methylcrotonyl-CoA carboxylase deficiency	*MCCC1, MCCC2*	X					
3-Hydroxy-3-methylglutaric aciduria	*HMGCL*	X					
Holocarboxylase synthase deficiency	*HLCS*	X					
β-Ketothiolase deficiency	*ACAT1*	X					
Glutaric academia type 1	*GCDH*	X					
Carnitine uptake defect/carnitine transport defect	*SLC22A5*		X				
Medium-chain acyl-CoA dehydrogenase deficiency	*ACADM*		X				
Very long-chain acyl-CoA dehydrogenase deficiency	*ACADVL*		X				
Long-chain L-3 hydroxyacyl-CoA dehydrogenase deficiency	*HADHA*		X				
Trifunctional protein deficiency	*HADHA, HADHB*		X				
Argininosuccinic aciduria	*ASL*			X			
Citrullinemia, type I	*ASS1, SLC25A13*			X			
Maple syrup urine disease	*BCKDHA, BCKDHB, DBT*			X			
Homocystinuria	*CBS, MTHFR, MTR, MTRR, MMADHC*			X			
Classic phenylketonuria	*PAH*			X			
Tyrosinemia, type I	*FAH*			X			

Continued

Table 1. *Continued*

	Gene(s)	Metabolic disorder			Endocrine disorder	Hemoglobin disorder	Other disorder
		Organic acid disorder	Fatty acid oxidation disorder	Amino acid disorder			
Primary congenital hypothyroidism	1				X		
Congenital adrenal hyperplasia	*CYP21A2*				X		
Sickle cell anemia	*HBB*					X	
B-thalassemia	*HBB*					X	
S,C disease	*HBB*					X	
Biotinidase deficiency	*BTD*						X
Critical congenital heart disease	2						X
Cystic fibrosis	*CFTR*						X
Classic galactosemia	*GALT*						X
Hearing loss	3						X
Severe combined immunodeficiencies	4						X
Conditions Not Included in Original RUSP (2006) because of No Available Screening Method							
Hyperbilirubinemia[1]	5						X
Familial hypercholesterolemia[1]	6						X
Carnitine palmitoyltransferase 1B (muscle)[1]	*CPT1B*		X				
Ornithine transcarbamylase deficiency[1]	*OTC*			X			
Severe combined immunodeficiencies[1]	4						X
Type 1 diabetes mellitus[1]	7						
Guanidinoacetate methyltransferase deficiency[1]	*GAMT*						
Wilson disease[1]	*ATP7B*						X
Arginine:glycine amidinotransferase deficiency[1]	*GATM*						X
Neuroblastoma[1]	8						X
Turner syndrome[1]	9						X

Continued

Table 1. *Continued*

	Gene(s)	Metabolic disorder			Endocrine disorder	Hemoglobin disorder	Other disorder
		Organic acid disorder	Fatty acid oxidation disorder	Amino acid disorder			
Carbamylphosphate synthetase deficiency[1]	*CPS1*						
Biliary atresia[1]	10						X
Fragile X syndrome[1]	*FMR1*						X
Congenital disorder of glycosylation type Ib[1]	*MPI*						X
Smith–Lemli–Opitz syndrome[1]	*DHCR7*						X
Adrenoleukodystrophy[1]	*ABCD1*						X
Mucopolysaccharidosis 1H[1]	*IDUA*						X
Fabry disease[1]	*GLA*						X
Creatine transporter defect[1]	*SLC6A8*						X
Lysosomal storage diseases[1]	11						X
Pompe disease[1]	*GAA*						X
Krabbe disease[1]	*GALC*						X

1. Most cases are sporadic but 15%–20% are genetic with *DUOX2, PAX8, SLC5A5, TG, TPO, TSHB*, or *TSHR* gene mutations
2. Multifactorial; most genetic causes unknown
3. Multiple etiologies including genetic and environmental; multiple genes associated with nonsyndromic and syndromic forms
4. 15 known genes associated; 14% of cases of unknown cause
5. Most causes are nongenetic
6. Most common genetic causes include *APOB, LDLR, LDLRAP1*, and *PCSK9* gene mutations.
7. Most multifactorial with rare single gene causes
8. Most sporadic; mutations in *ALK*, *KIF1B*, and *PHOX2B* in rare familial cases
9. Chromosome disorder
10. Multifactorial, most genetic factors unknown
11. Large number of disorders under this category with known genetic causes

Of the 31 conditions in the current RUSP, 27 have identified genetic etiologies, whereas the remaining four (congenital hypothyroidism, hearing loss, critical congenital heart disease, and severe combined immunodeficiencies) are frequently caused by identifiable genetic defects.

[1]Adapted from ACMG Newborn Screening Expert Group (2006). Table adapted from www.hrsa.gov/advisorycommittees/mchbadvisory/heritabledisorders/recommendedpanel.

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and despite being ranked highly in terms of their clinical significance, availability and efficacy of treatments and potential for avoidance of serious sequelae through early detection, could not be added to the recommended panel due to lack of an available screening test. Many have an underlying genetic etiology, with the causative genes identified and, therefore, could be detected through molecular techniques. In the list of 23 conditions not included in the recommended screening panel because they lack an accurate screening method (ACMG Newborn Screening Expert Group 2006), 21 are detectable in some or all cases with molecular genetic analysis and, therefore, have the potential to be added to NBS panels (see Table 1). Of 52 types of inborn errors of metabolism considered for inclusion in recommended NBS panels, all have the potential for detection with sequencing. These include Fabry disease, familial hypercholesterolemia, Wilson disease and the Creatine Deficiency Syndromes that cannot be detected with standard NBS methods.

Sequencing offers the ability to expand this list beyond inborn errors of metabolism. The list could include, for example, genes associated with early childhood cancer such as multiple endocrine neoplasia type IIB due to mutations in the *RET* gene, inherited channelopathies that lead to potentially preventable cardiac arrhythmias, and primary ciliary dyskinesia (PCD), a rare, genetically heterogeneous disorder resulting in a range of manifestations including situs inversus, neonatal respiratory distress at full-term birth, recurrent otitis media, chronic sinusitis, chronic bronchitis that may result in bronchiectasis, and male infertility.

Other conditions have not been included in recommended screening panels due to lack of treatment by traditional definitions. Individuals with conditions leading to intellectual disability might derive benefit from early detection and intervention, including earlier enrollment in developmental intervention services and avoidance of the diagnostic odyssey. In 2006, Alexander and van Dyck challenged the traditionally held belief that NBS should only include conditions with effective treatments and broadened the concept to include conditions with benefits to the family for reproductive decision making, the potential to participate in research or innovative therapeutics and avoidance of the diagnostic odyssey (Alexander and van Dyck 2006). On the other hand, genetic information predicting the inevitable development of an incurable genetic disorder may be unwelcome to some parents of otherwise apparently healthy newborns. The practice of mandatory screening for such nonmedically actionable conditions, if widely accepted by public health screening programs, would dramatically alter the nature of the screening program, potentially undermining the currently accepted practice of screening without obtaining explicit consent.

ETHICAL CONSIDERATIONS

Unlike many kinds of medical tests, which provide information of a transient, temporal nature, genetic testing typically can reveal information about an individual's past, present, and future medical conditions; this information may also have immediate implications for family members. These characteristics, combined with the complexity of genetic information, which ranges from probabilistic to highly deterministic, has led many to view genetic information as somehow different from other kinds of biomedical information ("genetic exceptionalism"). Consideration of the ethical, legal, and social implications of genetic knowledge has been an inherent component of the Human Genome Project and other genomic research efforts (Greely 1998), and inexorable advances in genetic testing have been accompanied by an immense societal discussion about the most appropriate uses of this information in healthcare, in human subjects research, and even in the setting of personal genomic exploration (Bunnik et al. 2011). The thread of an individual's right to self-determination is woven tightly throughout the ethical considerations of genetic testing (e.g., Nyrhinen et al. 2009; Bunnik et al. 2013). Genetic testing in children raises additional complexities that could potentially alter the dynamic that currently exists in NBS.

Considerations of the benefits and risks of genetic testing are perhaps most acute in chil-

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dren because of their special status as minors, under the guardianship of their parents for a period of time, after which they may achieve independence and acquire their own right to self-determination (Lantos 2010). Although parents are given a significant amount of leeway in their decisions about how to raise their children, there are also limits on this guardianship regarding that child's future autonomy. Parents have the responsibility to act in their child's best interests, which is the primary consideration in most approaches to pediatric genetic testing. Further complicating matters, consideration of such testing inevitably occurs in the context of highly variable childhood developmental states and unique family settings (Fanos 1997).

Expert panels have put forth various guidelines to delineate appropriate uses of genetic testing in children (Wertz et al. 1994; ASHG Board of Directors, ACMG Board of Directors 1995; AAP Committee on Bioethics 2001; Ross et al. 2013b). These recommendations have traditionally been grounded in "best interests" being limited strictly to the impact of genetic information for the child in question. For example, it is generally agreed that testing children for adult-onset conditions should be avoided when the information would not directly impact medical management during childhood. This recommendation typically envisions the scenario in which a condition is known to exist in a family and the at-risk child will be able to make an informed decision about genetic testing when they reach adulthood. The recommendation to avoid predictive genetic testing is based in the idea that such testing will not alter medical management of the child, and that there could be psychological harms associated with learning one's mutation status. The ACMG recommendations regarding the return of adult-onset clinically actionable incidental findings in children (Green et al. 2013) appears to be at odds with these restrictions on testing for adult-onset disorders, except that in the case of a child undergoing diagnostic genome-scale sequencing or NBS, there may be no knowledge in the family about a clinically actionable adult-onset disorder (e.g., a 25-year-old mother with no family history of early-onset breast cancer who inherited a *BRCA1* mutation from her father). Such a finding, if not reported, could lead to irreparable harm to the child caused by the early death of a parent from a condition that might have been prevented. This type of incidental finding could therefore have direct psychological benefit to the child and be in the "best interests of the child," even though the revelation of the information may obviate that child's "right not to know" later in life. However, opinion seems to be split regarding the justification of revealing information about adult-onset clinically actionable conditions in a child when the benefits are theoretical and less certain to accrue than the discovery of a condition with direct medical implications for the child (e.g., Strong et al. 2014; Yu et al. 2014). Clearly, there is equipoise about the balance of benefits and harms in this situation. There is a view that testing for carrier status for recessive disorders is not likely to benefit the child and should thus be deferred until the individual is considering reproduction. However, it should be noted that substantial counter arguments have been made on behalf of informed decision making by parents, despite these concerns (Pelias 2006; Rhodes 2006).

In the recommendations made by the ACMG and AAP regarding the uniform screening panel (ACMG Newborn Screening Expert Group 2006), the expert group made three recommendations that could have profound implications for NBS via genome-scale sequencing.

First, the expert group recommended that 25 additional "secondary" targets be examined and reported. Although the original intent of NBS was to detect only the specific condition screened for, such as PKU, it has long been recognized that some with positive newborn screens had less medically significant conditions, such as hyperphenylalaninemia, raising concerns that this would cause some children to undergo unnecessary treatments and cause parents undue anxiety (Gurian et al. 2006). These 25 conditions are "clinically significant and revealed by the screening technology but lack an efficacious treatment" and the expert group "thought it was important that such findings be communicated to the health care service community and

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to families" (ACMG Newborn Screening Expert Group 2006). The direct implication of this recommendation is that any clinically relevant finding from NBS should be reported, which in the case of genome-scale sequencing would include essentially any genetic condition. Presumably, the expert group was not envisioning the use of a genome-scale sequencing test for NBS when they made these recommendations, because many families would refuse such testing if they knew there was a possibility of learning about findings without any related preventive measures (Bombard et al. 2014), thus jeopardizing the immense value of the newborn screen at the population level.

Second, the expert group recommended that states "mandate . . . reporting of any abnormal results that may be associated with clinically significant conditions, including the definitive identification of carrier status" (ACMG Newborn Screening Expert Group 2006). In practice, carrier results for cystic fibrosis and sickle cell disease are routinely returned as part of NBS. Expanding this recommendation for all conditions detectable by genome-scale sequencing would essentially convert NBS from a program that detects rare, preventable disorders in a small minority of cases into a carrier screening test for all recessive disorders that would yield a handful of findings in every individual screened. Again, the expert group was likely not considering the implications of this recommendation for genome-scale sequencing being used in NBS.

Finally, the expert group recommended that states "consider that the range of benefits realized by NBS includes treatments that go beyond an infant's mortality and morbidity" (ACMG Newborn Screening Expert Group 2006), which seems to imply that there is value to NBS beyond preventable conditions—That personal utility (or utility as perceived by the family unit) is just as valid a consideration in determining what information to divulge as the traditional values of improving the health of the individual child. This recommendation is somewhat similar to the ACMG incidental findings recommendations, in which the benefit to the child is indirect and related to the overall well-being of the child's family members. That being said, the expert group's recommendation, if taken to the logical extreme, could be interpreted as meaning that any genetic information that is desired by the parents is justifiable if considered beneficial by the parents.

Most would likely agree that when genetic information is available, parents should have a reasonable ability to learn such information if desired, and also to refuse information that they do not want. The challenge is in defining what is "reasonable"—Herein lies the equipoise when considering the use of genome-scale sequencing in NBS. It can be argued that parental prerogative is the primary consideration—Parents are responsible for their child's health care, and learning (or refusing) information about any genetic condition could be considered part of this responsibility. On the other hand, some information could have damaging effects on the child's own well-being if that knowledge interferes with parental bonding, creates family stress including divorce, or leads to abuse or abandonment. In addition, even the decision making process could lead to strife between parents if they are unable to agree about whether or not to learn such information.

The mainstream consensus of the bioethics community appears to be that adult-onset disorders with no effective prevention or treatment should be off-limits to parents and are most appropriate for informed decision-making by the individual when he or she becomes an adult. That being said, some argue that even these disorders fall within a parent's responsibility to raise their child to the best of their ability and prepare them for any eventuality, that the theoretical harms are less significant than initially supposed (Malpas 2008) and that parents are in the best position to make decisions relative to their child's best interests (Robertson and Savulescu 2001). Furthermore, in the case of a disabled child who will likely never be able to make an informed decision, parents could reasonably expect to make such decisions on that child's behalf.

Clearly, the application of genome-scale sequencing in NBS raises a host of ethical, legal,

and social implications (Tarini and Goldenberg 2012). Challenges related to the use of genomic sequencing in newborns, both technical and ethical, will need to be overcome to establish a widely accepted NGS-based platform to augment NBS.

CONCLUSION

The possibility of a significant expansion of NBS raises a number of concerns, including the lack of evidence-based efficacy studies, the need for informed consent, the challenge of providing information and support for families, and the ethical, legal, and social issues associated with such scenarios as disclosure of carrier status or genetic susceptibility to future disease (Taylor and Wilfond 2004; Botkin 2005; Bailey et al. 2006). There are major objectives that need to be addressed to incorporate use of genomics and other technological advances in NBS. Public and professional education will be required, and the expert infrastructure for dealing with children who screen positive will need to be improved significantly (Alexander and van Dyck 2006). Some consideration should be given to whether the long-held criteria for screening should be changed, by broadening the concept of benefit from screening for the child to include the family.

With these challenges in mind, pilot projects to examine these issues are being funded by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) and the National Human Genome Research Institute (NHGRI) of the National Institutes of Health (NIH) under the Genomic Sequencing and Newborn Screening Disorders research program. Use of genome-scale sequencing in NBS will require careful consideration and informed decision making by parents and education of providers as they use this technology.

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Potential Uses and Inherent Challenges of Using Genome-Scale Sequencing to Augment Current Newborn Screening

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RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART A

Experiences With Obtaining Informed Consent for Genomic Sequencing

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Despite the increased utilization of genome and exome sequencing, little is known about the actual content and process of informed consent for sequencing. We addressed this by interviewing 29 genetic counselors and research coordinators experienced in obtaining informed consent for sequencing in research and clinical settings. Interviews focused on the process and content of informed consent; patients/participants' common questions, concerns and misperceptions; and challenges to obtaining informed consent. Content analysis of transcribed interviews revealed that the main challenges to obtaining consent related to the broad scope and uncertainty of results, and patient/participants' unrealistic expectations about the likely number and utility of results. Interviewees modified their approach to sessions according to contextual issues surrounding the indication for testing, type of patient, and timing of testing. With experience, most interviewees structured sessions to place less emphasis on standard elements in the consent form and technological aspects of sequencing. They instead focused on addressing misperceptions and helping patients/participants develop realistic expectations about the types and implications of possible results, including secondary findings. These findings suggest that informed consent sessions should focus on key issues that may be misunderstood by patients/participants. Future research should address the extent to which various stakeholders agree on key elements of informed consent. © 2015 Wiley Periodicals, Inc.

Key words: genetic counseling; genetic testing; genomic sequencing; informed consent; qualitative research

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INTRODUCTION

Genome sequencing is increasingly being performed in both clinical and research settings. Currently, clinical sequencing primarily aids in the diagnosis of individuals who have conditions suspected to have a genetic etiology [Biesecker and Green, 2014]. In such situations, sequencing identifies a pathogenic variant in about 25% of cases [Lee et al., 2014; Yang et al., 2014], occasionally leading to significant changes in clinical management [Worthey et al., 2011; Milligan et al., 2014]. Although currently not in widespread use, clinical genomic sequencing can guide cancer therapy selection and monitoring [Garraway, 2013; McLeod, 2013; Van Allen et al., 2014] and is being applied in many other clinical situations [Bowdin et al., 2014; Dewey et al., 2014]. Despite predicted clinical utility, experts have identified factors that preclude its rapid clinical adoption, and limitations that should be addressed in the informed consent process [Burke et al., 2013; Evans and Khoury, 2013; Manolio et al., 2013; McLeod, 2013;

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Biesecker and Green, 2014; Dewey et al., 2014; Vrijenhoek et al., 2015]. To briefly summarize, technical limitations prevent the identification of all disease-associated variants, and the reliance on incomplete and error-prone variant databases prevents the unambiguous classification of many variants that are identified. Despite efforts to improve and streamline variant capturing and calling, data on genotype-phenotype correlation may be unavailable, and controversy remains about which variants, or categories of variants should be returned to patients. Additional barriers to clinical adoption include limited evidence of clinical validity and utility of test results; the uncertain nature and frequency of adverse psychosocial outcomes resulting from testing; and concern that patients and providers might not understand results or act appropriately on them.

The informed consent process can be used to help patients understand the implications of possible results and the limitations of testing, and make decisions about the return of secondary findings. Lessons learned from experiences in obtaining consent and returning results could guide best practices, potentially preventing some of the possible harms if and when sequencing gains widespread use.

The National Institutes of Health through the Clinical Sequencing Exploratory Research (CSER) Consortium has spearheaded efforts to collect evidentiary data for the successful clinical integration of genomic sequencing. CSER consortium projects offer genome or exome sequencing to participants with a variety of clinical indications to investigate the efficacy, impact and outcomes of testing [Gray et al., 2014]. Cohorts represented in the various CSER consortium projects include healthy adults; adults seeking preconception carrier screening; adults who previously participated in genetic research; and children and adults with suspected genetic conditions, including various types of cancer, cardiomyopathies, or intellectual disabilities. In each of these projects, participants are offered, or randomized to be offered, some secondary findings. Additional information about the CSER projects can be found at https://cser-consortium.org/projects.

Although the offer of sequencing in these projects is governed by site-specific research protocols, there are many direct links to clinical activities. For example, participants are usually recruited by the patients' clinicians; the confirmed, diagnostic results may be entered into the participant's medical record; and clinicians who either assume responsibility for acting on results or making appropriate referrals are members of the research team [Burke et al., 2014]. Thus, the content of the consent process in these projects must address elements relating to research participation as well as the expected risks, benefits and limitations of learning clinically-relevant and non-relevant results, including secondary findings [Scollon et al., 2014].

Policies are also being developed that address clinical integration of genomic sequencing, including informed consent [Manolio and Green, 2014]. The American College of Medical Genetics and Genomics (ACMG) has defined a minimum list of "actionable" genes for which laboratories should report pathogenic variants identified as secondary findings because appropriate clinical action can significantly reduce disease risk [Green et al., 2013]. The initial ACMG statement recommended that, regardless of the clinical indication for sequencing, laboratories should analyze and report mutations found in 56 genes associated with 24 Mendelian conditions [Green et al., 2013]. The revised statement acknowledged that patients should be able to decline secondary findings [ACMG Board of Directors, 2014].

The ACMG has also outlined points to consider for the informed consent process for genomic sequencing [ACMG Board of Directors, 2013]. Recommended content of informed consent includes the likelihood and types of primary and secondary findings that might be returned; the risks, benefits and limitations of testing; potential implications for family members; whether identifiable results are provided to databases; and policies for re-contacting as new knowledge is gained about the clinical implications of a variant. The ACMG guidelines are thus broad and primarily address the content, rather than the process, of informed consent. Early reports of the consent process for research genomic sequencing described the complexity [Ormond et al., 2010; Rigter et al., 2014], and warned of the large amount of time needed to obtain informed consent [Mayer et al., 2011; Tabor et al., 2012]. It has become clear that, in order to make the process scalable and manageable to both patients and clinicians, alternative strategies are needed that provide important information without overwhelming the recipient [Hooker et al., 2014].

As a first step to examining which content was deemed important to relay, the consent forms being used by the 9 U01 CSER projects were compared [Henderson et al., 2014]. The content showed considerable heterogeneity, an unsurprising finding in light of the differences in the types of patients recruited, the inclusion of results in the medical record, the results eligible to be returned and the degree to which participant preferences influence this decision [Henderson et al., 2014]. It was also noted that the consent forms were generally long (mean number of words was 4588) and used language demanding a high reading level (median Flesch-Kincaid grade level was 10.8). However, the content of consent forms may not be associated with one's level of understanding, as many participants (and patients) sign consent forms without reading them [Joffe et al., 2001; Desch et al., 2011; Robinson et al., 2013]. Even when relatively simple and recognizable language is used in consent documents, gaps in understanding remain [Morgenstern et al., 2014]. The potential discrepancy between consent form content and patient comprehension highlights the critical role played by interpersonal interactions to promote understanding, autonomy and shared decision-making [Schenker et al., 2011; Nishimura et al., 2013; Presidential Commission for the Study of Bioethical Issues, 2013].

Genetic counselors have played a central role in interpersonal interactions during the consent process for genetic testing because of their expertise in educating patients and families about genetics, and counseling them about the risks, benefits and limitations of genetic tests [Markel and Yashar, 2004]. Settings offering genomic sequencing both clinically and under research protocols in the United States rely on genetic counselors to obtain informed consent and participate in the return of results [Iglesias et al., 2014; Rigter et al., 2014; Williams et al., 2014]. Some CSER projects also use research coordinators trained by genetic counselors to obtain consent.

We conducted semi-structured interviews with individuals in the U.S. who are currently among the most experienced at obtain-

ing consent: genetic counselors and research coordinators for the CSER-sites and genetic counselors obtaining consent for clinical sequencing. In this paper, we describe the content and process of informed consent for genomic sequencing as reported by these experienced professionals, and describe factors that influenced the way they conducted consent sessions. These data can be used to inform the development of guidance on the content and process of informed consent for genomic sequencing.

MATERIALS AND METHODS

Participants

We recruited study participants in two ways. First, we contacted a principal Investigator (PI) or Co-PI from each of the 9 NIH-funded U01 CSER projects or from other projects in the CSER consortium that offer genomic sequencing, to request names and contact information for 1-3 individuals (study coordinators, genetic counselors or physicians) with the most experience conducting informed consent sessions for their genomic sequencing project. Second, investigators identified 5 large clinical centers in the United States known to the study team for their experience offering clinical genomic sequencing and contacted 1 genetic counselor at each center about participating in the project.

Recruitment and Data Collection

A study investigator sent an email describing the study to potential interviewees and scheduled a telephone interview. The study team developed a semi-structured interview guide that included open-ended questions followed by probes asking interviewees to describe their clinical experience and responsibilities; the process of consenting study participants and/or patients for genomic sequencing; common questions, concerns and misperceptions patients/participants had raised; how obtaining informed consent for genomic sequencing differed from obtaining consent for other types of genetic tests, and challenges that had arisen and how they responded to them. We also asked interviewees to describe a particularly challenging or memorable case; findings from that part of the interview have been reported elsewhere [Tomlinson et al., 2015].

Interviews were conducted by four study team members (three genetic counselors and one social worker). Each interviewer initially conducted one interview and the transcripts from those interviews were reviewed and discussed by team members. The interview guide was modified slightly after the initial interviews, and feedback was given to standardize the way each interviewer asked questions and the probes used to expand on interviewee responses. After obtaining verbal consent, we conducted digitally recorded phone interviews between March and July, 2014. Each interview lasted between 30 to 80 min, and was later transcribed.

Data Analysis

We reviewed the transcribed interviews to check for accuracy, completeness, and to remove any identifiable information. We imported de-identified interview transcripts into QSR International's NVivo 10 software for coding and content analysis. Study investigators met after reviewing a subset of transcripts to develop a coding system through an iterative process standard for content analysis [Miles et al., 2013]. Initial codes related directly to questions asked during the interviews (for example, common questions that participants/patients raised; length of sessions, challenges, etc.). Eight transcripts were independently coded by two investigators, both of whom are experienced qualitative researchers with considerable coding experience, and differences in coding were resolved by consensus. Because there were so few discrepancies in coding, the remaining transcripts were coded by a single coder. New codes were added to the codebook as needed, after discussion with the study team. After all transcripts were coded, the investigators reviewed the coded data to identify dominant themes. Anticipating that obtaining informed consent in a research setting might differ in important ways from that obtained in a clinical setting, we noted the setting and whether the interviewee was a genetic counselor or a research coordinator while reading the transcripts and summarizing findings. We selected representative quotes to illustrate pertinent findings.

The study protocol was classified as exempt by the Institutional Review Board of the University of Pennsylvania.

RESULTS

Twenty-nine of 35 potential interviewees contacted completed interviews for an 83% participation rate. The majority of interviewees were genetic counselors; about half had at least 6 years' experience (Table I). Thirteen had experience obtaining informed consent for sequencing in clinical settings and nearly all had

TABLE I. Interviewees (n = 29)

	N	%
Profession		
Research coordinator	8	27.6
Genetic counselor	21	72.4
Years of professional experience		
0–2	6	20.7
3–5	8	27.6
6–10	4	13.8
>10	8	27.6
Not reported	3	10.3
Sequencing consenting experience		
Research only	16	55.1
Clinical only	2	6.9
Clinical and research	11	37.9
# Patients/participants personally consented		
<20	5	17.2
21–50	13	44.8
>50	10	34.5
Not reported	1	3.4
Population consented for sequencing		
Children only	5	17.2
Adults only	14	48.3
Children and adults	10	34.5

conducted at least 20 consent sessions for exome or genome sequencing.

We have organized the results of the interviews into findings related to the contextual issues interviewees considered as they structured a consent session, differences between obtaining informed consent for genomic sequencing and for other types of genetic testing, the *process* of consenting, and the *content* of consent sessions. The individuals interviewed are referred to as "interviewees". We use the term "patients/participants" in cases where interviewees referred to patients or research participants interchangeably. Otherwise, those offered sequencing are referred to as either patients or participants. When interviewees referred to consenting a child for sequencing, we frequently use the term "family" because the parents were the primary participants in the informed consent discussion.

Contextual Issues Influencing the Consent Process

Interviewees identified a variety of contextual factors that influenced their approach to conducting consent sessions (Table II). Interviewees considered these factors when structuring sessions and anticipating how different families might weigh the risks and benefits in deciding whether or not to agree to sequencing and/or opt to learn secondary findings. First, interviewees considered whether sequencing was being offered as a part of a research protocol or as a clinical service, and ensured that the patient understood the difference, especially because research testing was frequently offered by the participant's own clinician during the course of a clinic visit. As one research coordinator explained:

> "The very first thing that we do is we make it extremely clear that the clinical visit is over—anything that was discussed in the clinical visit is over and completely separate. We say: "We are now going to embark on a research topic". We stare them right in the face and make sure they get that. (03-2)

Interviewees pointed out that discussion of the return of secondary findings also differed according to the context for offering testing. With research testing, the research protocol dictates which, when and how secondary results would be returned. When testing is offered in a clinical setting, discussion of return of results, including secondary findings is frequently based on policies of the laboratory performing the sequencing. When genomic testing is offered to children in both research and clinical contexts, the fact that some results might be available on parents when trios are tested became an important contextual issue that interviewees considered when obtaining consent. Interviewees also discussed how they varied the consent session when consenting a healthy adult in a research protocol, for whom all findings would be secondary, as opposed to testing an affected child whose parents may have spent years seeking the cause of their child's condition. In those cases, parents frequently have an inflated expectation for an answer from exome or genome sequencing. Additionally, in families with an acute illness, such as cancer, interviewees pointed out that there is an unrealistic expectation that sequencing will lead to modifications in treatment. Because of their preoccupation with their child's serious illness, such families may fail to attend to discussion of secondary findings, or may make decisions about return of such findings based on limited consideration of risks and benefits. Interviewees also explained that they approached informed consent differently depending on how much time the family could devote to the session, how much time they had already spent at the hospital that day, and the amount of time that had elapsed after a diagnosis was made or suggested. When families had limited time available, or were already overwhelmed, interviewees indicated that they might decide to obtain informed consent over two visits. Finally, interviewees considered how much experience a patient or family has had with genetic testing. In families new to genetic testing, for example, when a child is diagnosed with cancer, more explanation of genetics might be included in the informed consent session. When offering genomic sequencing to the parents of a child who had already had multiple genetic tests, interviewees would spend minimal time discussing the basics of genetics, but more time explaining the difference between whole exome sequencing and other tests previously performed, such as chromosomal microarray analysis or testing for mutations in single genes.

How Obtaining Consent for Genomic Sequencing Is Different

The genetic counselors interviewed were asked how consenting for genomic sequencing differs from obtaining informed consent for other types of genetic tests. Nearly all interviewees indicated that there are distinct differences, primarily relating to the broader scope of possible results available from genomic sequencing, as well as the greater potential for obtaining uncertain results. As one genetic counselor explained:

> "The scope is so much broader. . .. the possibility [of a VUS] is so much greater and there's so much more that we don't know than we do." (05-1)

Genetic counselors discussed having to change their usual approach of providing in depth education about potential results that might be obtained when testing for single genes or small panels of genes. One interviewee explained how she modified her typical approach:

> "When we first started thinking about doing sequencing, we were overwhelmed just because we had been trained to consent for a single gene test or a panel of tests and since there was less information to talk about, we did a really good job explaining every piece of that. But with sequencing, you can't possibly explain every single outcome. You don't know every single outcome" (07-3)

Given the possibility of secondary findings results, interviewees pointed out that unlike testing for a single condition for which there is a family history, patients/participants may have no experience with the types of conditions they might learn about through genomic sequencing. One genetic counselor said:

TABLE II. Contextual Issues Considered When Approaching Informed Consent

Issue	Reason for importance	Illustrative quotes
Research vs. clinical testing	Expectation of benefit; discussion of study-related procedures; confusion about research testing in clinical setting; types of results returned	*"In clinic, the message is clearer because you're just talking about the test; you're not also talking about all the complexities of research." (10-2)* *"Because we're doing this research project in the clinic. . . they feel like they're getting a medical test"(03-1)*
Pediatric vs. adult	Assent for pediatric testing; availability of results on parents when children are tested (trio testing)	*"So we want to always try to obtain assent when we should, but sometimes they're playing video games. I need to really make sure that they are part of the conversation. " (01-1)* *"Even though we're doing the test on the child we can find out information about the parents." (01-3)*
Healthy vs. affected	All results are incidental in healthy individuals; those affected expect an answer/treatment	*"I think the healthy population usually has more concerns—maybe just because they have more to lose." (07-2)* *"When people are so wanting the information and wanting a potential genetic diagnosis, does that cloud the ability to truly think about secondary findings or to truly think about potential risks that could come from it?" (20-7)*
Type of illness	Individuals with acute illness may expect testing will lead to treatment; In families with an acute illness, genomic testing may be a low priority; diagnostic odyssey for those with chronic illness; less concern about risks for those with terminal illness	*"These parents are concerned with their kid with cancer. That's their number one thing. That's so overwhelming in itself that they really don't stress out, or think so much, about the genetic testing." (01-1)* *"Most of the patients, participants that we see, are coming in so excited about the study that they don't want to listen to any of it, they just want to sign the consent form. Like, okay, where do I sign? I'll sign now." (8-2)* *"Our patients are terminal, and so their motivations for enrolling in these kinds of projects might be different from other groups because they have nothing to lose, at this point." (09-2)*
Timing	Amount of time family has been in clinic; poor attention/comprehension if individual recently became ill/received diagnosis	*"A lot of times they've got four more appointments and they're trying to run to their next appointment, so we definitely go with the flow and are flexible." (01-2)* *"A very common answer would be 'I got my hands full. I'm looking at chemotherapy and I can't handle this.'" (03-2)*
Previous experience with genetic testing	Level of knowledge about genetic testing; need to differentiate exome/genome sequencing from other genetic tests	*"For most people in the study, this is not their first genetic test so they've already been consented for some clinical genetic testing" (10-2)* *"So I think that the biggest challenge is that it does require a pretty in-depth knowledge of genetic information. We're fortunate that the people who are getting to us have often gone through some of that process already, so the baseline level is a little bit higher." (20-8)*

"The hardest part is when you're counseling for a specific gene, people come in with some idea of what this might mean for them because they had some experience with the condition in question. . .[with sequencing] they may have a finding that doesn't make sense to them at all because they don't have any personal experience with it." (09-3)

Because of this, the decision-making process about undergoing testing may differ, as this interviewee explained:

"So it seems like they're almost more thoughtful when it comes to a single gene disorder, which they may experience in their family...I think it's harder, sometimes, to deal with the implications of a known quantity than an unknown quantity" (08-2)

Since patients/participants do not necessarily expect to learn results that are unrelated to their primary indication for testing, several interviewees discussed how they try to prepare patients for

thinking about the broader scope of genomic sequencing before they obtain consent. One genetic counselor offering clinical sequencing explained:

> "It's tough with the optional pieces and they have a lot they need to decide, so I do try to get them ahead of time, before we want to order the test, and give them some time to discuss it with family or think about it instead of right there when they're in front of me". (01-2)

Process of Consenting Patients/Participants

When asked to describe the process by which they obtained consent, interviewees reported a variety of approaches. In clinical settings, the consent process was conducted by a genetic counselor with or without a clinical geneticist and occurred as part of a single clinical visit, or in 2 sessions when insurance pre-authorization was needed. The consent process was integrated into the genetic counseling session to include a discussion of the risks, benefits and limitations of testing. In the research settings, the process of obtaining consent was largely influenced by the specific research design and protocol. As shown in Table III, a physician, usually the patient's clinician, most often introduced the study and explained what participation would entail. In all projects, a research coordinator and/or a genetic counselor explained study components and obtained informed consent. In nearly all studies, patients who enrolled interacted with, or were given the opportunity to interact with, a genetic counselor. In 2 of the 9 CSER sites, participants were always given the consent form or educational materials before the study visit. In 3 additional studies, this access sometimes occurred.

Many interviewees explained that they initially had conducted sessions by closely following the order of topics in the consent form but, as they gained more experience, they began to summarize the main topics and re-order topics discussed according to the desires of participants. Both genetic counselors and research coordinators reported modifying their sessions in this way. This change led to much less rigidly structured sessions guided largely by the individual patient/participant's level of knowledge, interests and concerns. As one interviewee said:

> "When I first started, I stuck to the consent form more. Now I've developed my own way to explain it in an easier way to understand. It also depends on the participant; I kind of change the way I speak based on how informed they are on the topic." (07-2)

This restructuring allowed for more family engagement in the discussion. Also, with more experience, interviewees reported that they gained more of a sense of the kinds of questions a family might be expected to ask, and guided families to ask them if they were not voiced during the session. One interviewee explained:

> "I'm able to say 'some people want everything back; some people don't want anything back.' Just having experienced some questions or concerns that other families have brought up before, I can incorporate that into the session if the families aren't really talking much or if they don't have a lot of questions." (06-3)

Interviewees reported session length varying between 10-70 minutes with 30 minutes being the most common length reported. In general, informed consent sessions for sequencing offered as a part of a research protocol were slightly longer than those for clinical sequencing because of the need to discuss the procedures involved with research participation. In both settings, the factor most often reported to increase the session's duration was a family's increased interest and engagement. Other factors associated with longer sessions were less familiarity with the genetic testing process, lack of participants' previous exposure to the consent form and/or the educational materials, less previous discussion about the study or about sequencing by clinical or study personnel, the presence of a disruptive child, and the need for a language interpreter.

Content of Informed Consent Sessions

The content of sessions also varied and was largely determined by the context for testing and patient/participants' questions and concerns, and their underlying knowledge and expectations of their potential sequencing results. Most interviewees indicated that

TABLE III. Components of the Informed Consent Process Used by CSER U01 Projects

	Study number								
Informed consent component	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
MD introduces study	+	+	+	+	+	+	+	+	
IC form/educational materials sent ahead	+/−	+/−		+		+/−			+
RC contact by phone before IC session		+/−		+	+				+
RC consents		+							
RC consents/GC provided					+				
RC consents/GC offered								+	
GC and/or MD consents	+					+			+
GC or RC consents			+						
GC and RC consent				+			+		

IC, Informed consent; RC, Research coordinator; GC, Genetic Counselor. + = always done in study +/− = sometimes done in study.

the main educational challenge to obtaining informed consent for genome sequencing stemmed from the patient/participants' unfamiliarity with the broad scope of results that could be returned, including multiple variants of uncertain clinical significance (VUS), and their blurring the distinction between diagnostic and secondary findings related to health. In addition, the participant's or parents' need to make decisions about whether or not to learn about various categories of secondary findings led to consent sessions that were different from those addressing other types of genetic testing. Interviewees observed that many patients/participants clung to the unrealistic expectation that their results would illuminate not only the condition for which sequencing was indicated, but also any possible future health problems. This commonly-held belief led interviewees to emphasize the limitations of sequencing in order to help patients/participants to develop realistic expectations about the types and utility of results that might be learned.

Interviewees who conducted research consent sessions described the difficulty of maintaining participants' attention as they tried to review the content of the consent document, facilitate understanding of the types of results that could be returned, and help participants make decisions about which secondary findings to request. With more experience, both genetic counselors and research coordinators began to paraphrase or only briefly review study-related items contained in the consent document. They also placed less emphasis on educating participants about genomics and sequencing techniques, focusing instead on describing the kinds of results that could be learned and their implications. Much of the variability of the content stemmed from differences between research protocols about topics such as how results would be returned, which types of secondary findings could be learned, and the inclusion of results in the medical record. For example, in some CSER projects, results were returned spanning two separate visits, in some, participants could choose to learn about many types of secondary findings, and in some projects, all or a portion of results were automatically included in the medical record.

The Patient/Participants' Perspective—Which Questions, Concerns and Misperceptions Influence the Content of the Consent Session?

The most common patient/participant questions, concerns and misperceptions reported by interviewees are shown in Table IV. Other than questions about practical aspects of research participation or testing, the majority of research participants raised few questions spontaneously during the consent sessions. Interviewees attributed the scarcity of questions to: patients'/participants' previous experience with genetic testing or with research participation; the extent to which they had already interacted with study personnel; their access to study materials, including educational pamphlets and the consent form prior to the session; being overwhelmed by the informed consent process or by their or their child's current illness; and/or the novelty of genomic testing. Although some patients/participants raised concerns about privacy, confidentiality and the potential for insurance discrimination, in most cases, questions or concerns were raised only after these topics had been introduced. Interviewees frequently attributed the apparent lack of concern about risks of testing or study participation to the patient/participants' primary focus on getting an answer to the health problem prompting sequencing, or because the option of sequencing had been introduced by a trusted physician.

Most of the misperceptions reported related broadly to patient/participant naiveté about the limitations of genomic sequencing. One genetic counselor explained that many patients have high expectations that genomic sequencing will provide a great deal of clinically useful information:

> "I think sometimes people think we have trust in our ability to interpret the genome more than they should. So they believe that this information will be really useful to their healthcare or provide them with information that could change their lives." (07-2)

Another genetic counselor pointed out that patients/participants frequently believe that sequencing will provide a definitive answer about the cause of their own or their child's condition:

> "They believe that if we don't find an answer maybe it's not genetic or that if it's genetic we should find an answer every time. I think it's probably hard for a lot of people to understand how much we don't know." (10-1)

The Professional's Perspective—What Content Information Should People Understand in Order to Provide Informed Consent?

In an open-ended question, interviewees were asked to identify the elements that they believed were most important for patients/participants to understand in order to provide informed consent. Twenty elements were mentioned by at least one interviewee (Table V). The most common ones included promoting understanding about the types of results that could be returned, the limitations of testing, especially when negative results were returned, and the implications of the results for the individual. Less commonly mentioned were implications for relatives, the requirements of study participation, privacy protections, and the potential for genetic discrimination. Research coordinators were more likely than genetic counselors to mention research-related items as important.

Several interviewees who were genetic counselors stated that, as they gained more experience reviewing, interpreting, and returning results, they modified their consent sessions to provide more specific and explicit descriptions of the range, prevalence and examples of possible results. As one genetic counselor stated:

> "We're not finding secondary findings in every family, and so that's something I've started making more clear during informed consent...from going through the variants and seeing the types of results that we're giving back also can give me some examples that I use when I'm talking about types of result that we give back." (04-1)

TABLE IV. Common Patient/Participant Questions, Concerns and Misperceptions

Common questions and concerns	Illustrative quotes
Practical details of study	*"I think, honestly one of the main things is the logistics of the blood draw for the kid and how – what kind of involvement that they need to have. (1-03)*
	"There are questions about does the child have to come back to the return visit? How long will it take? Will we get a copy of the results?" (04-1)
Probability of finding an answer	*"I think most commonly, "what are the chances. . .?". . .like "what is the chance that this is gonna find the answer?" (20-7) "*
Possible results	*"A lot of people are asking about kind of multi-factorial conditions. Like is this going to tell me about diabetes?" (08-1)*
Privacy/ confidentiality	*"Privacy issues—how is my information going to be kept private? Is it possible to keep it private? That kind of thing."* (07-1)
	"We also talk about sharing data with DbGap. . .a lot of people are concerned about privacy and aren't that comfortable sharing that information with this public database." (20-6)
Effect on other family members	*"Generally there are questions about what impact this might have for their family. . . "if you do find something, does that mean my family should come back in here?"* (03-2)
Anticipated response to results	*"There have been people who we've been worried about how they might respond to getting testing results back and have not enrolled because it just seemed like too big of a risk to their mental health" (05-1)*
Insurance discrimination	*"I find that most people have no idea about GINA even though it's in the consent form. . .And so that tends to be the thing that I bring up that actually does give people pause during the consent process" (05-1)*
Impact of results on management	*"The question comes up about if it is positive, is there a cure? Is there a treatment?" (06-3)*
Common misperceptions	
Negative results mean a "clean bill of health"	*"I think one misperception that I've heard is some people say well, I hope that this Genome Report tells me that I'm healthy, gives me a good prognosis." (07-3)*
Negative result means not genetic	*"When we're giving negative results, the idea that what they're doing here is kind of the ultimate genetic test that's gonna identify all genetic causes – if we don't find something, that it's gonna rule out genetic conditions, and mean that their child doesn't have one. (04-1)*
Report will contain many incidental findings	*"They're surprised when there's not anything to tell them. They're surprised if they get just a couple pharmacogenetic results. . . people think that their exomes, or genomes, are gonna be more interesting than they actually are. "* (03-1)
Sequencing will identify the cause of a condition	*"A lot of parents put so much hope into this, especially when their kids have been through so much and they've had so many different tests that they – their expectations are very, very high"* (20-2),
Expect incidental results to explain diagnosis in absence of diagnostic findings	*". . .so the biggest [misperception] is that incidental findings are either going to hold a secret to the answer for their diagnosis or are going to interact in a meaningful way with their diagnosis. ..folks definitely think that the incidental findings are going to be more medically meaningful for them than we think they have the potential to be." (10-2)*
Results will be certain	*"The idea that genetic information might give you a "due date" or something like that. . .or you'll get something back that'll say you're definitely gonna get stomach cancer or you're definitely gonna get Alzheimer's when you're fifty." (02-1)*
Genome will change over time	*"Some people will ask "well for the reanalysis do you have to take blood again?" because they think that things might change, or an answer might appear, because their genes have changed" (10-2).*
Results will be predictive of future health	*"I think that somehow they feel like we are going to open this Pandora's box and answer every possible question for them. . .They just think it's so exciting and that we can predict the future with it." (08-1)*

Testing limitations are important for participants to understand, but there was some consensus that patients/participants could gain a sufficient appreciation of these after a relatively minimal amount of education about the technical aspects of genomics and sequencing. One interviewee explained:

> "I give people the 20,000-foot view—that we're going to be looking at their genetic information, comparing it to a standard sequence, and we're looking for differences and changes between theirs and the standard and then trying to hone down on the changes that we think are relevant for their health. . .. And then I usually say 'I'm happy to talk about the details of how we do that, if it's important for you'. I've maybe had a handful of people who have said 'yes, I'd really like to understand that'." (5-1)

Interviewees reported that they used a variety of methods to assess the degree to which participants understood the content of

TABLE V. Elements of Informed Consent Mentioned as Most Important for Patients/Participants to Understand

Informed consent element	# Interviewees mentioning
Results	
Limitation of testing/meaning of negative result	13
Implications of results for individual tested	10
Which results are non-optional	5
Implications of results for family members	4
Which results are placed in medical record	3
Possibility of uncertain results	3
Re-annotation of sequence data	1
Research-related items	
"Everything" included on consent form	5
What participation involves (surveys, interviews, etc.)	5
Study goals	2
Participation is voluntary	3
Study/testing risks	
Privacy	6
Genetic discrimination	6
Psychological risks	3
Discovery of non-paternity	1
Understanding of test	
How sequencing is different from other genetic tests	3
What is genome/exome sequencing?	2
What is an exome?	1
Rationale for requesting parental samples	1

the consent discussion. Several genetic counselors noted that they used their traditional genetic counseling skills to obtain consent and gauge participant understanding. Interviewees assessed participant engagement through non-verbal cues such as eye contact or head nods, or through the number and types of questions asked by families. Some interviewees assessed understanding by asking personalized questions, or by doing understanding "checks" during sessions, such as this research coordinator who said she asks participants:

> "If you did join this research, why would you?"... And if they say it's because they don't want to get breast cancer and they think that this will help them, then we've gone south some place and need to regroup." (03-2)

DISCUSSION

The general population has been observed to exaggerate the benefits of genomic sequencing [Caulfield et al., 2013; McGowan et al., 2013; Wade et al., 2013], a belief most likely driven by media reports that hype both the predictive and therapeutic value of genomic information [Caulfield et al., 2013]. Through our interviews with professionals experienced in conducting informed consent sessions in both clinical and research settings, we learned that many patients and research participants being offered genomic sequencing held these same beliefs. As a result of this widespread misperception of the likely benefits of sequencing, obtaining informed consent requires the adoption of strategies to manage unrealistic expectations about the range and utility of information that may be learned.

The need to modulate expectations led most of the professionals we interviewed to structure consent sessions by engaging patients/participants in a wider discussion to emphasize the types of results that they might learn and what a "negative result" really means in light of technological limitations of sequencing. The process and content of the sessions were influenced by a number of contextual issues. One factor was the extent to which the patient/participant was cognitively and emotionally prepared to discuss the testing, which in turn was influenced by previous contact with study or clinical staff, exposure to the consent document and/or educational materials, and/or previous experience with genetic testing. Other contextual factors influencing sessions were whether the person being sequenced was an adult or a child, the indication for sequencing, the current state of their illness, and the timing of the consent session. Interviewees reported that, during their initial consent sessions, they generally followed the order of the content of the consent forms fairly closely. As they developed strategies to promote family participation in sessions and as they became familiar with the range of questions, concerns and misperceptions held by patients or participants about genomic sequencing, interviewees reported that they began to conduct sessions in a less structured and more conversational manner, a style that they believed promoted better understanding and engagement.

Consistent with the conclusions of previous research [Joffe et al., 2001; Robinson et al., 2013], interviewees recognized that most patients and participants cannot attend to, let alone understand, all of the information contained in the consent documents. Interviewees recognized that it would not be feasible to devote two to six hours to informed consent sessions, as had been previously reported [Tabor et al., 2012; Rigter et al., 2014], nor was this amount of time necessary. Interviewees came to restructure the session to focus on communicating content that they learned through experience was most important for patients or most likely to be misunderstood. What became key information was the explication of the types of results that could be returned and their implications. This informational focus loosely aligns with recommendations of the ACMG relating to informed consent [ACMG Board of Directors, 2013], but study interviewees were quick to point out that session content varied considerably according to individual patient and family needs, as recommended by Siegal et al. [2012] so as to shift control of the informational process to patients.

By contrast, unless explicitly requested by the patient/participant, interviewees generally spent less time discussing genomic principles or technological aspects of sequencing, except for what they believed was necessary for patients/participants to understand how the results were generated and interpreted, including the meaning of negative results. Interviewees' experiences in returning results led to their providing more explicit examples about the types of diagnostic results and the range and characteristics of secondary findings that could be returned. With increasing expe-

rience, the verbal content of the sessions tended to become much more personalized and responsive to the patient/participant's informational needs with a corresponding diminished emphasis on some content. Importantly, as recommended by Merrill and Guthrie [2015], rather than providing the type of in-depth pre-test counseling about a specific condition that occurs when testing for a single gene, interviewee provided more global counseling before testing, and more in-depth counseling after testing, based on test results.

Consensus from a variety of stakeholders, including patients and members of the general public will be needed to outline which kinds of information should be presented to patients or participants to provide valid informed consent and to resolve the potential discrepancy between the views of patients/participants and those of scientists or IRB members [Beskow et al., 2010]. Required elements of informed consent for research participation as summarized by Joffe et al. [2001] include items such as an explanation of the purpose of the research; a description of any benefits to others; a description of confidentiality of records; an explanation that medical treatments are available if injury occurs; and an explanation of whom to contact for answers to pertinent questions about the research. Although considered essential by regulatory bodies, interviewees generally did not consider these elements essential for them to verbalize during the session in order to obtain informed consent. As a way to address similar discrepancies, Beskow et al. [2014] recently used a Delphi process to enable a diverse group of expert stakeholders, including biobank participants, to identify a concise set of key points to be included in consent documents and consent sessions that prospective participants should understand in order to provide informed consent for biobank participation. A similar exercise could be done with potential and past patients/participants to identify a minimum set of information that they would want before consenting to genomic sequencing. The initial list could include the elements identified here with additions from other experts and patients/participants [Ayuso et al., 2013].

Because patient/participants' increased familiarity with information about genetic testing or study participation resulted in shorter session lengths, future research should identify innovative ways of providing different levels of details about genome sequencing and its potential outcomes and impacts as desired by individual patients/participants. Ideally, this would lead to a personally tailored approach to informed consent in which patients identify and select the information that is important to their decision-making process [Siegal et al., 2012]. In addition, because decision aids can support decision-making about genetic testing [Kaphingst et al., 2010], more study is needed to assess the extent to which the use of decision aids improves understanding and align decisions with personal values and preferences [Khan et al., 2015].

It should be noted that none of the interviewees in this study reported doing any formal assessment of patient/participant understanding as a part of the informed consent process. Although an instrument to assess genomic knowledge has been developed [Kaphingst et al., 2012] additional tools are needed to help clinicians assess patient/participants' priorities and values, and their understanding of other critical pieces of information [Beskow et al., 2010; Khan et al., 2015]. It is especially important to develop ways to judge understanding of topics that often do not surface unless specifically raised by the clinician, such as the potential for the emergence of unexpected genetic information and the implications of results for obtaining long-term care, disability or life insurance, or for other forms of genetic discrimination [Prince and Roche, 2014].

Limitations

This study represents a description of the current process and content for obtaining consent for genomic sequencing as practiced by individuals with extensive experience conducting consent sessions. However, several limitations should be acknowledged. First, even though we interviewed a substantial subgroup of the genetic counselors and research coordinators conducting consent for genome sequencing in both research and clinical settings in the U.S., they may not represent the experiences of others doing such work, including those in countries outside the U.S. In addition, we did not study actual visits where informed consent was obtained, and we did not seek out the viewpoints of patient/participants. Thus, the list of elements of informed consent mentioned by interviewees as the most important for patients/participants to understand is not intended to be a comprehensive or an ordered list.

CONCLUSIONS

Despite these limitations, because we interviewed a group of professionals with considerable experience conducting informed consent sessions, our findings have important implications for the development of guidelines for informed consent for genomic sequencing as it moves into clinical care. In our study, a subset of key items emerged to become the main focus of informed consent sessions. Our interviewees independently chose the potential results from sequencing to be the main focus of the session. They placed special emphasis on elements relating to this central topic that were likely to be misunderstood including the range and uncertainty of information that could be learned, and the implications of both positive and negative results for the patient. Topics such as sequencing techniques and genomics were relegated to supplementary roles. Future research should address the views of various stakeholders on the key elements of informed consent that this study has identified, and link the process and content of informed consent with outcome measures, such as participant understanding, response to sequencing results, decision satisfaction and utilization of healthcare resources after results disclosure.

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Technologies for Genomic Medicine

CANVAS and AnnoBot, Solutions for Genomic Variant Annotation

A RENCI TECHNICAL REPORT
TR-14-04

renci

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List of Technical Terms and Websites

1000 Genomes Project, www.1000genomes.org

AnnoBot (Annotation Bot)

BioPython software, biopython.org/wiki/Main_Page

BLAT (BLAST-like Alignment Tool), www.blat.net

CANVAS (CAroliNa Variant Annotation Store)

ClinVar (Clinical Variants Resource database), www.ncbi.nlm.nih.gov/clinvar

dbSNP (Single Nucleotide Polymorphism Database), www.ncbi.nlm.nih.gov/SNP

ESP (Exome Sequencing Project), evs.gs.washington.edu/EVS

gbff (GenBank flat file) format, www.ncbi.nlm.nih.gov/Sitemap/samplerecord.html.

HGNC (HUGO Gene Nomenclature Committee), www.genenames.org

HGMD® (Human Gene Mutation Database), www.hgmd.cf.ac.uk/ac/index.php

PostgreSQL (Structured Query Language) database, www.postgresql.org

python™ modules, www.python.org

RefSeq (Reference Sequence Collection), www.ncbi.nlm.nih.gov/refseq

SQLite3 database, www.sqlite.org

Introduction

Genomic medicine holds great promise to transform the medical profession and individualize health care. Technological advancements such as massively parallel genomic sequencing have made it possible to produce large amounts of genomic data within a reasonable timeframe and at a relatively low cost (Mardis, 2008; Horvitz and Mitchell, 2010; Koboldt et al., 2010; Kahn, 2011).

Projects such as the ClinVar and ClinGen initiatives, funded by the National Institutes of Health (NIH), are expanding our understanding of the clinical significance of genomic data through the adjudication of genomic variants and the methodical annotation of the genome (NIH Staff, 2013). Yet challenges remain in how best to interpret, reuse, and share the data (Ahalt et al., 2014; Global Alliance to Enable Responsible Sharing of Genomic and Clinical Data, 2013; Data and Informatics Working Group, NIH BD2K Initiative, 2012).

Those challenges include the need for new technologies to capture, store, and update annotations to provide critical clinical interpretations of genomic data and metadata to attribute provenance or "ownership" and the history of a given data set (e.g., biological sources, laboratory processing steps, transformation and analysis steps, estimates of validity and reliability, etc.).

Herein, we describe two solutions—CAroliNa Variant Annotation Store (CANVAS) and Annotation Bot (AnnoBot)—that together provide version-controlled annotation and metadata to aid in the clinical interpretation of genomic variant data.

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About RENCI

RENCI is an institute of the University of North Carolina at Chapel Hill that develops and deploys advanced technologies to enable research discoveries and practical innovations. The institute was launched in 2004 as a collaborative effort involving UNC Chapel Hill, Duke University, and North Carolina State University. For more information, see www.renci.org.

*Christopher Bizon serves as the technical lead on CANVAS and AnnoBot; Kirk Wilhelmsen serves as Principle Investigator and Director of RENCI's Biomedical Research division, which is leading the development of CANVAS and AnnoBot; all other team members are listed alphabetically

CANVAS[1]

CANVAS was developed initially to support a National Institutes of Health–funded research project, entitled "North Carolina Clinical Genomic Evaluation by NextGen Exome Sequencing" (NCGENES; Foreman et al., 2013). NCGENES is based at the University of North Carolina at Chapel Hill (UNC) and aims to explore the use of whole exome sequencing data for genomic research and clinical care. In order to achieve the study aim, RENCI needed to develop methods and approaches to: (1) match genomic variant data derived from NCGENES with reference genome data derived from publicly available databases; and (2) store the variant data with complete, up-to-date, version-controlled annotation derived from the reference genome data and other sources of variant annotation.

CANVAS was developed as a solution to these challenges. It is an open source, relational PostgreSQL database that stores genomic variant data with its associated annotation and metadata. CANVAS is designed as a relational data representation system that supports the management, query, and analysis of gigabyte- to terabyte-sized data sets from patient-level genomic sequencing data. CANVAS consists of ~85 tables organized into various schemas (Figure 1), including var (research project–specific variant data), refseq (reference genome data derived from RefSeq), and a collection of schemas for capturing additional variant annotation. Those annotation schemas are derived from several updateable data sources: dbSNP; the 1000 Genomes Project; ESP; HGNC; HGMD®; and ClinVar.

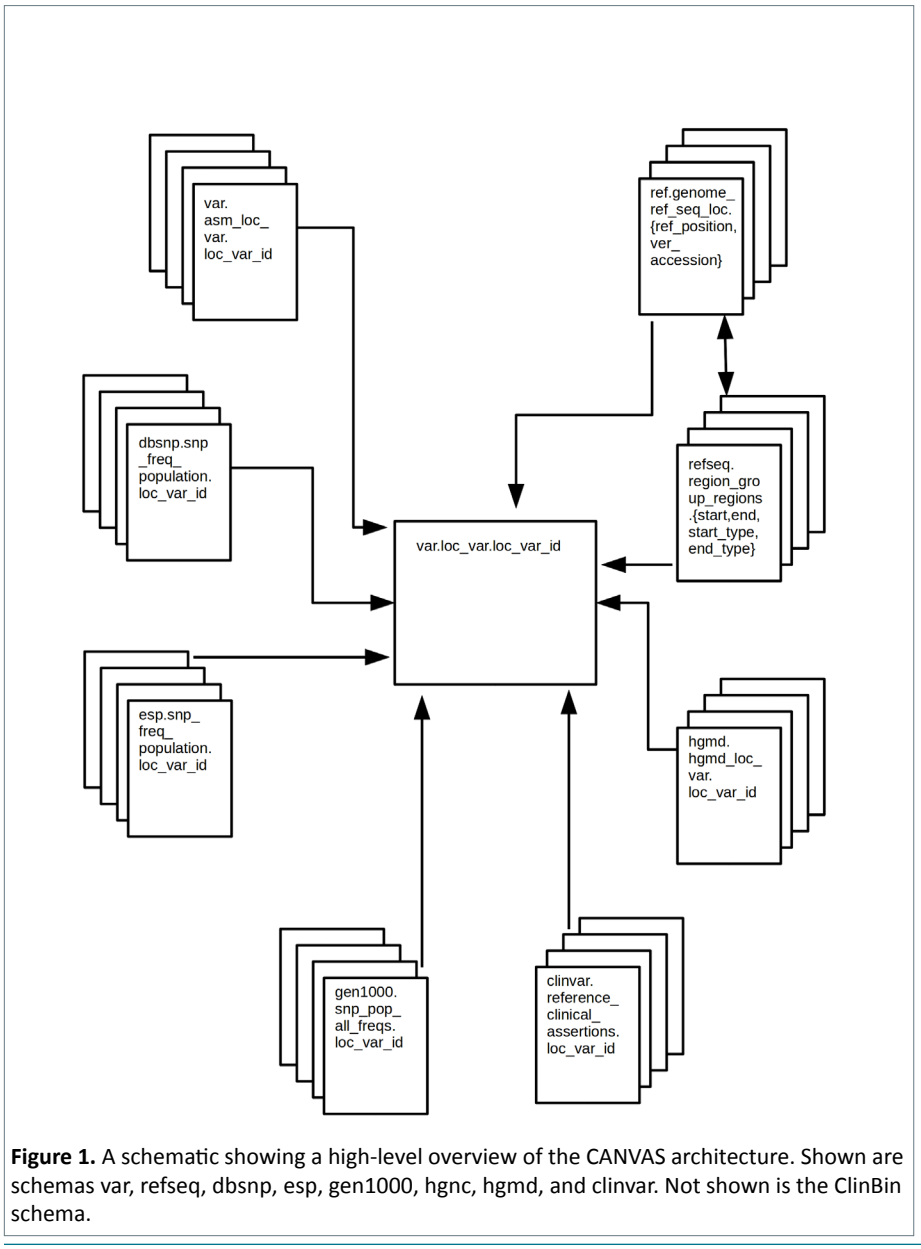

Figure 1. A schematic showing a high-level overview of the CANVAS architecture. Shown are schemas var, refseq, dbsnp, esp, gen1000, hgnc, hgmd, and clinvar. Not shown is the ClinBin schema.

Note that not only are there multiple annotation sources, with annotations organized and presented differently across sources, but the annotation sources are frequently updated as new information becomes available; this presents a challenge in how to ensure that the annotation in CANVAS is current and that all previous versions of annotation remain accessible in order to guide interpretation of current and past findings. AnnoBot (described below) was developed to monitor these external data sources for updates, extract any new annotation, and add that annotation to CANVAS. AnnoBot adds versioning information to all annotation to ensure that interpretations of genomic variant data are based on known data sources.

[1]CANVAS was formerly termed VarDB (Variant DataBase).

CANVAS also contains a schema ClinBin (not shown in Figure 1), which is used for NCGENES-specific computation to determine whether variants should be sorted into the diagnostic bin (DxBin) or incidental bin (IncidentalBin). DxBin includes variants that were targeted for a given patient/subject on the basis of a defined phenotype, have established clinical validity and utility, and thus are used for clinical diagnosis; in contrast, IncidentalBin includes incidental findings, or variants that were identified as a result of the sequencing effort but are believed to be unrelated to the disease phenotype or diagnostic goals and are thus used for research purposes only (Shoenbill et al., 2014, Foreman et al., 2013). ClinBin contains definitions of the variants in each bin, with annotation on the sources of those definitions, as well as data on patient-specific variants and their clinical significance. Of note, the variant data that are pushed into DxBin and IncidentalBin contain metadata on the origin of the variant annotation (i.e., they are version-controlled).

Storing annotation in CANVAS

Variant annotation is stored in Table var.loc_var, which contains loc_vars or variants located with respect to a specific reference sequence and described as follows:

- loc_var_id: an arbitrary integer identifier (surrogate key) assigned to the variant by the database
- pos: the position of the variant on the chromosome
- ref_id: the identifier of the reference sequence
- ref_ver_accession: the chromosome accession number
- ref: the reference allele
- alt: the alternate allele
- type: the variant type (i.e., single nucleotide polymorphism [SNP], insertion, deletion, substitution).

Acquiring output on genomic variants

An example of some of the query output provided by CANVAS for annotation on a given variant is shown below. Note that CANVAS provides approximately 70 fields of data on each variant, including data on the reliability and validity estimates for both the project's sequencing results and any reference data derived from public sources (see Owen et al., 2014 for additional fields and visual displays of output).

- **Variant id**: 57483
- **Chromosome:** 11
- **Position:** 1308393-1308394
- **Reference:** NCBI build 37.1
- **Analysis type:** incidental
- **Variant Class:** SNP
- **Variant:** A/T
- **Strand:** reverse
- **Minor Allele Frequency:** unknown
- **Zygosity:** heterozygous
- **Protein-coding effect:** missense
- **Gene:** thromboxane A2 receptor
- **Phenotype:** unknown
- **dbSNP ID:** rs5743
- **RefSeq ID:** NG_013363.1

Defining a variant's chromosomal position

CANVAS defines a variant's position on a chromosome as the physical location of a base in the reference sequence that is affected by the variant. A variant position p is the position between the physical locations p – 1 and p, for all p > 1; variant position p = 1 is the position preceding physical location 1. The bases displaced by a variant are those immediately following the variant position.

Reconciling ambiguous insertion/deletion variants

CANVAS invokes several functions to handle ambiguity in a variant's position. For example, consider a reported deletion of CAG from the beginning of the reference sequence CAGCAGCAG, which produces CAGCAG. Note that a deletion of AGC from the second position of the reference sequence, or a deletion of GCA from the third position, also produces CAGCAG. Because of this ambiguity, CANVAS describes the variant in a general or canonical form as a deletion replacing CAGCAGCAG with CAGCAG. This function is described as var.generalize_variant(). When implemented, the reference sequence is scanned to the left and the right of the ostensible variant location to determine if alternative candidate variants could produce the same sequence (i.e., ambiguity). If alternative candidate variants exist, then a single insertion/deletion is produced in the canonical form.

Adding variants to CANVAS

The database function var.loc_var_register() is used to add a variant to CANVAS. If the variant is an insertion or deletion, and if there could be ambiguity about its location due to sequence repeats, then this function expands the variant to a longer canonical form through the function var.generalize_variant(). If the variant already exists in the database, then the database returns its loc_var_id; otherwise, the variant is added to CANVAS, and the database generates a new loc_var_id.

AnnoBot

AnnoBot is a set of python™ modules and software driver code that are designed to automatically monitor targeted databases for updated information, extract new or revised annotations, and add those annotations to CANVAS. As noted above, the data sources that are currently monitored are: dbSNP, 1000 Genomes, ESP; HGNC; HGMD®; ClinVar; and RefSeq (Figure 1). AnnoBot can be extended to monitor additional databases as they become available.

AnnoBot implements the following python™ modules:

- Downloader: identifies and downloads new or edited annotations from external database sources
- Processor: transforms the data using BioPython
- Dbloader: uploads the data into CANVAS
- Mapper: maps the data to the genome using BLAT
- Maploader: filters the mapping before uploading it into CANVAS.

Describing AnnoBot's functionality using RefSeq as the primary external data source

RefSeq contains genomic sequencing data derived from many different species and stores this information as gbff files. The RefSeq Downloader reads each gbff file in this directory and identifies the ones derived from human data using the regular expression ORGANISM*Homo sapiens. RefSeq version numbers are indicated in the file names, and the Downloader captures this information as well.

The gbff files in RefSeq are hierarchical and similar to xml files. The RefSeq Processor invokes BioPython software to process the data files. BioPython parses the gbff files and transforms them into database-appropriate formats.

The RefSeq Dbloader implements a process similar to OR (Object Relational) mapping (Ambler, undated) to upload the transformed data into CANVAS. The Dbloader executes specific tasks, such as autoincrement counting, checking for existing rows, and maintaining links between the different tables in CANVAS.

The RefSeq Mapper uses BLAT to map the variant transcripts to the reference genome. BLAT conducts a gapped alignment; gaps in the alignment correspond to introns in the variant sequences. The RefSeq Maploader then uploads the mappings into CANVAS.

The AnnoBot Driver is used to manage the python™ modules and is comprised of software driver code and a SQLite3 database. The SQLite3 database organizes the state of each module, using hard versioning. The driver code continuously loops over the modules, processes any unprocessed data, uploads new processed data to the SQLite3 database, and invokes the Mapper to upload the new data to CANVAS.

A key feature of CANVAS and AnnoBot is annotation version control. As AnnoBot pulls updated annotation from external databases into CANVAS, the new annotation is stored in parallel with the older versions within the CANVAS schema. AnnoBot also pulls the version of the source data from which the annotation was derived. Version control allows the user to compare results across data and annotation sources—a feature that is missing from most other annotation systems.

Conclusion

CANVAS and AnnoBot work synergistically to provide a comprehensive solution to the challenges involved in maintaining a detailed, up-to-date, version-controlled record of genomic variant annotation, including metadata to record provenance and the history of a given data set.

Key Features:

- Architecture is open source
- Annotations are updated automatically
- All annotation is versioned and stored in parallel
- Queries are rapid and return rich output
- The system is modifiable and extendable
- The approach is generalizable to non-genomic annotation systems

Underlying Software and Technologies:

- BioPython
- BLAT
- PostgrSQL database
- python™
- SQLite3 database

Impact

- Currently supports variant annotation for the following research programs: (1) National Institute on Drug Abuse–funded NIDASeq, "Deep Sequencing Studies for Cannabis and Stimulant Dependence," (Dr. Kirk Wilhelmsen, PI), which is conducting whole genome sequencing of ~5,500 patient samples; (2) National Human Genome Research Institute–funded NCGENES, "North Carolina Clinical Genomic Evaluation by NextGen Exome Sequencing" (Dr. James Evans, PI), which is conducting whole exome sequencing of >2,000 patient samples drawn from multiple disease categories; (3) National Institute of Child Health and Development–funded NC Nexus, "North Carolina Newborn Exome Sequencing and Newborn Screening Disorders" (Dr. Cynthia Powell, PI), which aims to conduct whole exome sequencing on 400 patient samples; and (4) UNCSeq, which applies tumor sequencing technology for >2,000 patient samples in order to identify mutations that are amenable to targeted treatments.
- Also supports the NIH-funded ClinGen and ClinVar initiatives (Dr. Jonathan Berg, Site PI), which involve a national effort to develop

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Points to Consider: Ethical, Legal, and Psychosocial Implications of Genetic Testing in Children and Adolescents

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In 1995, the American Society of Human Genetics (ASHG) and American College of Medical Genetics and Genomics (ACMG) jointly published a statement on genetic testing in children and adolescents. In the past 20 years, much has changed in the field of genetics, including the development of powerful new technologies, new data from genetic research on children and adolescents, and substantial clinical experience. This statement represents current opinion by the ASHG on the ethical, legal, and social issues concerning genetic testing in children. These recommendations are relevant to families, clinicians, and investigators. After a brief review of the 1995 statement and major changes in genetic technologies in recent years, this statement offers points to consider on a broad range of test technologies and their applications in clinical medicine and research. Recommendations are also made for record and communication issues in this domain and for professional education.

Introduction

In 1995, the American Society of Human Genetics (ASHG) and American College of Medical Genetics and Genomics (ACMG) published a joint statement titled "Points to Consider: Ethical, Legal, and Psychosocial Implications of Genetic Testing in Children and Adolescents."[1] This publication was influential in guiding clinicians and families during an era in which a number of new genetic tests, particularly predictive or predispositional testing, were being introduced into clinical medicine. Since 1995, clinicians have gained substantial experience with genetic testing in a number of clinical contexts, and research has improved the evidence on which professional recommendations can be developed. The ASHG determined that a new statement addressing genetic testing in children was timely, both because of the continuing evolution of genetic testing and because of the special considerations raised in the care of children. The purpose of this statement is to provide guidance on a variety of different genetic testing approaches for children in both the research and clinical contexts.

The ethical, legal, and social issues in genetic and genomic testing have been subject to special scrutiny for several reasons. First, for some heritable conditions, genetic testing can provide powerfully predictive information about the individual's future health status. Professionals, and society more broadly, have been concerned about the impacts of such predictive power on the psychological well-being of those found to be at increased risk, as well as concerns about stigma and discrimination. Second, genetic information about one individual provides presumptive information about other "blood" relatives. The family or kindred nature of genetic information poses ethical, legal, and social challenges for the appropriate management of that information in clinical and research contexts. Third, genetic and genomic information is complex, and health risks associated with this information are often probabilistic. This means that special care and expertise are important in ordering and interpreting many genetic tests. Finally, genetics has a troubled history, evident during the first half of the twentieth century, when genetic concepts were misunderstood and misused to the detriment of vulnerable groups in society. Genetic and genomic tests are not uniquely challenging with respect to ethical, legal, or psychosocial considerations, but these features justify careful thought and an element of caution as we assess the benefits and risks of these evolving technologies.

This statement is focused on the use of these technologies with children. Children also warrant special consideration for several reasons. Informed consent to genetic and genomic testing is a core principle for which there are few exceptions. Young children lack decision-making capacity, so decisions about testing must be conducted through surrogates, usually the parents, and must be done with the child's best interest at heart. The notion of "best interest" is intended to place the child's welfare foremost in medical decision making. However, given the

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subjective nature of the interests of those who cannot speak for themselves, defining an individual child's "best interest" is often complex and controversial, particularly in medical circumstances involving burdensome treatments and profound disabilities. Surrogate decision making is also an ethically freighted concept, because although parents are the appropriate surrogates for their children in almost all cases, controversies arise when parents make decisions that seem contrary to the best interest of their child.

As children age, they gain decision-making capacity and experience with health conditions. Therefore, including children to various degrees as they age in genetic- and genomic-testing decisions and responses is important but challenging. Finally, because children are young, decisions for them, and by them, might have implications for the course of their lives.

As genetic and genomic tests become more accurate and their use becomes more common, these ethical, legal, and psychosocial challenges will become more familiar and less worthy of statements of this sort. In many contexts, genetic and genomic tests are no different than other forms of testing. But in the contexts outlined below, the ASHG believes that these recommendations will assist families, clinicians, investigators, and policy makers in maximizing the benefits offered by these evolving forms of genetic and genomic testing.

A Summary of the 1995 ASHG Report

In 1995, the ASHG and ACMG issued a joint report that offered points to consider for genetic testing in children. The clinical context of that report focused on decisions about testing for single-gene disorders in response to either a family history or within-population screening programs. The social context of that report included limited data about the psychosocial impact of such testing in children. The ASHG and ACMG recommended that clinicians and parents consider timely medical benefits related to diagnosis, prognosis, and interventions as the best justification for testing in the child. Additionally, the report recommended that the potential psychological benefits to adolescents who request such testing also be considered. The report suggested that in the absence of timely medical benefits to the child, or the expressed wishes of adolescents, testing should be deferred until adulthood, particularly for adult-onset conditions or for carrier status for reproductive decision making. However, the report acknowledged that there was limited information about the benefits and risks of genetic testing in children. The report recommended deferral of testing in the face of this uncertainty, yet it also recommended deference to parents in some circumstances. The report has been influential in encouraging caution and reflection regarding testing children but often has been over-interpreted as a stricter prohibition of predictive testing in children for adult-onset conditions than was intended.

Recent Changes in Genetic and Genomic Technologies

Cytogenetics and molecular diagnostics have both undergone several revolutions since the fields began in 1959 and in 1976, respectively.[2,3] Cytogenetics started with chromosome analysis and matured with increasingly detailed banding and then fluorescence in situ hybridization. Most recently, the field has seen the introduction of chromosomal microarray analysis (CMA) for deletions and duplications (formerly done by cytogenetics). Molecular diagnostics has transitioned from hybridization-based techniques to Sanger sequencing with the increasingly common utilization of next-generation-sequencing-based techniques. In both fields, the increased coverage and increased resolution of the current technologies confer high analytic validity, but both platforms create problems with interpretation. First, a significant challenge is the difficulty in distinguishing between pathogenic variants and rare polymorphisms, resulting in the identification of "variants of uncertain significance." Second, there are difficulties in interpreting variants and copy-number alterations whose significance is incompletely understood because of reduced penetrance or a lack of sufficient data on clinical associations. Third, these technologies result in the identification of variants unrelated to the indication for testing (secondary or incidental findings). These challenges arise from our evolving understanding of the fine structure and variation in the human genome. At the present time, the contrast between our ability to identify genetic variants and our ability to fully interpret the information gives rise to many of the ethical issues in this domain.

Predictive Genetic Testing in High-Risk Families

In the 20 years since the first ASHG-ACMG pediatric-testing statement, there has been a modest volume of clinical research about the impact of predictive testing in high-risk families. To date, this limited research has not found evidence of significant psychosocial harms in children.[4] Perhaps the most significant finding is that, even without testing, children and many families create narratives about a child's genetic status. That is, some families simply assume that their children are destined to have, or not have, the familial condition. Further, the baseline uncertainty about risk status can cause psychosocial distress in the absence of genetic testing. Over the last two decades, there has been a general shift toward greater parental discretion in the face of clinical uncertainty about the best interests of the child.[5] This broad shift is not exclusive to genetics but has implications for genetic testing.

As parents consider the best course of action regarding genetic testing of their children, it remains important for parents to be aware that informed adults make a range of choices about predictive and reproductive testing, and thus many adults decline such testing. Deferring testing to adulthood allows children the opportunity to make their own decisions. This is especially important for the small subset of conditions where a minority of at-risk adults opt

for genetic testing, such as for Huntington disease. Approaching parents (and children, when appropriate) with respectful but directive recommendations, along with acknowledging flexibility, might be an effective approach to forging a therapeutic alliance with families. Encouraging families to consider such decisions over a period of time might convince some families that testing will be helpful in their particular context, or it might become clear that it will be most appropriate to defer testing until adulthood. The ASHG offers the following recommendations:

- Unless there is a clinical intervention appropriate in childhood, parents should be encouraged to defer predictive or pre-dispositional testing for adult-onset conditions until adulthood or at least until the child is an older adolescent who can participate in decision making in a relatively mature manner.
- Adolescents should be encouraged to defer predictive or pre-dispositional testing for adult-onset conditions until adulthood because of the complexity of the potential impact of the information at formative life stages.
- Providers should offer to explore the reasons why parents or adolescents are interested in predictive or pre-dispositional testing for adult-onset conditions. Providers can acknowledge that, in some cases, testing might be a reasonable decision, but decisions should follow thorough deliberation.

Adolescents should be provided the opportunity to discuss these issues without the presence of their parents, although parents should be involved in, and supportive of, any final decisions for testing. A referral to genetic counselors and mental-health professionals is appropriate if the clinician and family need additional support for decision making or in assessing the psychosocial dynamics.

- Facilitating predictive or pre-dispositional testing of children for adult-onset conditions can be justified in certain circumstances. For example, after careful deliberations with the family and older child, testing can be justified to alleviate substantial psychosocial distress or to facilitate specific life-planning decisions. The impact of predictive testing on children and families remains uncertain and therefore can be justified in specific cases when it is requested by families after informed deliberations and when the testing is not clearly inconsistent with the welfare of the child.
- Empirical research on the psychosocial impact of predictive or pre-dispositional testing in children is necessary for future policy recommendations. Genetic testing of children for adult-onset conditions in the research context can be ethically justified because of its social importance and when risks are minimized by appropriate counseling and support and when appropriate parental permission and child assent are obtained.

Genome-Scale Sequencing in Children

The technology to enable whole-exome sequencing and whole-genome sequencing has become more accurate, more efficient, and less expensive. For the purposes of this statement, we use the term "genome-scale sequencing" to mean either whole-genome or whole-exome sequencing. The cost of genome-scale sequencing is coming down progressively, and there is some confidence that "the $1,000 genome" will be achieved in the next few years. These cost estimates are for the generation of sequence data and do not include the clinical interpretation of the information. Given these technical improvements, genome-scale sequencing can be considered in a variety of clinical and research contexts. These include diagnostic testing, predictive testing for childhood-onset conditions, pharmacogenetic testing, and testing in children with cancer to inform diagnosis or therapy.

Genome-scale sequencing creates a tension between the need to generate a comprehensive analysis of an individual's genome to address a clinical challenge and the need to limit problems created by a wealth of data, including secondary findings and findings of uncertain clinical significance. Yet, the improving coverage, accuracy, sensitivity, and cost effectiveness of genome-scale sequencing will eventually equal that of testing a single gene or performing targeted gene panels, meaning that genome-scale sequencing might become an attractive choice for interrogating a single gene or targeted set of genes. The ASHG recognizes the current debate regarding the obligation, if any, to search for selected variants with high clinical validity and clinical utility when conducting genome-scale sequencing.[6] The ASHG makes an important distinction between using genome-scale sequencing as the method of choice for searching broadly for a diagnosis and choosing genome-scale sequencing with analysis restricted to a limited number of genes when a more targeted strategy is indicated. The recommendations below reflect ASHG's assessment that targeted tests, or selective sequence analysis, is usually preferable to less-discriminate data acquisition when the clinical challenge can be addressed through a targeted approach.

- When clinically indicated, the scope of genetic testing should be limited to single-gene analysis or targeted gene panels based on the clinical presentation of the patient.
- Targeted testing using genome-scale sequencing, but restricting analysis to a limited set of genes relevant to the clinical indication, is an acceptable alternative to a single-gene analysis or targeted gene panel in certain circumstances. When genome-scale sequencing is performed but the analysis is restricted to a limited set of targeted genes, ASHG finds it ethically acceptable for the laboratory to limit the analysis to the genes of clinical interest.
- ASHG recommends that, in the context of diagnostic testing for a child with a most likely genetic disorder, genome-scale sequencing is appropriate when prior,

more limited genetic testing failed to identify a causative mutation. Depending on the clinical presentation and on the quality and availability of appropriate targeted testing, comprehensive testing such as genome-scale sequencing might also be indicated in certain circumstances, even in the absence of prior, more limited genetic testing.

- At the present time, genome-scale sequencing is not indicated for screening in healthy children. Accordingly, genome-scale sequencing is not indicated for the purposes of clinical newborn screening at this time. In the research setting, genome-scale sequencing in newborns for screening purposes can be justified as part of carefully developed protocols for better understanding the potential benefits and risks of this technology in this context.

Secondary Findings

The move from targeted genetic testing to genome-scale sequencing has led to a vigorous debate about the ethics of managing massive amounts of individual-level genetic data.[7] (It should also be noted that although secondary findings are a significant problem for genomic medicine, they are by no means unique to this field; other disciplines, particularly radiology and pathology, have been grappling with similar concerns for decades. See, e.g., Berland et al.[8] and Orme et al.[9]) The generation of a patient's genomic sequence data radically increases the probability of discovering incidental or secondary findings.[10] For consistency, throughout this statement we use "secondary findings," defined as clinically relevant information unrelated to the condition for which the sequencing was originally ordered.

Secondary findings might have a clinical utility for a child or his or her family members. Therefore, there will be cases in which it is acceptable to return Clinical Laboratory Improvement Amendments (CLIA)-validated information derived from a child's sequence when such information has important clinical implications for the child or someone in the child's family.

Parents or guardians should have a clear understanding of when secondary findings might be generated and of the circumstances, if any, under which they can expect to be offered results. Children should be included in the informed-assent or -consent process to the extent that they are capable.

- ASHG recommends that clinicians offer to disclose secondary findings for a child to the child's parents or guardians only when the information has clear clinical utility for the child and/or his or her family members.
- In any clinical genomic endeavor that has a substantial likelihood of generating clinically relevant secondary findings, ASHG recommends that there should be a robust informed-consent process.
- If genome-scale sequencing is performed in somatic tissue, such as in tumor tissue in children with cancer, it is usually necessary to also conduct germline sequencing on the patient to adequately interpret the tumor sequence.[11] Therefore, ASHG recommends that the same considerations in the management of secondary findings be undertaken for both somatic-tissue sequencing and germline genome-scale sequencing.

Parents have wide decision-making authority, but in cases where the clinical response to a secondary finding will most likely prevent serious morbidity or mortality for the child, it can be appropriate to override a parental decision not to receive this information.

- ASHG recommends that, in general, parents should be able to decline to receive secondary findings in advance of genetic testing.
- However, when there is strong evidence that a secondary finding has urgent and serious implications for a child's health or welfare, and effective action can be taken to mitigate that threat, ASHG recommends that the clinician communicate those findings to parents or guardians regardless of the general preferences stated by the parents regarding secondary findings.

There is an ongoing debate about the extent to which researchers are obligated to disclose secondary findings to research participants. Research and clinical care have distinct characteristics, and the responsibility of a clinician necessarily differs from that of a researcher.[12] Clinicians have a primary obligation to act in the best interest of their patient; researchers must protect the welfare of subjects but are primarily charged with the production of generalizable knowledge. Although they are generally distinct, the line between research and clinical care is often blurry, particularly in the context of genomics.[13] Institutional review boards (IRBs), perhaps with expert consultation, are in the best position to determine whether and how to disclose secondary findings in a given research setting.

- When secondary findings are likely to be generated in the conduct of pediatric research, ASHG recommends that investigators develop and follow an IRB-approved plan to manage such findings.

Questions about whether there is a duty to look for secondary findings have been actively debated.[6] As analytic tools make searching for a limited list of high-value variants more efficient, the benefits of actively searching for such variants in the clinical context are likely to outweigh the costs and adverse consequences. However, more data, experience, and debate are necessary for defining the most ethically appropriate approach in the clinical pediatric context regarding an obligation to look for secondary findings. In the research context, the ethical responsibilities and risk-benefit considerations differ from the clinical

context. Therefore, actively searching for secondary findings in research involving genome-scale sequencing might be ethically acceptable in certain circumstances (with the informed consent of parents) but should not be considered ethically required at the present time.[7,14]

- In the clinical and research contexts, ASHG recommends that it be considered ethically acceptable, but not required, to search for secondary findings that are not relevant to the clinical or research indication for sequencing.

CMA

The transition from chromosome analysis by karyotype to the utilization of CMA has transformed genetic diagnostics.[15] CMA is now a standard diagnostic test for a wide variety of conditions, including developmental delay with and without dysmorphic features, autism spectrum disorders, and multiple congenital anomalies, in the pediatric population.[16] Use of these arrays has increased the utility of cytogenetic testing by increasing the rate of positive diagnoses (allowing the identification of much smaller deletions and duplications than cytogenetics alone), and with increasingly precise definition of breakpoints and gene content for deletions and duplications, it has allowed the identification of many new syndromes.[17] However, these tests also allow the identification of copy-number alteration of disease-associated genes unrelated to the initial reason for study, allow the identification of excessive homozygosity indicating potential consanguinity or incest, and have a significant likelihood of identifying a variant of uncertain significance. CMA also has the potential to identify secondary findings. Therefore, CMA, like sequencing, raises ethical considerations that warrant obtaining informed consent from the child's parents, a practice that has not been routine for traditional chromosome analysis.

- The ASHG recommends that work be conducted for assembling a list of genes in which duplications or deletions are clearly associated with clinically important diseases. This list could function as a secondary-findings list with implications for what should and should not be reported back to families.
- Clinicians and parents should be adequately informed about the complexities of CMA testing before CMA testing is ordered and results are provided to patients. Clinicians should understand the concepts of variants of uncertain significance, variable expressivity, and reduced penetrance and the potential need to consider testing of other family members.
- The ASHG recommends that practice guidelines be established for using CMA testing.

Carrier Testing of Adolescents

Carrier testing of adolescents has historically been controversial, and professional statements generally do not support routine carrier testing of adolescents outside of pregnancy or reproductive planning.[18,19] Hypothetical concerns include stigma, discrimination, and potential confusion over affected versus carrier status.[4] It is notable that a significant body of literature addresses carrier screening in adults. Outside of some specific populations (e.g., Orthodox Jewish individuals), there is little documentation of discrimination around carrier status in recent years, and most adult carriers without a family history do not appear to have significant short- or long-term differences in anxiety. In contrast, adult siblings of individuals affected by recessive or X-linked conditions often have strong views on whether or not they wish to know their carrier status and how it might affect their reproductive decision making. Some studies have reported that siblings show transient anxiety and depression after carrier testing.[20–23]

Most studies assessing adolescent or childhood carrier testing are small and address individuals with a family history of X-linked conditions (e.g., Duchenne muscular dystrophy, hemophilia, and fragile X syndrome) and autosomal-recessive conditions; Borry et al. provide a summary of some of the early literature in this area.[18,24] These small studies documented high short-term recall and a number of potentially beneficial psychosocial outcomes, including relief in those who are non-carriers, relief from uncertainty in both carriers and non-carriers, and positive reappraisal of self-esteem and self-image. Additionally, these studies also suggested that adolescents found to be carriers felt able to plan for future parenthood and that most were open about the condition and their carrier status, sharing with family, and planning to tell partners.[25–29]

- On the basis of the evidence indicating potential benefits and a low risk of harm, ASHG neither recommends nor discourages offering carrier testing to adolescents who desire such testing in the setting of a positive family history. Adolescent assent and parental consent should be obtained for carrier testing, and genetic counseling might be appropriate in some circumstances.

Carrier testing could be performed on children in other less well-studied settings, including institutional settings such as high school, college, or athletic programs. Outcome studies in this area are somewhat limited and generally describe carrier testing offered in high schools in Canada, Australia, and the Netherlands. These studies, performed over 20 years, have shown high uptake rates and have not demonstrated adverse psychological consequences.[30,31] Ross summarizes many of these early studies and discusses potential concerns—including those about potential coercion, confidentiality, and the informed-consent process—with similar implementation in the US.[32]

- ASHG recommends that carrier testing in children and adolescents not be performed through institutional or population-based approaches at this time.

Research projects to further evaluate adolescent carrier testing in institutional contexts is appropriate with carefully drafted protocols.

Direct-to-Consumer Testing

Direct-to-consumer genetic testing (DTC GT) refers to genetic testing that bypasses the involvement of healthcare providers and is sold directly to consumers. DTC GT is marketed to consumers primarily via the internet and was initially limited to paternity and ancestry testing. However, DTC GT has in recent years been expanded to offer testing for potential health-related claims.[33] Several concerns have been raised about DTC GT, and they include the lack of high-quality pre-test and post-test counseling and clinical interpretation of test results, the lack of adequate validation of some tests, and the testing of children for adult-onset conditions.

DTC GT offers individuals the opportunity to have access to personal genetic information.[34] Yet, there is a strong tradition in genetics that in many clinical circumstances, testing involves pre- and post-test counseling from a qualified health-care provider, meaning a genetic counselor or a medical geneticist.[35] It is clear that some clinicians who provide genetic-risk assessment of DTC GT results to patients lack the knowledge or background for appropriate interpretation. In one study of interviews conducted with clinicians who offered genomic-risk assessment to patients, the clinicians appeared to have learned most of what they know about genomics directly from the commercial laboratories.[36] In the absence of professional counseling and interpretation, there are concerns that consumers might make misguided changes in their health care or lifestyle.[37] Fortunately, empiric studies of DTC GT to date have shown little or no evidence of inappropriate changes in lifestyle or health-related behaviors.[38–46]

DTC GT provides information of variable accuracy and clinical validity.[47] Some companies that offer DTC GT have made poorly validated claims regarding the health impact of their testing. In response to such marketing claims, the FDA prohibited 23andMe from selling its personal-genome service in November 2013.[48] However, this does not prevent overseas companies from marketing or providing services or US-based companies from moving overseas.[49] It also does not prevent companies from offering genetic testing services without associated clinical interpretation. Other countries have passed legislation that regulates DTC GT.[50]

DTC GT has additional implications in children, given that many of these tests are intended to diagnose or identify risk for adult-onset disorders, such as breast cancer, ovarian cancer, and Huntington disease. One study surveyed companies that offer DTC GT, and only 13 responded. Ten of those 13 companies performed testing of minors in response to requests from parents or legal guardians. Three companies would consider testing if it was requested by a minor.[51]

Finally, there is no consistency regarding the information provided on DTC GT websites regarding consent for testing. Information on DTC GT websites might not be balanced with regard to how they present risks and benefits. Users of the test might consent to testing without understanding the full consequences of the results.[52,53]

- The ASHG recommends that DTC GT be discouraged in children until such a time when companies that provide DTC GT can assure quality, accuracy, and validity of their testing and assure that there is adequate pre- and post-testing counseling.
- The ASHG recommends that DTC GT in children be performed with the appropriate informed permission from a parent or legal guardian and the assent of the child when appropriate.
- The ASHG recommends that DTC GT not be performed in children for genetic conditions that have onset in adulthood or require surveillance beginning in adulthood.

Pharmacogenomic Testing

Pharmacogenetic testing in adults and in children has the potential to improve drug efficacy and reduce adverse events.[54] Testing might be indicated prior to the first use of a medication in order to guide drug choice and initial dosing or to evaluate adverse effects or non-responsiveness to prior drug treatments. However, research on pharmacogenetic testing in children has been limited, so there is little current evidence on the potential benefits and harms associated with this type of genetic testing. Further, pharmacogenetic data can account for some, but not all, variability in drug response and therefore should be considered in conjunction with other factors in clinical pharmacologic decision making. In particular, some enzymes known to have significant pharmacogenetic variability can be "metabolically immature" in newborns and infants.[55,56] This can result in clinical outcomes that are different from those predicted by genotype alone. CYP2C19, an enzyme that is involved in a number of commonly prescribed drugs, is one example in which genotypically predicted extensive (normal) metabolizers can have a poor metabolizer phenotype in the first few months of life.[57]

Clinical pharmacogenetic testing in children is strongly supported by evidence in some areas, such as TPMT testing in association with thiopurine therapy for childhood leukemia. Pharmacogenetic testing has been proposed for clinical use and is supported by varying levels of evidence in many medical specialties, including but not limited to oncology, rheumatology, psychiatry, HIV treatment, immunosuppression, and anticoagulation.[54–56,58–62]

- ASHG recommends that when there is a clear evidence base in the literature for clinical utility, pharmacogenetic testing in children might be appropriate.

- ASHG recommends additional evaluation of pharmacogenetic testing opportunities in the pediatric population in order to better demonstrate the utility and limitations of this form of testing.

Newborn Screening

Newborn screening (NBS) is one of the most effective public-health programs of the last century. The ASHG strongly supports NBS programs and encourages genetic professionals to support NBS in their communication with patients, colleagues, and policy makers.

NBS is conducted by state-based public-health programs in the US. For the first four decades of the programs, there was substantial variability between states on the conditions targeted.[63] In 2005, the ACMG published recommendations for a uniform panel composed of 29 primary conditions and a number of secondary conditions that will be identified through targeting the primary conditions.[64] These recommendations were supported by the American Academy of Pediatrics and the newly formed Secretary's Advisory Committee on Heritable Diseases in Newborns and Children (SACHDNC).

The SACHDNC was established in 2004 through federal legislation with the primary goal of establishing an evidence-review process to make recommendations for conditions on a uniform screening panel.[65] Although states determine the nature of their screening programs, currently all states screen for all conditions on the ACMG list.

Given the low prevalence of most conditions targeted by NBS, making informed policy decisions regarding the introduction of new tests is challenging. For this reason, the ASHG supports robust evidence-review processes, at the state and/or federal level, as an essential element to a state health department's policies and procedures for NBS programs.

- The ASHG recommends that state programs only introduce new conditions on a mandated NBS panel after a thorough review of the evidence on the benefits and harms, the impacts on systems of care, resources, and capacity, and input from relevant stakeholders.

State NBS programs are designed to both enable affected children to receive a prompt, accurate diagnosis and coordinate short-term clinical care for the condition. However, health departments do not typically collect data on the longer-term outcomes for children or their families. Further, the low prevalence of many conditions targeted through NBS makes it difficult to conduct outcomes research without large, multicenter projects. Therefore, data on the clinical outcomes of affected children, with or without NBS, is often limited.

- The ASHG supports conducting outcomes research on NBS and developing infrastructures for conducting outcomes research on these rare conditions. Such infrastructures would support the ability to assess outcomes and to conduct controlled trials of therapeutic options and evaluate support systems required for affected children and their families.

NBS is conducted on dried bloodspots collected from the infant within the first few days of life. Although all state programs provide information to parents about NBS, usually in the form of a brochure, the literature shows that most parents do not read this information. Accordingly, most parents have little awareness and understanding of NBS.[66] The literature also demonstrates that many primary-care physicians (PCPs) have a limited understanding of NBS and often feel poorly prepared to manage screen-positive infants and provide guidance to their parents.[67] Adequate information and education of parents and PCPs is important for maximizing the effectiveness of these programs. The literature demonstrates that parents want to be informed, but most only want basic facts about NBS programs.[66] However, research has been limited on how to effectively deliver information to parents about NBS. Public surveys, the American Academy of Pediatrics, the American College of Obstetrics and Gynecology, and commentators support NBS education in the prenatal time period.[68]

- The ASHG recommends additional research for improving the quality, delivery, and effectiveness of parental, public, and professional education regarding NBS.

NBS is conducted under state mandates in all but two US states or territories (Wyoming and the District of Columbia). However, 43 states permit parents to refuse NBS for either religious or philosophical reasons. The number of parents who opt out of NBS is exceedingly small.[69,70]

The role of parental permission in the conduct of NBS has been a topic of debate since the inception of the programs in the 1960s. State programs typically are strongly supportive of the current opt-out approach because a formal permission process is cumbersome, particularly if signed consent forms are required, and could increase the risk that newborns will not be screened. Nevertheless, a number of professional statements over the years support a parental permission process (an "opt-in" approach).[19,71] Surveys of public and professional attitudes regarding parental permission demonstrate that the public is evenly split on the appropriateness of opt-in versus opt-out approaches.[72,73] However, the public expects to be informed about NBS regardless of the permission model.

Obtaining truly informed permission for NBS during the postnatal period is challenging because of the hectic environment, the short hospitalization for many newborns, and the many competing priorities for parents and newborn-care providers. Further, signatures to document permission can be obtained in a perfunctory fashion, so

requiring signatures per se does not assure a meaningful informed-permission process. Under the assumption that parents are reasonably informed about the program and their rights under state law, both opt-in and opt-out approaches to NBS are ethically acceptable.

- Although the ASHG supports improved parental education about NBS, it does not advocate a change in most state programs that mandate screening but permit parental refusals.

When screening is conducted, programs obtain sufficient blood from infants to perform all testing and to conduct repeat testing when warranted. This means that most infants will have extra blood on the filter cards after screening. Traditionally, many states have saved these residual dried bloodspots (DBSs) for several purposes, including quality assurance (QA) for NBS laboratory services, forensic uses, and biomedical research.[63] The DBSs are particularly useful for research because they represent a tissue set on the entire population of newborns and can be used for genetic epidemiology and for exposure to prenatal infectious diseases and environmental toxins, among other applications. Although many states discard the DBSs after screening is complete, many states retain these DBSs for various lengths of time. The retention of DBSs became controversial in recent years when two state programs, those of Minnesota and Texas, were sued by parent groups for the lack of parental permission for this practice.

In the US and Canada, research on public attitudes regarding the management of DBSs demonstrates broad public support for the retention of DBSs for QA and biomedical research, contingent on parental education and choice.[72,74] Consistent with public and professional opinions on this issue, the ASHG supports the retention and research uses of residual DBSs under carefully developed, transparent public policies and practices. Prior to 2015, when used for biomedical research, residual DBSs were typically de-identified, or research was conducted under a waiver of parental permission. However, in late 2014, the Newborn Screening Saves Lives Reauthorization Act of 2014 (public law no. 113-240) was passed to require informed consent from parents for all Department of Health and Human Services-funded research using DBSs and to prohibit the waiver of consent. The impact of this law on NBS-related research remains to be determined. However, the ASHG considers the retention of DBSs strictly for quality-improvement activities for the NBS programs to be covered under the state mandate for screening. Therefore, parental permission should not be necessary for the use of DBSs for QA purposes.

- The ASHG encourages states to retain DBSs for QA purposes. Retention for QA purposes should be considered integral to the NBS program and should not require specific permission from parents.
- The ASHG encourages states to retain DBSs and to make specimens available to investigators and to public-health programs under carefully developed guidelines.
- Parents should be informed of state policy and practices regarding the retention and use of DBSs.[75]
- Parents should be offered a choice regarding the retention and use of their child's DBSs for purposes beyond the clinical NBS program and QA uses. This choice ought to be clearly separated from the decision to participate in NBS.

NBS can also provide benefits to a newborn's family by alerting parents to their reproductive risk for future pregnancies and can benefit society more broadly by advancing the understanding of disease. Information relevant to reproductive risk is also provided by the generation of results related to carrier status. Disclosure of carrier status through NBS raises challenges because this information is not typically available without informed consent and is not usually provided to minors.[76–78] However, recent guidelines and studies have suggested that reproductive benefits might represent an important goal of NBS because carrier detection can inform family planning.[79–82] Many NBS programs disclose carrier results to families. However, there is limited evidence to support the utility and impact of disclosing carrier results to families. A stronger evidentiary base is required to inform evidence-based decision making and recommendations.

- The ASHG recommends additional research for assessing the utility of disclosing carrier results generated from NBS for reproductive decision making and cascade testing, as well as the impacts on systems of care and resources in the context of engagement with relevant stakeholders

Adoption, Consanguinity, and Paternity

Adoption

In the US, approximately 2% of children are adopted, and many children are living in foster care. Prospective adoptive parents might want genetic information about a child to inform their decision on whether or not to adopt. But previous consensus statements of the ASHG and ACMG have advocated that indications for pre-adoption testing closely parallel the indications applied to children living with their biological parents.[83] The rationale for these recommendations rests on concerns that harms might come to the child without sufficient benefit to balance the scales. If such concerns are valid for children living with their biological parents, then the standards for genetic testing should be the same for all children. The "principle of equity" articulates the idea that prospective adoptive parents are entitled to no more information at the time of taking custody of a child than the child's birth parents could obtain.[84]

A countervailing argument has been raised to the principle of equity. It has been suggested that it is in the interest of the child to be placed with families who are optimally capable of taking care of their medical needs.[85] Adoptive parents are already subjected to additional scrutiny to ensure that they have the capability to serve as suitable parents.[86] To some extent, the child's background might also influence these decisions. A commonly held view is that it would disadvantage the child to be placed with some adoptive parents and that even factors such as cultural and ancestral education should be considered.

It is possible that a child with an untreatable genetic disorder would be better off with parents specifically chosen because of their ability to deal with this difficult circumstance. An obvious objection is that knowledge of the disorder might so restrict the pool of willing parents that the child is made "unadoptable." Another concern is that adults responsible for the placement of adoptive children most likely do not have the specialized genetics knowledge that would be required for assigning children to "matched" families.

Another argument for matching is that prospective, adoptive parents' interests would be harmed by failure of the adoption agency to make the best possible choice of home on the basis of the full range of relevant information about the child. However, there is no assertion of a parallel responsibility of the prospective parents to undergo genetic testing themselves. The argument of matching creates the possibility that some parents might find themselves to be genetically unsuitable to adopt.

- The ASHG recommends that both children awaiting adoption and adopted children be given the same consideration in genetic testing as children living with their biological parents. We endorse and affirm the previous recommendations of the ASHG.
- All genetic testing of newborns and children in the adoption process should be consistent with the tests performed on all children of a similar age for the purposes of diagnosis or of identifying appropriate prevention strategies.
- Because the primary justification for genetic testing of any child is a timely medical benefit to the child, genetic testing of newborns and children in the adoption process should be limited to testing for conditions that manifest themselves during childhood or for which preventive measures or therapies can be undertaken during childhood.

Consanguinity

Inbreeding, including first-degree relative relationships, could be detected in genome-wide assays including but not restricted to SNP genotyping, whole-exome sequencing, and whole-genome sequencing.[87] It is possible to find long segments of chromosomes lacking expected heterozygous variation—called runs of homozygosity or absence of heterozygosity (AOH). If AOH is confined to a single chromosome, the cause could be a chromosome replication or segregation abnormality (uniparental isodisomy [UPD]). In UPD, the person undergoing testing has received identical copies of one parental homolog for part or all of a chromosome. The length of the homozygous segment will usually distinguish UPD from autozygosity—identical chromosome segments inherited from the mother and father as a result of a recent shared ancestor. In contrast, if there are multiple long AOH segments with AOH involving many or all of the chromosomes, the most likely explanation is that the parents are close biological relatives. The ACMG has published guidelines for diagnostic laboratories to distinguish UPD from consanguinity.[88] With the accumulation of extensive genomic data in diverse human populations, we can expect further refinement and improved specificity in methods of interpreting tests.[89]

In some ways, detection of extensive AOH is a secondary finding. The motivation for genetic testing might be to detect a diagnostically important DNA copy-number abnormality or single-gene disorder. But the finding of AOH cannot be considered purely incidental because UPD detection is a formal reason for diagnostic testing. UPD or autozygosity can be a necessary condition for imprinting defects or homozygous recessive disorders. Disclosure of the results should, therefore, be guided by the same principles as those for other diagnostic testing.

The detection of extensive long segments of AOH is most consistent with reproduction between close relatives. In the absence of a history of assisted reproduction, this implies incest. The central concern for practitioners is the possibility of sexual abuse of a minor. Sexual relations between close relatives are illegal in most jurisdictions, but the specifics of the laws vary in how relatedness is specified.[90] The detection of a consanguineous relationship by itself does not engender a duty to report it to the authorities. Physician-patient confidentiality must be respected in most circumstances. An important exception is the circumstance in which the health-care provider suspects that a child is being abused. Physicians are obligated to report suspected child abuse without exception.

It does not necessarily follow that the possibility of discovering information that could lead to a suspicion of child abuse should be presented in pre-test counseling. For most patients, this information will be irrelevant but could cause unnecessary anxiety and could even lead to the refusal to allow a diagnostic test.

- The ASHG recommends that laboratories adopt data standards and analytical methods that allow reliable detection of incest. Practitioners should develop procedures for case management when genetic laboratory results are consistent with incest involving a minor. Practitioners have a duty to report suspected child abuse. Health-care providers do not have a responsibility to report incest involving consenting adults, even though this might be illegal in their jurisdiction.

Parentage

Misattributed parentage could be detected when biological relatives undergo genetic testing. Genetic testing, and especially genomic testing, of children and their parents can lead to results inconsistent with the assumed social inheritance relationships. The most commonly encountered problem is misattributed paternity. With estimated rates of 1%–10% from various studies, non-paternity is relatively common and is therefore highly likely to be encountered in routine practice and in research.[91–93] However, with the increased use of assisted reproduction, rare occurrences of misattributed maternity have been described. Misattributed parentage (where neither the mother nor the father is biologically related to the child), albeit very rare, would be quickly recognized with many forms of modern genetic testing. Clarifying the pattern of inheritance of pathogenic variants is a key goal of genetic testing; therefore, it is recommended in all cases that evidence of segregation of potentially disease-causing alleles and parental test results be examined to conclusively demonstrate de novo mutation.

Arguments in favor of full disclosure of paternity findings center on issues of a patient's right to know, avoiding paternalism, and the duty of physicians to be truthful. A broad answer to these concerns is that it is not possible for either mothers or fathers to truly exercise their autonomy if the options are not presented before testing has taken place. Given the intuition that there could be extensive harm, health-care providers following a plan of non-disclosure could be exercising prudence in avoiding interference in the family relationships.

Specific recommendations for the disclosure of misattributed parentage have been made, but opinions expressed in the literature are diverse and unsettled.[94] Although the mother and father (both social and biological) have an undoubted stake in the outcome of parentage information, there is an asymmetry of risk. Only the fidelity of the mother is at stake in the test result. For this reason, it is common practice to disclose only to the mother. For example, the Institute of Medicine produced a report advocating disclosure of misattributed paternity only to the biological mother.[71] This has been countered with arguments pointing out that both the integrity of the physician-patient relationship and professional responsibility involve both the mother and father.[95] Intentional deception is contrary to fundamental values in medical practice. In her critique, Ross strongly advocated for full disclosure to both parents. Although the risk is asymmetric prior to testing, the post-test results involve both the mother and father. Lack of disclosure to the father could involve either misleading interpretations with consequent misleading counseling or outright deception. These are departures from standards of full disclosure, non-directiveness, and respect for autonomy.

More recently, it has been suggested that information about parentage should not be part of routine genetic test reporting and counseling unless it is specifically requested by the parents in advance of the test. Arguing in favor of such an approach, Palmor and Fiester conclude that health-care professionals have no legitimate right to decide about a matter with such high potential for harm to so many individuals in both the close and extended family.[96] They suggest that providers inform clients that although misattributed parentage could be detected in the testing, it will not be disclosed to either the mother or the father. They further argue that parents wishing to investigate parentage should pursue specific testing.

Given the unsettled nature of the debate, it is essential that health-care providers develop a consistent plan for dealing with parentage and ancestry questions of all types. Parents should be informed before the test is performed about the risk of detection of misattributed parentage, and as with other forms of incidental findings, pre-test counseling should be provided. Because the risk in misattributed paternity is asymmetric, an approach for pre-test counseling could include confidentially informing the mother of the potential detection of non-paternity.

- The ASHG recommends that parents be given information about the possibility of detecting misattributed parentage during pre-test counseling. While honoring their broad responsibility to be truthful with patients and their families, we recommend that health-care providers avoid disclosure of misattributed parentage unless there is a clear medical benefit that outweighs the potential harms.

Record and Communication Issues

Quality clinical genetics practice begins and ends with good communication, and evidence indicates that patients value clear communication from medical providers. Because of the complexity of the information, genetic test results have the potential to be misunderstood and to cause harm. Examples include NBS false-positive results, over-interpretation of carrier status or variants of uncertain significance, and the nuances of "negative" results in the face of a suspected genetic disorder.

- The ASHG recommends that providers of pediatric genetic testing have appropriate training and expertise in the interpretation and communication of genetic information.
- The ASHG recommends that diagnostic laboratories develop reports that are detailed and accurate but also facilitate comprehension by providers.

Communication of genetic test results in the pediatric setting is complicated by the potentially long timeline of transition from childhood to adulthood, during which parents act as decision makers on behalf of the child, and the differing capacity of individual children at different development stages to participate in such decisions and to contemplate the meaning of the results. Genetic

information can also have important implications for siblings and other family members.

- The ASHG recommends that genetic testing in children should include a long-term communication plan for all results, including consideration of who should be involved in the communication of information and the staging of information sharing on the basis of age, maturity, and capacity to understand.

Unlike medical tests that measure temporary aspects of an individual's anatomy or physiology, genetic tests provide information of a permanent nature about an individual and potentially their family members. However, maintaining knowledge of genetic results over long periods of time can be challenging. Even though basic information might be recalled (such as the fact that a genetic work-up was performed), the specific details about childhood genetic test results and their implications might not be accurately remembered many years later. This loss of retention severely impairs their subsequent utilization by clinicians, patients, or patients' family members and can lead to unnecessary repeat genetic testing and thus a waste of resources. Modern electronic medical records have the potential to maintain information with much greater fidelity over the lifespan of the individual.

- The ASHG recommends that standards be developed for permanent storage of genetic data in electronic health records or other secure electronic systems to facilitate the provision of genetic information in patient portals.
- The ASHG also recommends the development of mechanisms for sharing family history and genetic results with family members.

As genetic testing modalities become more comprehensive and generate large amounts of raw data, genetic test results will challenge the current model of storing laboratory results. Most genetic variation will be of unclear clinical significance but might become interpretable over time with continual advances in medical science. However, current electronic medical records are not typically designed to manage storage or re-analysis of genome-scale information, and it is not clear whether it would be desirable for them to do so. Recent federal regulations provide for laboratory results to be the property of the patient, raising questions about how much genomic information should be placed in the medical record, particularly in the case of genetic variation that does not have well-established clinical implications. Furthermore, with some notable exceptions, a key limitation of the typical interface between the clinical laboratory and the medical record is that it involves a single instance of data transfer that does not permit re-interpretation of genetic results over time.

- The ASHG recommends the development of uniform guidelines to standardize medical-record capabilities and management of interpreted results and raw genetic sequence data.
- The ASHG also recommends developing novel models for molecular laboratory and interpretive services on the basis of prospects for the re-analysis of genetic information over time.

Professional Education

If health-care providers are to adhere successfully to the recommendations in this report, they must have appropriate knowledge and skills related to genetic and genomic testing, interpretation of test results, communication of results to patients and families, and basic genetic counseling. In addition, the health-care system will require adequate numbers of trained medical geneticists and genetic counselors to assist in the role of specialty testing and interpretation of results. With the expected expansion of genetic and genomic testing, all health-care providers will need (1) educational programs that target relevant scientific, clinical, ethical, legal, and social topics and (2) support systems that address structural and systemic barriers to the integration of genetic medicine into clinical practice.

Providers' Understanding of Genetic Medicine

Previous studies have clearly documented that health-care providers have knowledge gaps that constitute a rate-limiting step in the incorporation of genetics and genomics into mainstream health care.[97–99] Guttmacher et al.[97] and McInerney et al.[98] summarized some of the central deficiencies related to clinicians' understanding of genetic medicine as follows:

> Misconceptions about genetics: many health-care providers still believe that genetic medicine is defined by rare, Mendelian disorders and circumscribed by pediatrics and obstetrics, when in fact genetics increasingly is concerned with the common, chronic diseases that are the daily focus for most health professionals.
>
> Lack of knowledge and confidence about genetics: surveys of practicing health professionals demonstrate a lack of basic knowledge about genetics and, often, a lack of confidence to deal with genetics-related issues that arise in the clinical setting.

Deficiencies in genetics education extend from the preservice training of most health-care professionals to postgraduate internships, residency and fellowship training, and continuing medical and professional education for actively practicing health-care professionals. Notable efforts exist in various organizations across the US to integrate genetics and genomics into formal education and to increase the genetics content of certifying exams.[100–104] Many of those efforts are driven by the development of competencies that focus on content knowledge and related clinical skills.

Equally important is the challenge of training those health-care providers currently in practice. A 2012 report from the UK's Human Genomics Strategy Group[105] captures the situation concisely:

> Ensuring that genomics is an integral part of initial medical/health education and training will be an important step towards developing the work force. But for the next 15 years at least, the majority of staff who will have to cope with the movement of genomics into mainstream clinical work will be those who are already trained and accredited. That is why the bigger educational challenge is to close the skills gap within the existing work force, via continuing professional development (CPD) arrangement.

The highly diverse disciplines, clinical settings, and motivations reflected in this vast health-care work force will require equally diverse educational approaches, all of which must involve the end user from the initial planning through implementation and evaluation.[98] Again, some good models for CPD are in place or in development in the US, but implementation, evaluation, and scaling from local to broader application remain as significant challenges, and addressing them will require material and personnel resources.[106–108]

Structural and Systemic Barriers

The practice model in health care evolves constantly, and just as the development of antibiotics in the twentieth century and medical imaging in the late twentieth and early twenty-first centuries changed the practice of medicine, genetics and genomics are changing medical practice today. Education of practicing clinicians and the application of new knowledge and skills highlight some of the systemic challenges to incorporating genetic medicine into health management, for example:

> Lack of management and referral guidelines in genetics and genomics: the paucity of evidence-based guidelines related to genetic medicine, and the slow dissemination of those that do exist, impede clinicians' attention to genetics and raise questions about clinical utility.
>
> A dearth of genetics professionals: the low numbers of medical geneticists and genetic counselors in the USA and elsewhere limit the provision of genetic services directly and, furthermore, limit the extent to which other providers have formal and informal access to genetics expertise.[109]

Haga et al. reported that in a survey of US PCPs, "more than half (53%) of respondents indicated they do not have access to genetics expertise." The authors of the study suggest "a hybrid model of education and support for PCPs and access to specialist consultation when needed."[110] Hamilton et al., using diffusion of innovation theory and focusing on clinical genetic services in the Veterans' Administration, have elaborated some of the factors that promote or impede the integration of genetics into various types of primary and specialist practice.[111] In assessing factors such as complexity, compatibility with existing services, and relative advantage ("added value … when compared to existing practice"), the authors found that study participants "indicated that benefits did not outweigh the costs of genetic services," and they conclude that uptake of genetic services "by simple diffusion" will not work. "Instead," they assert, "adoption of clinical genetic services will require development of targeted organizational supports to strengthen the likelihood of adoption and implementation."

Even these few examples demonstrate the complexity of the challenges facing the education of health professionals and the subsequent integration of genetics and genomics into practice. Information does not equal education, especially when the objective is to change clinical behaviors and improve patient outcomes.

Although it is not ASHG's responsibility to direct change in this complex system of formal and informal education from pre-clinical training to continuing education, it can help to promote change by supporting the recommendations below.

- ASHG recommends that the genetics community work closely with appropriate educational institutions, governing bodies, and professional societies to develop and deliver programs that provide the knowledge and skills health-care providers need to apply the recommendations herein in their own practices.
- ASHG recommends that the introduction of genetics-related content and case examples should emphasize the extension of existing knowledge and skills and should not portray genetics as a discipline that requires wholly new approaches to clinical care.
- ASHG recommends that those developing educational programs be cognizant of the structural barriers that impede the integration of genetic medicine—or any other clinical innovation—into routine practice and attempt to address those barriers in program content and implementation strategies.
- ASHG recommends that educational programs for health-care providers include well-designed evaluation plans that assess the efficacy of content, instructional approaches, and implementation strategies. Evaluation plans should be in place before program development begins and should reflect carefully developed educational objectives and outcomes.
- Because a well-informed public presumably will make better individual and collective decisions about the issues elaborated in this report, the genetics community should support efforts to improve public genetic literacy and scientific literacy in general.
- The inevitable and significant increase in the number and use of genetic tests will require more genetic

counselors and more genetically competent nurses, physician assistants, and physicians. The ASHG recommends an increase in the number and size of training programs and the provision of funds to support this expanding training infrastructure.

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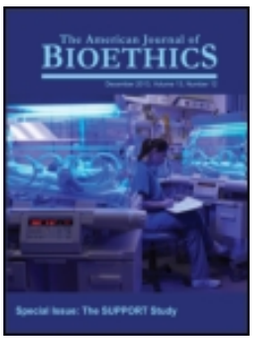

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Addressing the Ethical Challenges in Genetic Testing and Sequencing of Children

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Target Article

Addressing the Ethical Challenges in Genetic Testing and Sequencing of Children

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American Academy of Pediatrics (AAP) and American College of Medical Genetics (ACMG) recently provided two recommendations about predictive genetic testing of children. The Clinical Sequencing Exploratory Research Consortium's Pediatrics Working Group compared these recommendations, focusing on operational and ethical issues specific to decision making for children. Content analysis of the statements addresses two issues: (1) how these recommendations characterize and analyze locus of decision making, as well as the risks and benefits of testing, and (2) whether the guidelines conflict or come to different but compatible conclusions because they consider different testing scenarios. These statements differ in ethically significant ways. AAP/ACMG analyzes risks and benefits using best interests of the child and recommends that, absent ameliorative interventions available during childhood, clinicians should generally decline to order testing. Parents authorize focused tests. ACMG analyzes risks and benefits using the interests of the child and other family members and recommends that sequencing results be examined for additional variants that can lead to ameliorative interventions, regardless of age, which laboratories should report to clinicians who should contextualize the results. Parents must accept additional analysis. The ethical arguments in these statements appear to be in tension with each other.

Keywords: ethics, pediatrics, exome sequencing, genome sequencing, risks, benefits, interests of child and family, best interests of the child

The debate about predictive genetic testing of children for adult-onset disorders has been cast in a new light by the release of two sets of recommendations, in February and March 2013, respectively, both endorsed by the American College of Medical Genetics and Genomics (ACMG). The first set of recommendations, as part of its overarching consideration of the ethical and legal issues raised by pediatric genetic testing and screening in a range of contexts, addressed whether it is appropriate to test children for a mutation typically associated with adult-onset disease already known to be present in the family and for which there is no intervention in childhood (American Academy of Pediatrics [AAP] and ACMG 2013). This document accompanied a technical report on pediatric genetic testing generally and was issued jointly with the American Academy of Pediatrics (AAP) (Ross et al. 2013) (both hereinafter referred to as the AAP/ACMG statements). One month after the issuance of these recommendations, the ACMG issued a second set of recommendations (Green et al. 2013; hereinafter referred to as the ACMG ES/GS [exome sequencing/genome sequencing] statement), followed shortly by a clarification (Incidental findings 2013), addressing the return of findings from clinical exome- and genome-wide sequencing that are beyond those needed to answer the clinical question for which sequencing was sought. In this article, members of the CSER Pediatrics Working Group, some of whom were involved in developing the documents just described, describe and compare these recommendations and the

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ethical arguments underlying them as they pertain to testing children for adult-onset disorders for which ameliorative interventions are not available during childhood.

The two sets of recommendations on predictive testing, which address somewhat distinct but potentially overlapping clinical contexts, differ in how they approach genetic testing of children for adult-onset conditions. The AAP/ACMG recommendations, affirming previous professional consensus (American Society of Human Genetics Board of Directors and American College of Medical Genetics Board of Directors 1995; Borry et al. 2006; Ethical issues 2001) and citing the best interest of the child, take the position that predictive genetic testing for adult-onset conditions that cannot be ameliorated in childhood—testing that is sometimes requested by parents—generally should not be performed, with rare and carefully considered exceptions when diagnostic uncertainty poses a significant psychosocial burden to the family. While the AAP/ACMG statement did endorse genetic testing for disorders that could occur or be ameliorated during childhood in families known to be at risk, it did not address the appropriateness of looking for or reporting such variants when children are being tested to address another clinical issue.

In contrast, the ACMG exome sequencing/genome sequencing (ES/GS) recommendations proposed that when a child undergoes testing for a specific clinical indication using exome or genome sequencing, the laboratory should also analyze and interpret the child's genomic data looking for known pathogenic mutations—and for certain genes, for variants that are expected to be pathogenic—in 57 (a number since reduced to 56) genes associated with 24 genetic conditions. The 56 genes on the ACMG list were selected because, in the view of the statement's authors, they are associated with phenotypes for which "preventive measures and/or treatments [are] available and disorders in which individuals with pathogenic mutations might be asymptomatic for long periods of time." This recommended analysis, applied irrespective of age, included adult-onset disorders for which measures to modify risk are unavailable during childhood or can safely be deferred to adulthood as well as those for which intervention during childhood is warranted. The ACMG ES/GS recommendations stated that the clinician is "expected ... to contextualize [these findings] for the patient in the light of personal and family history, physical examination, and other relevant findings." The ACMG ES/GS recommended that while patients and parents should have a right to refuse GS or ES, if they do authorize testing, they should not be given the choice to opt out of analysis and reporting to the clinician who ordered the test of identified pathogenic mutations in the 56 genes.

The two sets of recommendations differ somewhat in their audiences. The AAP/ACMG recommendations on predictive genetic testing are directed primarily at clinicians who are considering whether to order a single gene test for a child with a positive family history. The ACMG ES/GS recommendations address both laboratories and clinicians regarding a secondary analysis of an exome or genome sequence that was ordered to diagnose a disorder in the child. These sets of recommendations therefore raise two questions: (1) How does each set of guidelines characterize and analyze the locus of decision making as well as the risks and benefits of testing? (2) Are the guidelines in conflict, or have they come to different but compatible conclusions because they consider different testing scenarios?

The discussion presented in this article proceeds in two parts. First, we lay out the AAP/ACMG recommendations about pediatric genetic testing and the ACMG's more recent recommendations about genomic sequencing and analysis of 56 additional genes, along with the justifications provided, relying heavily on the documents' language. Second, we present a side-by-side comparison of issues raised by AAP/ACMG and ACMG ES/GS statements, identifying questions for further discussion in light of this comparison. The authors of this article hold widely divergent views about whether the two sets of recommendations can be reconciled (and if not, about which represents the more appropriate approach). This article thus does not seek to draw conclusions about which sets of recommendations, or which parts of which sets, are preferable, but rather to elucidate the range of frameworks, assumptions, and values in the two documents as a prelude to further discussion.

THE TWO SETS OF RECOMMENDATIONS AND THEIR ETHICAL JUSTIFICATIONS

AAP/ACMG Recommendations

There has been a long-standing consensus that the primary and strongest justification for genetic testing of children exists when the results will clarify the cause of current symptoms, when the onset of the condition may occur during childhood, or when the information will be used to embark on a course of care that must start during childhood to prevent or ameliorate later symptoms (American Society of Human Genetics Board of Directors and American College of Medical Genetics Board of Directors 1995). The last, for example, is the justification for newborn screening. The broad consensus has been that minors who are known to be at risk of adult-onset disorders should not undergo genetic testing for a condition unless the results would lead to altered medical management during childhood that improves outcome (e.g., familial adenomatous polyposis), in part so that these young people can make their own choices about testing once they reach adulthood. Although at-risk adults are more likely to refuse predisposition genetic testing when no therapeutic or preventive interventions for the condition in question exist, some decline testing even when such interventions are available (de Snoo et al. 2008; Glenn, Chawla, and Bastani 2012; Kinney et al. 2006; Melnyk and Shepperd 2012; Ramsoekh et al. 2007). The February 2013 statement of the ACMG and AAP concluded that

> Predictive genetic testing for adult onset conditions generally should be deferred unless an intervention initiated in childhood may reduce morbidity or mortality. An exception might

> be made for families for whom diagnostic uncertainty poses a significant psychosocial burden, particularly when an adolescent and his or her parents concur in their interest in predictive testing.

In the accompanying technical report, "The AAP and ACMG continue[d] to support the traditional professional recommendation to defer genetic testing for late-onset conditions until adulthood," citing more than two dozen previous statements by national and international professional organizations. They went on, however, to state that

> Predictive genetic testing may be appropriate in limited circumstances. [cit. om.] In deciding whether a child should undergo predictive genetic testing for late-onset conditions, the focus must be on the child's medical best interest; however, parents and guardians may also consider the potential psychosocial benefits and harms to the child and the extended family. [cit. om.] Extending consideration beyond the child's medical best interest not only acknowledges the traditional deference given to parents about how they raise their children [cits. om.] but also recognizes that the interest of a child is embedded in and dependent on the interests of the family unit. In some families, the psychosocial burden of ambiguity may be so great as to justify testing during childhood, particularly when parents and mature adolescents jointly express interest in proceeding. Some parents may seek predictive genetic testing for adult-onset conditions even when children are unable to participate in the decision-making process because of immaturity or cognitive impairment. After careful genetic counseling, it may be ethically acceptable to proceed with predictive genetic testing to resolve disabling parental anxiety or to support life-planning decisions that parents sincerely believe to be in the child's best interest. [cits. om.]

ACMG Recommendations Regarding Results of Additional Analysis of Genomic Data

Genome-based tests, such as genome and exome sequencing, which make it possible to assess variants in nearly all genes, are now beginning to be used in all age groups for refining cancer diagnoses and therapies. Of particular relevance to pediatrics is the growing importance of these approaches for ascertaining the causes of previously undiagnosed genetic conditions, particularly neurodevelopmental disorders and multiple congenital anomaly syndromes. Often these studies are done on parent–child trios to facilitate analysis of inheritance for recessive disorders and to identify de novo mutations. As the use of these technologies increases, a great deal of sequence data on children (and their parents) is being generated, raising the question of which parts of the data, if any, need to be analyzed and reported beyond that needed to answer the presenting clinical question. The ACMG ES/GS recommendations identified 56 genes that have pathogenic mutations that can be acted on, at times well into the future, to prevent or mitigate later symptoms. They recommended that laboratories analyze these 56 genes, and interpret and report identified pathogenic mutations to the ordering clinicians, for both adult and pediatric patients. The ACMG ES/GS statement reaffirmed prior ACMG guidance (Points to consider 2012) that informed consent should be sought for genomic testing after appropriate pretest counseling, including discussion of the possibility of findings from additional analysis, but "did not favor offering the patient a preference as to whether to receive" the findings of additional analysis.

A major driver of the ACMG ES/GS recommendations was concern that patients and their parents, and by extension other family members, would not otherwise learn about these mutations, since genome-wide tests are not currently broadly available. A related motivation was the possibility that these mutations may be present even in the absence of a positive family history that might prompt targeted diagnostic testing. The authors of the recommendations explained that

> at this moment in the evolution of clinical sequencing, an incidental finding relevant to adult disease that is discovered and reported through clinical sequencing of a child may be the only way in which that variant will come to light for the parent. . . . The Working Group also felt that the ethical concerns about providing children with genetic risk information about adult-onset diseases were outweighed by the potential benefit to the future health of the child and the child's parent of discovering an incidental finding where intervention might be possible.

In a subsequent clarification, the ACMG reasoned that identifying pathogenic mutations in children would benefit the children by enabling their parents to obtain medical management for the risk to their own health, as well as providing the children with information about a predisposition about which they might not otherwise learn at any point prior to the development of clinical manifestations. They further reasoned that any risk of altered parental nurturing as a result of receiving information is outweighed by the increased ability of the child to recognize the need to obtain medical care in the future. The ACMG in its clarification stated:

> The ACMG affirms its recommendation not to perform diagnostic testing for an adult-onset condition in children but believes that reporting an incidental finding of a severe, actionable, pathogenic mutation falls outside this recommendation.

In comparing the two documents, questions remain about whether these sets of recommendations do in fact conflict and if so, to what extent their differences can and should be reconciled.

POTENTIAL DIFFERENCES BETWEEN THE SETS OF RECOMMENDATIONS

Nature of the Test and the Reason It Is Performed

In the scenario contemplated in the AAP/ACMG statements, parents request that their child undergo predictive testing for a mutation associated with adult-onset disease known to be present in the family but for which effective early intervention in childhood is not available. The only question is whether to do the test or not, and these organizations concluded, as have many before and since (Points

to consider 2012; van El et al. 2013), that such tests should be discouraged because they fail to protect and promote the child's best interests. Although few data are available regarding the impact of such tests on children, either for good or for ill (Malpas 2008; Mand et al. 2012; Wade, Wilfond, and McBride 2010), the rationale is that children may be harmed during childhood by being tested for adult-onset disorders. The harm of such testing that has raised the greatest ethical concern is foreclosure of the child's ability to decide for him- or herself about whether and when to be tested after reaching adulthood—an opportunity loss that is relevant since some adults who know they are at risk choose not to pursue testing (de Snoo et al. 2008; Glenn, Chawla, and Bastani 2012; Kinney et al. 2006; Melnyk and Shepperd 2012; Ramsoekh et al. 2007). If testing is deferred, then assuming that their parents share the risk information with them in an appropriate and understandable manner and they are referred to competent providers (Aktan-Collan et al. 2011), children will be able to make their own decisions about testing on reaching adulthood. The AAP/ACMG statements acknowledged that it may be appropriate in some cases to proceed with testing during childhood, but only after detailed conversations between the provider and family that take into account the family's motivation, context, and understanding.

In the scenario contemplated in the ACMG ES/GS statement, by contrast, the child is undergoing genome-wide or exome-wide sequencing in order to address a current medical problem such as cancer or an undiagnosed genetic disorder. The ACMG ES/GS recommendations are predicated on the assumption that the family whose child is undergoing testing would be unaware of their child's and their family's risk for an additional condition that could be uncovered by further analysis of the sequence data (in some cases, however, the family may already be aware of the familial risk of one or more conditions being evaluated by additional analysis). The ACMG concluded that mutations in the 56 genes are "incidental findings [that] are inextricably part of exome and genome analysis, and that such results should be returned to clinicians" who can then "contextualize" the results for patients and families as noted in the following. The existence of these data—data that are not obtained in order to answer the question for which sequencing was ordered—led the ACMG to recommend that sequence information be analyzed for pathogenic mutations in these 56 genes, and to conclude that failure to do so may even be "unethical" (Incidental findings 2013). In recommending that these genes be analyzed, the ACMG was influenced by the fact that genome sequencing and exome sequencing are at present not widely available. In addition, if the family of a child with a pathogenic mutation in one of these genes is unaware that its members are at risk, family members likely will not otherwise have reason to seek to learn whether they have one of these mutations, precluding or delaying the possibility of seeking appropriate medical management for the child's relatives, even if no intervention was warranted for the child prior to adulthood.

Whose Interests Are to Be Taken Into Account?

The ACMG/AAP documents focused on the best interests of the child, with the family's interests being pertinent primarily insofar as they affect the child. While the ACMG ES/GS recommendations similarly addressed the interests of the child, they also considered the potential health benefit to parents or other family members as a factor in deciding which results to seek and disclose to the clinician. The ACMG ES/GS authors argued that disclosure will benefit the child both directly and indirectly—directly by learning of a significant health risk that she or he may choose to address as an adult, and indirectly by having parents and other biological relatives who might be healthier by virtue of having been given an opportunity to address their own, perhaps previously unsuspected, risk.

Weighing Risks and Benefits

The potential benefits of testing just described are categorized differently in the two sets of recommendations and are also weighed differently against the potential risks to the child. In assessing the impact of predictive genetic testing for an adult-onset disorder for which the child is known to be at risk, the AAP/ACMG statements focused on averting the risks to the child of learning that he or she is at risk, including the risk that the parents may treat the child differently. They identified as relatively minor the benefit of reducing uncertainty through testing of the child, and as major the benefit of deferring to permit the child to make a decision after reaching adulthood. By contrast, the ACMG, in its recommendations about reporting the specific results of additional analysis of genomic data, placed a higher value on the benefit to the family and to the child of identifying and reporting these mutations, which in the ACMG's view outweighs the child's interest in making his or her own decision in the future based on the information available at that time.

Who Decides What?

Finally, the sets of recommendations diverge in who is involved in decision making and the roles they play. The details of these differences are set forth in Table 1 and summarized here. The AAP/ACMG recommendations established a strong presumption that, unless ameliorative interventions are available during childhood, children should not undergo testing for predispositions to adult-onset conditions and clinicians should generally decline to order testing. The recommendations did, however, allow for circumscribed exceptions to this presumption, and accorded decision-making discretion to the child's clinician and parents (and, if appropriate, the child, especially in adolescence). In the context of clinical sequencing, by contrast, the ACMG recommended which types of mutations laboratories should report to clinicians, with parents and clinicians given the choice only between sequencing plus reporting findings in 56 additional genes or forgoing sequencing

Table 1. Roles of Potential Decision Makers

Potential decision makers	AAP/ACMG pediatric genetic testing for adult onset disorders	ACMG ES/GS Results of additional analysis of genomic data
What is the scope of parental decision making?	Parents may ask clinician to test the child for a mutation known to exist in their family	With acceptance of ES/GS for the primary indication, parents accept analysis of the additional 56 genes
What is the role of the adolescent?	Greater presumption for testing if desired by both adolescent and parents	Not addressed
What is the scope of decision making for clinician?	Clinicians should decline to test children for adult-onset disorders unless preventive or therapeutic interventions are available during childhood. Testing after careful counseling may be permissible in unusual cases to relieve anxiety or permit life planning.	Clinicians working with families are responsible for contextualizing results or making referrals; "clinicians . . . have a fiduciary duty to prevent harm by warning patients and their families" about these findings.
What role do professional organizations play?	Set forth ethical guidance for decision making by parents and physicians, including strong presumption against genetic testing of minors for predisposition to adult-onset condition unless ameliorative interventions are available in childhood.	Define list of genes that must be analyzed by laboratories with pathogenic and predicted-to-be-pathogenic mutations returned to clinicians; provide ethical arguments for their recommendations.

altogether. According to the ACMG ES/GS statements, "The rationale for our recommendations was that **not** reporting a laboratory test result that conveys a near certainty of an adverse yet potentially preventable medical outcome would be unethical." (There is ongoing debate in the genomics community about whether all 56 of the conditions included in the ACMG's list reach this evidentiary standard, but that topic, which will require additional research to resolve, is beyond the scope of this article.) The ACMG stated that the child's clinician should "contextualize" the additional results, but also said that "clinicians and laboratory personnel have a fiduciary duty to prevent harm by warning patients and their families about certain [results of additional analysis of genomic data] and that this principle supersedes concerns about autonomy" (Green et al. 2013, 11). (Some readers may argue that the ACMG recommendations technically do not direct clinicians to disclose results to patients or parents, but rather, only recommend that laboratories report those results to clinicians, who then may then separately decide whether or not to report them to patients. However, once the results have been placed in a medical record, as will occur in many medical practices, it may realistically be difficult to prevent the patient or parent from seeing them, especially given the mandate of the Health Insurance Portability and Accountability Act (HIPAA) and the new requirements of meaningful use of electronic health records (EHRs), which gives patients a legal right to access to their own medical records. A full discussion of this issue, however, is also beyond the scope of this article.). Table 1 lays out in parallel the positions in the documents about the roles of various participants in decisions about testing.

In summary, our reading of the AAP/ACMG and ACMG ES/GS recommendations supports the conclusions that their ethical justifications differ and appear to be in tension with each other and that therefore the statements differ with regard to whose interests should be taken into account, how benefits and risks should be weighed, and the decision-making roles of clinicians and parents. Additional deliberation involving a broad range of stakeholders that carefully considers some of the issues identified here and the many nuances that they raise points to the need for additional research in this area. This research, over time, should lead to the development of ever more sophisticated, comprehensive, internally consistent, and ethically sound guidelines for genetic testing of children.

Language From ACMG Clarification regarding Incidental Findings

> We believe, however, that the disclosure of incidental findings such as a *BRCA1* gene mutation is justified for the following reasons: 1) If the child carries a pathogenic mutation there is a high probability that one parent does as well. Given that this is an incidental finding, it is fair to assume that the presence of this variant in the family has not been previously recognized based on clinical findings or family history. In this circumstance, and since only medically

actionable variants highly likely to be pathogenic would be reported, the child does benefit by potentially preventing a severe adverse health outcome in a parent. 2) The recommendation that children not be tested for an adult-onset disorder is typically invoked in circumstances where there is a known family history of risk, with the expectation that the child will be offered testing at an age when he or she can make an informed decision about testing. If there are no other clinical or family history indications, as might be the case for an incidental finding, that opportunity may not occur, potentially until the child is affected. 3) There is also some concern that the nurturing of the child might be adversely affected by the parent's knowledge of the child's future risk and the need to decide when to reveal that to the child. We believe, however, that the ability to identify a significant medical risk for the child that could avoid future morbidity takes precedence over this possible risk. ACMG affirms its recommendation not to perform diagnostic testing for an adult-onset condition in children, but believes that reporting an incidental finding of a severe, actionable, pathogenic mutation falls outside this recommendation.

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One co-author (L.S.F.R.) was the lead author of the joint American Academy of Pediatrics/American College of Medical Genetics and Genomics policy statement and technical report on the Ethical and Policy Issues in Genetic Testing and Screening of Children (AAP and ACMG 2013; Ross et al. 2013) and another co-author (L.G.B.) was a co-author of ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing (Green et al. 2013).

Ellen Wright Clayton wrote the first draft of this article and made all revisions in response to comments and suggestions made by members of the Clinical Sequencing Exploratory Research (CSER) Consortium Pediatrics Working Group. She approved the final article as submitted.

Laurence B. McCullough, Leslie Biesecker, Steven Joffe, Lainie Friedman Ross, and Susan M. Wolf made substantial contributions to the article's analysis; they reviewed and revised it critically for important intellectual content, and they ultimately approved the final article as submitted. ■

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Stress in African American Pregnancies: Testing the Roles of Various Stress Concepts in Prediction of Birth Outcomes

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ABSTRACT

Background: *The persistently higher rates of adverse birth outcomes among African American women are a major public health concern.* ***Purpose:*** *The purpose of this study was to explore the relations among psychosocial stress, socioeconomic status, and birth outcomes in African American women.* ***Methods:*** *A prospective survey research design was used to measure stress exposure, subjective responses to stressors, including intrusive effects of life events, and medical and sociodemographic variables in a sample of 178 pregnant African American women. Birth outcomes were obtained from medical charts.* ***Results:*** *Life event exposure was high, but levels of perceived stress and negative emotional responses were low to moderate. Lower income African American women reported significantly greater pregnancy undesirability than higher income African American women. Educational attainment was not related to any of the stress variables, and neither income nor educational attainment was significantly related to birth outcomes. Number of stressful life events significantly predicted 3% additional variance in gestational age after controlling for potential confounders. Psychosocial stress variables altogether accounted for 7% additional variance in gestational age-adjusted birth weight, with event distress and intrusive thoughts concerning severe life events emerging as the significant independent stress predictors.* ***Conclusions:*** *These results contribute to our understanding of the complex etiological processes involved in African American birth outcomes and set the stage for further research into their reproductive health status.*

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INTRODUCTION

The reproductive health status of African American women is a major public health concern. African American infants have two times the rate of infant mortality (1), two times the rate of preterm delivery (< 37 weeks gestation), four times the rate of very preterm delivery (< 28 weeks gestation), two times the rate of low birth weight (< 2,500 g or 5 lb 8 oz), and three times the rate of very low birth weight (< 1,500 g or 3 lb 4 oz) of their White counterparts (2). With the U.S. Surgeon General's call to eliminate health disparities across social groups in the new millennium, understanding why African American women have such comparatively poor birth outcomes is a critical issue (3).

Stress and African American Birth Outcomes

Psychosocial stress may be a key factor in understanding African American women's poorer reproductive outcomes. In the pregnancy literature, there is mounting evidence that psychosocial stress influences birth outcomes (4–6). Various stress concepts, including stressful life events, event distress, perceived stress, state anxiety, and pregnancy-related anxiety have been linked to both earlier delivery (7–10) and lower birth weight

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(11,12). Further studies show that African Americans carry a greater burden of stress than do other groups in terms of frequency, quantity, and severity of exposure to stressors (13–15).

A few investigators have studied stress and pregnancy in African Americans with mixed results. Barbosa (16) did not find an association between life events and gestational age in her sample of nearly 500 low-income African American women, and Murrell (17) reported that daily hassles were not a significant predictor of gestational age or infant birth weight in her study of 147 low-risk pregnant African American women. In their predominantly African American sample, McCormick et al. (18) did not observe a relation between stressful life events and birth weight either. However, other studies have demonstrated a significant effect of stressors on birth weight. Orr et al. (19) conducted a prospective investigation of stress and pregnancy, using a sample of 1,861 urban pregnant women, and found that measures of both acute life events and chronically stressful life conditions significantly predicted low birth weight, but only in the African American subsample. Reeb, Graham, Zyzanski, and Kitson (20) and Collins et al. (21) also found that stressful life events were significantly related to low birth weight in African Americans.

Besides objective stressors, studies have also examined African American women's emotions and emotional responses to stressors during pregnancy. Norbeck and Anderson (22) reported that effects of state anxiety on birth weight held only for the African American women in their sample, not for Whites or Hispanics. Zambrana, Dunkel-Schetter, Collins, and Scrimshaw (23) documented that life event distress and perceived stress mediated the relation between ethnicity and birth weight in their study of pregnant African American and Mexican-origin women. Although they did not explore relations with birth outcomes, Stancil, Hertz-Picciotto, Schramm, and Watt-Morse (24) found that pregnant African American women's levels of perceived stress predicted blood pressure at 32 to 36 weeks gestation.

Thus, in the preponderance of available studies, various aspects of stress have been linked to African American birth outcomes (birth weight most consistently), although notable nonreplications exist. It is unclear which stress variables pose the greatest threat to African American pregnancies, though, and few studies have compared the power of multiple stress indicators to predict risk. Such comparisons must consider assessment of stress exposures, stress responses, general chronic stress, and context-specific stressors such as pregnancy-specific stress. In addition, traumatic stress and its effects have not been investigated adequately, although traumatic experiences have been linked to pregnancy outcomes (25,26). There are indications that traumatic stressors, and the psychological distress they cause, may be high in African Americans (27,28).

Role of Socioeconomic Status

The poor are more likely to experience both acute life events and chronically stressful life conditions (29). As a result, the poor are more likely to suffer from psychological distress and disorder (30). It is also well established that lower socioeconomic status (SES) is related to adverse health outcomes (31). Compared to Whites, African Americans are three times more likely to be poor (32), and have lower average incomes, smaller net worths, and fewer net financial assets (33). Although socioeconomic indicators have been found to be related to pregnancy outcomes in African American women (17,34), not all investigations have noted such a relation (19,35). Despite the lack of consistent findings, SES constitutes an important factor to consider in studying issues of race, stress, and birth outcomes.

This Study

It has been argued that the focus on comparative investigations of various ethnic or racial groups has slowed our understanding of ethnic health disparities by assuming the groups under study are relatively homogenous (36). Intragroup analyses, on the other hand, focus on the variability within particular population groups and are therefore able to uncover distinctive patterns of risk. The specific aims of this study were to (a) assess stress exposure and stress responses in pregnant African American women, (b) explore socioeconomic differences in stress and birth outcomes, and (c) determine whether stress predicts birth outcomes in this group, controlling for medical and sociodemographic risk factors. Several psychosocial variables were assessed and tested in this study, including measures of exposure to stress, stress responses such as state anxiety, chronic stress, and context-specific measures such as anxiety about pregnancy and degree of undesirability of the pregnancy. A measure of response to traumatic stress, intrusive thoughts concerning severest life events, was also included.

The study hypotheses were that higher levels of stress would be associated with lower SES and poorer birth outcomes, especially lower birth weight and growth retardation in utero. Although in the general stress and pregnancy literature psychosocial stress has been more consistently related to gestational age (4,5), we expected stronger effects of stress on birth weight because prior work has pointed more to birth weight or intrauterine growth effects in African Americans. Orr et al. (19) presented two possible mechanisms by which stressors could influence African American birth weights: indirectly, through clinical and behavioral risk factors such as higher rates of smoking, and directly, through physiological stress responses, such as elevated levels of catecholamines, which may decrease blood flow to the uterus. They further suggest that African Americans may be more vulnerable to the negative effects of stress on health, which may help to explain long-standing Black/White disparities in birth outcomes. The hypothesis that lower SES would be related to poorer outcomes is consistent with broader theories of SES and health and is examined here within an African American sample varying in SES. This provides clarification of the distinct roles of SES and ethnicity that is frequently not available.

METHOD

Design

The Behavior in Pregnancy Study was a 3-year prospective investigation of stress in pregnancy and its effects on birth outcomes. Women in the Los Angeles, California area who

spoke either English or Spanish fluently, were 17 years of age or older, and were less than 20 weeks gestation were recruited from private, public, and HMO prenatal clinics. Trained interviewers and research nurses collected psychosocial and medical data three times prenatally (Time 1: 18–20 weeks, Time 2: 24–26 weeks, Time 3: 32–36 weeks) and once 6 to 8 weeks postpartum.

Of the 609 women in the overall sample, 234 self-identified as "Black or African American." Of these 234, this investigation examined a subset of 178 who were U.S.-born, gave birth to a live infant, and had complete data on all study variables. The 56 Black women excluded from this sample were compared on all study variables to the 178 who were retained. The only difference between the two groups was age; excluded women were significantly younger (M = 25.30, SD = 5.58) than women included in the sample (M = 27.25, SD = 5.24), $F(1, 228) = 5.296$, $p < .05$. Age was not associated with the significant stress predictors or birth outcomes, however.

Stress Variables

In assessing psychosocial stress, we included a variety of measures to operationalize stress exposure (e.g., life events), stress responses (e.g., anxiety), and chronic stress (e.g., perceived stress). Instruments were extensively pilot-tested on a similar population and had been used in prior studies.

Stressful life events. A 24-item stressful life events inventory, completed at Time 1 and Time 3, was adapted from measures used in Lobel (6) and Zambrana et al. (23) to measure the number of stressful life events (SLEs) that participants experienced 1 year prior to, and during the course of, the pregnancy. The scores from Time 1 and Time 3 were averaged into a summary life events count.

Events distress. For each SLE that occurred, participants were asked to rate how undesirable it was for them personally on a scale ranging from 1 (*not at all*) to 4 (*very much*). Undesirability ratings were averaged across life events and across time points to obtain a summary events distress score.

Intrusive thoughts. Each participant reviewed her list of SLEs and selected the two that were the most distressing. She then answered five questions adapted from the seven-item Intrusion subscale of the Impact of Events scale (37), which is a valid and reliable measure (38,39), about each of these two particular events to assess subjective distress manifested as intrusive thoughts. A summary score was calculated by averaging scores across the two events and then across the two time points. This subscale exhibited very high internal consistency at each time point, with Cronbach's alphas ranging from 0.90 to 0.92.

Perceived stress. An eight-item shortened version of the Perceived Stress Scale (PSS) (40) was used to assess feelings of chronic stress "during the past week" at each of the three prenatal time points using a 5-point scale from 1 (*never*) to 5 (*almost always*). This measure has been used in previous studies of stress and pregnancy and has been shown to be psychometrically sound (10,11,23,41). A summary score was calculated by averaging responses across time points. The scale exhibited good reliability at each time point, with Cronbach's alphas in the low 0.80s.

State anxiety. General feelings of anxiety "during the past few days" were assessed on a 4-point scale from 1 (*not at all*) to 4 (*very much*) at each of the three prenatal time points using the 10-item shortened version of the Spielberger State–Trait Anxiety Inventory (STAI) (42). This measure is psychometrically sound and has often been used in pregnancy research (5,9). Scores were averaged over time points. This scale demonstrated good internal consistency at each assessment, ranging from 0.84 to 0.90.

Pregnancy-specific anxiety. Pregnancy-specific anxiety was assessed at all three prenatal time points with a set of items developed by the researchers to assess various affective responses to the pregnancy. Participants were asked how often in the past week they had felt anxious, concerned, fearful, and panicky about the pregnancy, using a 5-point scale from 1 (*never*) to 5 (*always*). Internal consistency estimates ranged from 0.51 to 0.69.

Pregnancy undesirability. Four questions were created for this study to assess whether the pregnancy was planned, whether the respondent had ever considered abortion or adoption, how the respondent currently felt about the pregnancy, and whether she ever wished she were not pregnant. Responses were standardized and summed into an index of pregnancy undesirability with higher scores indicating less desirable pregnancies. Cronbach's alpha was 0.68.

Sociodemographic and Medical Variables

Demographic information included age, employment status, and cohabitation status. SES was approximated with measures of educational attainment and income. Educational attainment was classified as no degree, high school diploma, more than high school but no 4-year college degree, and 4-year college degree or more. Annual household income was assessed using a 10-point scale ranging from less than $2,500 per year to over $80,000 per year. It was adjusted for household size by dividing the income score by the number of people in the home, yielding a per capita income score.

Medical risk was the number of 32 possible risk conditions from past obstetrical history, past medical history, and this pregnancy that were present. The list of conditions was based on previous research (43,44) and the consensus of the medical experts on the team. A complete listing is available from the authors. Weight gain, parity, and substance use also served as control variables.

Birth Outcomes

Birth weight in grams and gestational age in weeks were the outcomes of interest. Because birth weight varies significantly

with gestational age (Pearson's r in this sample was .70, $p < .01$), it was regressed onto gestational age, and the residual scores were used to represent gestational age-adjusted birth weight, an indication of fetal growth. This procedure is in line with previous investigations (45,46).

RESULTS

Statistical Procedure

Data analysis included univariate, bivariate, and multivariable techniques. Frequencies and descriptive statistics were used to summarize the data. Correlational analyses were used to test bivariate relations and to decide which variables to enter into regression models. Hierarchical multiple regression was used to test for significant predictors of birth outcomes.

Descriptive Statistics

The mean age of the sample was 27.3 years (SD = 5.24) with a range of 18 to 42 years. A little over 40% of the sample was employed outside of the home either part or full time. Although only 33% of the sample was married to the baby's father, two thirds were living with him.

Regarding highest degree attained, 12% had no degree, 65% had a high school diploma, 14% had post-high-school training but no college degree, and 8% had a 4-year college degree or better. According to 2000 census figures, 22.8% of African American women age 15 and older had less than a high school education, 32.2% had a high school diploma, and 13.6% had at least a 4-year college degree (47). Thus, in comparison to African American women in the general population, this sample of African American women was more likely to have completed high school, probably because the youngest person in the sample was 18 and not 15, but less likely to have completed college.

Median annual household income was $20,001 to $30,000, ranging from under $2,500 to over $80,000 per year. When compared to 2000 census figures for African American median household earnings (48) and poverty levels (49), the African American women in this study earned less money and were more likely to be living below the poverty level than the general African American population.

With regard to medical and health-related factors, 30% of the sample was nulliparous. The average number of medical risk conditions was 2 (SD =1.07) with a range of 0 to 3. Only 13% of the sample had no medical risk conditions. The sample gained an average of 12½ kg (28 lb) (SD = 15.09), during pregnancy, ranging from a loss of 5 kg (11 lb) to a gain of 35½ kg (79 lb). Twelve percent reported they smoked cigarettes, 23% that they drank alcohol, and 18% that they used illicit drugs. The African American women in this sample were less likely to smoke, but more likely to drink or use illicit drugs during pregnancy, than has been noted elsewhere in a similar sample of African American women (50).

Participants reported an average of 6.66 (SD = 2.89) stressful life events (range = 1–16). This is twice the number of life events that other researchers have thought indicated a stressful pregnancy (21,51). These events were deemed to be somewhat stressful overall (M = 3.17, SD = .58) with participants reporting on average that they sometimes (vs. *never/rarely* or *often/always*) had intrusive thoughts (M = 2.91, SD = .86) about their most distressing life events. Interestingly, everyone in the sample reported exposure to at least one SLE, and each event listed in the life events inventory was selected by at least one person as "the most distressing" when completing the intrusive thoughts measure. Participants perceived relatively little chronic stress (PSS: M = 2.38, SD = .55), were somewhat anxious in general (STAI: M = 2.04, SD =.54), and somewhat anxious about the pregnancy itself (M = 2.87, SD = .84). Nearly 70% of the sample did not intend to get pregnant, but only 7% seriously considered abortion or adoption, only 2% did not want to have a baby now that they were pregnant, and only 5% often or almost always wished they were not pregnant.

Infants were born around 39 weeks gestation (SD = 1.81), with 12% being born prematurely (< 37 weeks), a rate much smaller than the national average of 17.6% for African Americans (52). The average birth weight was 3,254.88 g (SD = 607.31). The 10.6% low birth weight (< 2,500 g) rate for this sample of African American women is slightly lower than the 11.4% national average for African Americans (52).

Socioeconomic Differences in Study Variables

Because both adjusted income (r = .27, $p < .01$) and education, $F(3, 174) = 6.92$, $p < .01$, varied significantly with age, socioeconomic differences in all the study variables were explored, controlling for age, using nested chi-square, nested F tests, and partial correlations (see Table 1). More highly educated women were more likely to be employed, having a baby for the first time, and less likely to use substances during the pregnancy. Women with higher per capita incomes were more likely to work (r = .49, $p < .01$) and to be giving birth for the first time (r = –.42, $p < .01$). Neither educational attainment nor income was even marginally related to stress or birth outcomes, controlling for age, except that higher income women had significantly lower pregnancy undesirability scores (r = –.28, $p < .01$).

Predictive Models

We used zero-order Pearson product–moment correlations to reduce the number of variables used in the regression analyses so that parsimonious models could be tested. Control variables and stress variables at least marginally associated ($p < .10$) with birth outcomes were retained. Weight gain, substance use, parity, event distress, intrusive thoughts, and state anxiety were tested as predictors of gestational age-adjusted birth weight, whereas medical risk, weight gain, and number of life events were tested as predictors of gestational age. Employment status, cohabitation status, adjusted income, education, perceived stress, pregnancy anxiety, and pregnancy undesirability did not reach marginal significance in bivariate tests with either outcome so these variables were not entered in the regression models. The intercorrelations of all study variables are shown in Table 2.

Gestational age-adjusted birth weight. To determine whether stress predicted gestational age-adjusted birth weight,

TABLE 1
Socioeconomic Differences in Study Variables Controlling for Age

	Educational Attainment				
	No Degree[a]	*High School*[b]	*Less Than College*[c]	*College or More*[d]	*F or* χ^2
Control variables					
Employed[e]	4.5%	45.7%	52.0%	60.0%	18.06**
Cohabitating	45.5%	57.8%	60.0%	86.7%	5.82
Nulliparous[e]	9.1%	34.5%	28.0%	40.0%	9.55*
Substance user	36.4%	43.1%	16.0%	33.3%	7.83*
Income (adjusted)	0.81 (0.41)	1.78 (1.19)	1.88 (1.37)	3.02 (1.45)	0.18
Age[e,f]	24.08 (5.25)	26.83 (5.00)	29.15 (4.69)	31.54 (5.07)	8.58**[g]
Medical risk	1.95 (1.09)	1.90 (1.08)	1.96 (1.02)	1.47 (1.06)	–0.04
Stress variables					
Stressful life events	6.91 (3.24)	6.21 (2.87)	5.82 (2.47)	5.40 (2.92)	–0.02
Event distress	3.14 (0.48)	3.10 (0.58)	3.01 (0.42)	3.30 (0.39)	–0.02
Intrusive thoughts	2.88 (0.90)	2.92 (0.83)	2.86 (1.04)	2.95 (0.83)	0.00
Perceived stress	2.59 (0.49)	2.34 (0.53)	2.31 (0.68)	2.26 (0.38)	–0.03
State anxiety	2.12 (0.43)	2.03 (0.51)	1.93 (0.58)	1.88 (0.49)	–0.01
Pregnancy anxiety	2.75 (0.91)	2.91 (0.76)	2.84 (0.83)	2.45 (0.60)	–0.03
Pregnancy undesirability[e]	0.20 (2.91)	0.01 (2.87)	–0.44 (3.12)	–1.10 (2.06)	–0.01
Birth outcomes					
Birth weight	3,395.32 (437.33)	3,192.04 (667.71)	3,469.96 (467.77)	3,200.67 (484.43)	-0.05[h]
Gestational age	39.44 (1.49)	38.75 (1.96)	39.32 (1.32)	39.24 (1.53)	–0.0

Note. Values are percentages or means with standard deviations in parentheses.
[a]$n = 22$. [b]$n = 116$. [c]$n = 25$. [d]$n = 15$. [e]Significantly related to adjusted income. [f]Post hoc tests showed that those with a college degree or better differed significantly in age from those with no degree and those with a high school diploma. [g]*F* value based on a one-way analysis of variance rather than a nested *F* test because age served as the control variable in analyses for the other variables. [h]*F* value for adjusted birth weight is –0.03.
*$p < .05$. **$p < .01$.

controlling for potential confounders, parity, weight gain, substance use, and age were entered together in Step 1 of the regression. Parity, weight gain, and substance use were significant predictors of adjusted birth weight, with the step accounting for 9% of the variance. Events distress, intrusive thoughts, and state anxiety were entered together in Step 2, accounting for a significant amount of additional variance, with intrusive thoughts the significant predictor (see Table 3). To estimate the separate effects of each of the three stress variables entered in Step 2, separate models were run for each variable because of their high intercorrelations. Results of these analyses showed that both intrusive thoughts ($\beta = –.25$, $p < .01$; *F* change = 12.47, $p < .01$) and events distress ($\beta = –.19$, $p < .05$; *F* change = 6.52, $p < .05$) were significant predictors of adjusted birth weight after controlling for parity, weight gain, substance use, and age, whereas state anxiety was not ($\beta = –.10$, $p > .10$; *F* change = 1.69, $p > .10$). Thus, African American women who had previously given birth, who did not use substances during the pregnancy, who gained more weight, and who had fewer intrusive thoughts and less distress concerning life events, had bigger babies.

Gestational age. Based on bivariate analyses, stressful life events was the only stress variable entered into the regression model predicting gestational age. Medical risk, weight gain, and age were entered together in Step 1 of the regression and stressful life events in Step 2. Step 1 accounted for a 5% of variance, with weight gain the significant predictor. Stressful life events accounted for a significant amount of additional variance over and above Step 1 (see Table 4). Therefore, African American women who gained more weight during the pregnancy and experienced fewer stressful life events had longer gestational lengths, controlling for medical risk and age.

DISCUSSION

This study examined the relations among stress, SES, and birth outcomes in a sample of African American pregnant women. Although a majority of their pregnancies were unintended and they experienced a high number of stressful life events, these women reported experiencing relatively low amounts of subjective stress and anxiety. This discrepancy may be due to a variety of factors operating individually or in combination, such as a general tendency, which has been noted in African Americans, to deny stress by not disclosing it (53). It may also reflect the cultural expectation that a "strong, Black woman" skillfully shoulders life's myriad demands (54–56) or the availability of strong psychosocial resources, such as social support and mastery, which have been shown to have beneficial effects on the birth outcomes studied here (9,45,57).

We hypothesized that higher SES African American women would report less stress than lower SES African American women. In past research, SES indicators have been associated with stress in pregnant African American samples (17,24). However, there were no significant stress–SES relationships in

TABLE 2
Intercorrelations of Study Variables

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. Adjusted BW	—															
2. GA	.01	—														
3. Employed	–.09	.10	—													
4. Cohabitating	–.07	.01	.10	—												
5. Parity[a]	.17**	.01	–.31***	–.01	—											
6. Substance use[a]	–.21***	–.01	–.16**	–.06	–.03	—										
7. Adj. income	–.08	–.02	.49***	.13*	–.42***	–.12	—									
8. Age[b]	–.04	–.11	.12	.10	.11	.03	.27***	—								
9. Wt gain[a]	.22***	.23***	–.16**	–.18**	–.14	–.22***	–.02	.34***	—							
10. Medical risk[a]	–.10	–.14*	–.16**	.02	.20***	.13*	–.05	.14*	–.11	—						
11. SLEs[a]	–.04	–.23***	–.07	–.17**	.15*	.15**	–.03	–.06	–.00	.21***	—					
12. Distress[a]	–.19**	–.03	–.09	–.01	.16**	–.02	–.05	.17**	–.07	.16**	.32***	—				
13. Intr thts[a]	–.24***	.03	–.06	–.07	.14*	.10	–.08	–.04	–.06	.04	.35***	.32***	—			
14. PSS	–.07	–.09	–.10	–.03	.11	.00	–.11	–.10	.12	.18**	.32***	.27***	.19**	—		
15. STAI[a]	–.13*	–.09	–.09	.04	.09	.14*	–.12	–.16**	–.04	.09	.34***	.24***	.37***	.59***	—	
16. Preg anx	–.06	–.10	–.09	–.03	–.01	.12	–.09	–.18**	–.14*	.04	.18**	.09	.28***	.21***	.55***	—
17. Preg und	.07	–.06	–.06	–.16**	.24***	.10	–.28***	–.07	–.02	.09	.24***	.11	.21***	.29***	.28***	.14*

Note. The correlation coefficients reported above are zero-order Pearson correlations. Pearson correlations between continuous variables and dichotomous variables are the same as point-biserial correlations. The dichotomous variables in the correlation matrix are employed (0 = not working outside of the home part time/full time, 1 = working outside of the home part time/full time), cohabitating (0 = not cohabitating with the baby's father, 1 = cohabitating with the baby's father), parity (0 = nulliparous, 1 = multiparous) and substance use (0 = did not use cigarettes, alcohol, or illicit drugs during the pregnancy; 1 = did use cigarettes, alcohol, or illicit drugs during the pregnancy). BW = gestational age-adjusted birth weight; GA = gestational age; adj. income = income adjusted for household size; wt gain = total weight gained during the pregnancy; SLEs = stressful life events; intr thts = intrusive thoughts; PSS = Perceived Stress Scale score; STAI = State/Trait Anxiety Scale score; preg anx = pregnancy anxiety score; preg und = pregnancy undesirability score.

[a]Predictor entered in regression models. [b]Age was also included as a predictor in the regression models at a reviewer's request.

*$p < .10$. **$p < .05$. ***$p < .01$.

TABLE 3
Predictive Model of Gestational-Age-Adjusted Birth Weight

Step and Variables	β	*Adjusted R^2*	*F Change*
Step 1		.09	5.49**
Multiparous	.18*		
Weight gain	.20**		
Substance use	–.18*		
Age[a]	–.03		
Step 2		.15	4.96**
Event distress	–.12		
Intrusive thoughts	–.22**		
State anxiety	.01		

[a]Age, although not significantly correlated with outcomes at $p < .10$, was included in the model at a reviewer's request. Whether age is included in the model or not, the effects of the other variables on outcomes are comparable.
*$p < .05$. **$p < .01$.

TABLE 4
Predictive Model of Gestational Age

Step and Variables	β	*Adjusted R^2*	*F Change*
Step 1	.05	3.79*	
Medical risk	–.06		
Weight gain	.20*		
Age[a]	–.08		
Step 2		.08	7.98**
SLEs	–.21**		

Note. SLEs = stressful life events.
[a]Age, although not significantly correlated with outcomes at $p < .10$, was included in the model at a reviewer's request. Whether age is included in the model or not, the effects of the other variables on outcomes are comparable.
*$p < .05$. **$p < .01$.

this study, other than the finding that lower income African American women were less likely to desire this pregnancy. With regard to birth outcomes, we expected lower SES to be related to poorer outcomes but did not confirm this. Although significant relations between income and birth weight (17) and education and low birth weight and preterm delivery (34) have been found in pregnant African American women, null effects of both education (19) and income (35) on birth weight and on gestational age (17), similar to this study, have also been reported.

Because SES effects, or the lack thereof, have been noted in some past research, our results may reflect the fact that using traditional indicators of SES—current income, education, and sometimes occupation—may be too simplistic an approach for capturing a true picture of the SES of African Americans. Not until the mid-1960s and the passage of the Civil Rights Act was middle-class status an attainable goal for many African Americans (58); thus, African Americans are more likely to be newly arrived into rather than generationally established in the middle class. Higher SES, as traditionally conceived, may have been gained too late to confer much positive health benefit. Considering length of time in social position may be a better method for capturing SES effects on African American women's health (59). Because SES and social position were not main foci in the parent study, we did not have extensive measures on them. Recent developments in the study of these important constructs can be very useful in further investigating the relation between SES and birth outcomes in African American women.

We further expected that higher levels of stress would be associated with poorer birth outcomes. We found partial support for this hypothesis. Life event exposure and subsequent distress were associated in bivariate tests to outcomes, and predictive models showed that stress was able to account for a significant amount of additional variance in outcomes over and above control variables. Specifically with regard to gestational age, the more life events a woman experienced, the shorter the length of the pregnancy, independent of medical risk and weight gain. Life events have been linked to gestational age in other samples as well (7,60,61). In addition, the more life event distress and the more intrusive thoughts a woman had about her most stressful life events, the smaller her baby, independent of how much weight she gained, whether she had previously given birth, and whether she reported that she used substances during the pregnancy. This intrusive thoughts finding is new to the literature, to our knowledge, and was not due to confounding of intrusion with depression, a concern that has been expressed about such effects (62).

Intrusive thoughts are unintended thoughts, images, and even strong waves of feelings (37) outside of the conscious control of the person that may occur in response to mild, moderate, or traumatic stressors (63). Along with avoidant behaviors, intrusive thoughts are part of the rumination process whereby one attempts to work through a crisis, to manage it, and to extract some meaning from it (64,65) by dealing with the traumatic situation as one feels able (66,67). Although this study focused only on the intrusive thought component of rumination, it may be that the sample was high in avoidance as well. This possibility merits follow-up in future research, especially given the finding that despite a high number of life events, this sample reported relatively little emotional distress. It may be a useful window into the true experiences of stress in African Americans—a glimpse beyond what is often masked by norms and coping processes.

It appears that intrusive thoughts were more potent than life event occurrence and anxiety in predicting gestational age-adjusted birth weight. However, anxiety has been identified as a risk factor in Latina and White pregnancies (9–11). An intriguing possibility is that different stress indicators may be associated with different outcomes (i.e., preterm delivery vs. fetal growth). In addition, these results suggest that different ethnic groups may have different psychosocial risk factors, a matter deserving much greater attention.

Not only does the intrusive thought process tap into a unique and potent aspect of stress response, it may also provide insight into the biological mechanisms by which stress negatively impacts outcomes of pregnancy. Glynn, Christenfeld, and Gerin (68) showed that ruminating about a stressful experience, especially an emotionally charged one, can significantly slow

physiological recovery immediately following exposure to a stressor and reactivate the physiological response process even when the stressor is not present. In pregnancy, elevated levels of the stress hormones CRH, ACTH, and cortisol have been associated with preterm labor and subsequent early delivery (10,69,70) as well as restricted fetal growth (71–73).

The findings discussed herein should be viewed in light of the study's limitations. Because all of the women initiated prenatal care prior to 20 weeks gestation, this sample may not be completely representative of lower income, pregnant African American women in the general population who tend to initiate care later. Financial, cultural, and systems barriers can all contribute to the later initiation (74). The women in this sample were insured, however, and thus it was assumed that they were receiving regular prenatal care throughout the pregnancy. Early and consistent prenatal care could reduce stress and anxiety about the pregnancy and would likely contribute to better birth outcomes.

The lack of SES effects could have been due to limited range or variability on the SES variables or to small sample size or to both. Although the mode for level of education was a high school diploma with some achieving higher levels than this, there was better range on income. With low-, middle-, and upper middle-income earners all represented, 50% of the sample fell below the poverty level, and 32% earned incomes at or above African American median household earnings reported in the 2000 census (48). Regarding the size of the sample, SES effects have been present in smaller samples and absent in much larger samples. For example, Murrell's (17) study of 147 low-risk African American pregnant women reported that income was a significant predictor of birth weight, whereas Orr et al.'s (19) study of 1,861 predominantly African American urban pregnant women did not find an effect of education on birth weight. Thus, SES may not be as major a factor within the African American pregnant population as in other groups because of historical disadvantages that affect all African Americans. On the other hand, this issue is a complex one and deserves further in-depth examination in the future.

Racism may be one source of stress of particular salience to pregnant African American women (75) that was not investigated here. Myers (15) maintained that the health of minority groups is inextricably linked to the high stress states created by a social system plagued by racial discrimination. Perceived interpersonal discrimination (76,77) residential segregation (78,79), political disempowerment, and economic disenfranchisement (80) are all forms of racism that have been associated with African American birth outcomes. For a complete assessment of stress in African American women's lives it is critical that future research efforts incorporate measures of racism as well.

CONCLUSION

The results of this study add to a small but growing body of literature that has investigated the impact of psychosocial stress on pregnancy outcomes among African American women. These findings highlight the need to further investigate stress processes in this group and to better understand how they contribute to the poorer relative birth outcomes of African Americans. By elucidating the unique psychosocial experiences of pregnant women from diverse social groups, we may gain a better understanding of the etiological factors driving persistent ethnic disparities in reproductive health.

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REVIEW | Genetics inMedicine

Social and behavioral research in genomic sequencing: approaches from the Clinical Sequencing Exploratory Research Consortium Outcomes and Measures Working Group

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The routine use of genomic sequencing in clinical medicine has the potential to dramatically alter patient care and medical outcomes. To fully understand the psychosocial and behavioral impact of sequencing integration into clinical practice, it is imperative that we identify the factors that influence sequencing-related decision making and patient outcomes. In an effort to develop a collaborative and conceptually grounded approach to studying sequencing adoption, members of the National Human Genome Research Institute's Clinical Sequencing Exploratory Research Consortium formed the Outcomes and Measures Working Group. Here we highlight the priority areas of investigation and psychosocial and behavioral outcomes identified by the Working Group. We also review some of the anticipated challenges to measurement in social and behavioral research related to genomic sequencing; opportunities for instrument development; and the importance of qualitative, quantitative, and mixed-method approaches. This work represents the early, shared efforts of multiple research teams as we strive to understand individuals' experiences with genomic sequencing. The resulting body of knowledge will guide recommendations for the optimal use of sequencing in clinical practice.

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The dramatic reduction in the cost of genomic sequencing coupled with the improved accuracy of genomic technologies has set the stage for routine use of whole-exome and whole-genome sequencing in medical care. Although sequencing holds the potential to improve patient outcomes, models for the optimal delivery of genomic care are lacking. To systematically investigate the impact of sequencing integration on individuals and health systems, and to foster collaboration in research on the major ethical, legal, and social implications of sequencing technologies, the National Human Genome Research Institute (NHGRI) formed the Clinical Sequencing Exploratory Research (CSER) Consortium. A principal challenge faced by CSER investigators is the need to accurately measure the factors that influence sequencing-related decision making and outcomes. To that end, investigators formed the Outcomes and Measures Working Group to harmonize some of the patient-centered outcome measures that will be used in CSER projects, provide a forum for discussing development of novel measures, and facilitate cross-study, data-driven analyses. Through an iterative process, we have identified and shared knowledge about measures that are in the public domain and discussed the pros and cons of outcome measurement in a variety of areas. In some cases, we have reached consensus on the measures that might be appropriate for use across our diverse study settings and populations. Although our group has identified many high-priority domains for investigation (**Figure 1**), we do not endorse specific measures. In this article, we outline priority areas for ethical, legal, and social implications research that working group members have identified to date. Although some CSER sites are also investigating provider perspectives on sequencing, that work will not be the focus of this article. Rather, this

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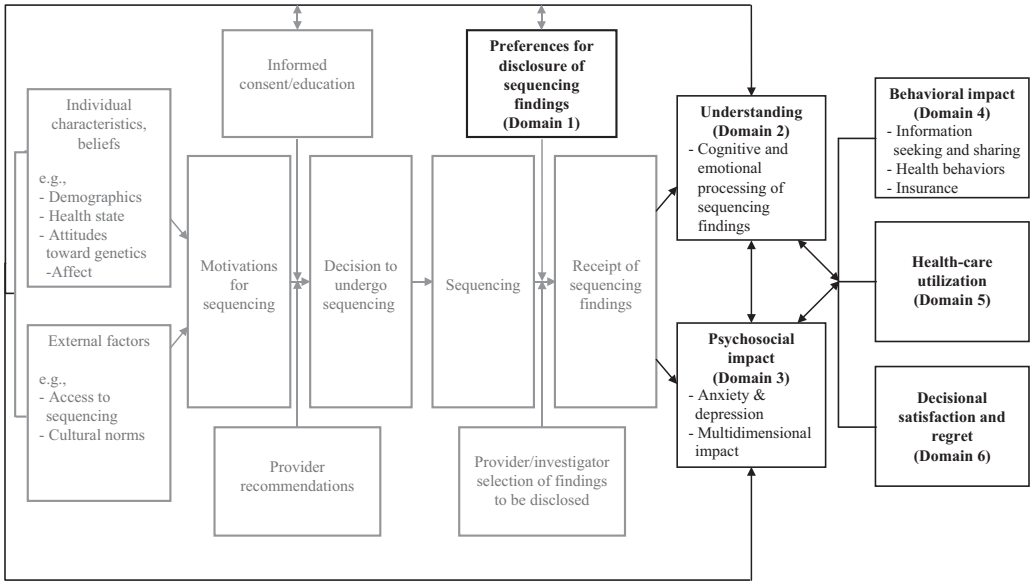

Figure 1 The process and potential outcomes of genomic sequencing. Dark boxes are outcomes that are featured in this article. Gray boxes represent steps in the clinical process.

research agenda serves as a first step in the development of a collaborative and conceptually grounded approach to studying participant outcomes in anticipation of future widespread sequencing adoption.

OVERVIEW OF THE CSER CONSORTIUM

The projects in the CSER Consortium investigate a diverse set of research questions in a variety of clinical and research contexts (**Supplementary Table S1** online). Briefly, the CSER Consortium includes projects funded through multiple mechanisms by the NHGRI, including U01 projects (RFA-HG-10–017), R01 projects (RFA-HG-11-003), R21 projects (RFA-HG-11-004), and investigator-initiated grants, as well as an NHGRI Intramural sequencing study. The U01 projects evaluate the integration of genomic sequencing in the clinical care of healthy individuals and adults with cardiomyopathy (Brigham and Women's MedSeq); in adults with metastatic lung and colon cancer (Dana-Farber Cancer Institute/Broad CanSeq); in adults who have clinical indications for testing for colorectal cancer/polyposis (University of Washington); in adults with cancer and cardiac disorders, children with dysmorphic findings, and adults and children with neurological disorders (University of North Carolina); in pediatric cancer patients (Baylor College of Medicine); and in children with heterogeneous disorders (Children's Hospital of Philadelphia). The investigators of the R01, R21, and investigator-initiated projects are studying issues related to the moral (The Children's Mercy Hospital) and legal duty to return sequencing research results (in pediatrics at Vanderbilt University and in newborn screening at Johns Hopkins University); the standards for sequencing-related informed consent (Columbia University); research participants' preferences for the return of research sequencing results (Seattle Children's Hospital, Columbia University, and Boston Children's Hospital); strategies for offering incidental findings to biobank research participants and deceased research participants' family members (University of California, San Francisco/Mayo Clinic/University of Minnesota); and attitudes and beliefs of patients and genetics professionals regarding the return of diagnostic genomic findings (Case Western Reserve University). The NHGRI Intramural program funds sequencing research on well-phenotyped adults (ClinSeq). Although the NHGRI has recently funded additional clinical sequencing U01 sites that are now part of the Consortium, investigators from those projects were not involved in the early work of the Outcomes and Measures Working Group that is detailed in this article.

DOMAINS OF INTEREST AND MEASUREMENT OF IMPACT

The Outcomes and Measures Working Group identified six major domains for coordination (**Table 1**). We considered a number of key factors when evaluating the domains for coordination, including whether the domains (i) are included in health decision-making or health behavior models that are being used at individual CSER sites, (ii) are being evaluated by at least two CSER projects, (iii) are the subject of prior genomics work, and (iv) were identified through clinical observation. For each area, we have identified compelling reasons for inclusion of the domain in Consortium studies, a brief overview of the published literature, a general description of how Consortium projects are addressing the domain, and challenges to domain measurement. Importantly, we recognize that measuring the impact of genomic sequencing is dependent on study design. Experimental designs—i.e., randomized controlled trials comparing patients who receive genomic sequencing with those who do not—may disentangle the effect of potential confounding factors. Observational

studies (i.e., non–randomized controlled trials) may provide more descriptive data about the impact of genomic sequencing on individuals. Both study designs have advantages and disadvantages and are represented across the various projects in the Consortium.

CONCEPTUAL DOMAINS

Preferences for information

There is much debate about what role patient preferences should play in the disclosure of genomic sequencing results. When asked hypothetically, most patients and research participants express interest in receiving all types of results, even those of uncertain significance.[1] Facio et al.[1] reported that 95% of participants in the NHGRI ClinSeq study wished to learn results from whole-genome sequencing and that intentions to learn results were higher for actionable findings and carrier status as compared with nonactionable findings and uncertain genomic results.[1] However, despite this general enthusiasm, the uptake of clinical genetic testing in the setting of single-gene studies in some cases is considerably lower,[2,3] suggesting that a subset of at-risk individuals hesitate to receive genetic information. Evidence from decision-making research demonstrates that intentions predict only a subset of health-related behaviors.[4] In addition, genetic testing uptake may be influenced by factors such as access to testing, insurance coverage, and provider knowledge.[2]

In July 2013, the American College of Medical Genetics and Genomics issued a recommendation that all laboratories conducting genomic sequencing seek out pathogenic or likely pathogenic variants in 56 genes associated with actionable conditions and that the results be disclosed to the ordering physician, irrespective of patient preferences.[5] These recommendations have generated substantial controversy. Although some Consortium projects do not rely on individual preferences to inform decisions about the return of results, others are specifically designed to assess and accommodate individual preferences.

A principal challenge in measuring preferences, whether hypothetically or in the context of actual decision making, is that there are few validated measures of preferences for the disclosure of genomic findings. Broader measures of preferences for shared decision making, such as the Shared Decision Making Questionnaire[6] and the Control Preference Scale,[7] provide insight into what roles participants would like to play in decisions about the communication of genetic test information. However, many projects are developing novel preference measures, posing questions about individuals' desires for information categorized according to key attributes (e.g., actionable versus nonactionable) and/or medical indications (e.g., pharmacogenomics, disease risk alleles). Qualitative methods can provide a nuanced understanding of individual preferences, by allowing investigators to probe participants on how they understand constructs such as actionability and uncertainty. Finally, many Consortium studies have decided that it is important to assess preferences for results both pre- and postdisclosure, in order to assess the stability of individual preferences over time and to determine the impact of disclosure on preference stability.

Table 1 Outcome measures coordinated across CSER sites by domain

Selected outcomes included in CSER projects[a]
Preferences
Preferred role of patient in medical decision making[7]
Novel preference measures under development by CSER sites
Elicitation of sequencing-related preferences through hypothetical vignettes
Elicitation of actual preferences for the disclosure of incidental information
Elicitation of preferences for the integration of sequencing results in medical records
Understanding
Knowledge about genetics and genome sequencing[16]
Novel understanding measures under development by CSER sites
Understanding of sequencing-related informed consent concepts
Comprehension of the implications of sequencing findings
Recall of sequencing results disclosed
Perceptions of uncertainty related to sequence information
Psychosocial impact
Anxiety and depression[28–30,72]
Multidimensional impact[34,35]
Behavioral impact
Information seeking & sharing[73,74]
Health information engagement and apprehension
Communication of sequencing findings to family members
Novel information seeking and sharing measures under development by CSER sites
Health behavior
Physical activity
Fruit and vegetable consumption
Smoking
Novel behavior measures under development by CSER sites (examples)
Changes in medical treatment
Vitamin, supplement, and medication use
Increased/decreased motivation to enact a variety of health-related behaviors
Health-care utilization
Hospital utilization and access to care[52]
Novel health-care utilization measures under development by CSER sites (examples)
Medical care visits (e.g., general practitioner, medical specialist, genetics provider)
Hospital-based care (e.g., acute care, planned care)
Medical tests/procedures (e.g., screening, diagnostic, surveillance)
Medication changes (e.g., prescription, over the counter)
Insurance (e.g., health, life, disability, long-term care)
Health behavior programs (e.g., smoking cessation)
Decisional satisfaction & regret
Decision regret[66]
Decisional conflict[75]
Novel decision satisfaction and regret measures under development by CSER sites
Satisfaction with communication of sequencing results
Satisfaction with sequencing results
Sequencing-related expectations satisfied

CSER, Clinical Sequencing Exploratory Research Consortium.

[a]Only measures that are utilized by two or more sites in the consortium are referenced. Citations do not provide a complete list of measures available for these domains, nor are they intended to be an endorsement of particular measures as the optimal measures in that domain.

Participant understanding: cognitive and emotional processing of sequencing findings

The Working Group devoted considerable attention to the challenges of measuring how individuals understand and process

information in the context of sequencing. "Understanding" in the setting of genomic sequencing can be conceptualized in many ways and assessed at multiple points in the testing process. Furthermore, there is a lack of consensus across CSER sites about how understanding should be defined and operationalized. Although assessment of baseline understanding (e.g., genetic knowledge), understanding after an informed consent session, and understanding of the limitations of sequencing technologies are essential—and are therefore key independent variables in a number of projects—we focus the present discussion on the understanding of disclosed sequencing results (e.g., health implications of testing). Accurately measuring how individuals process and understand disclosed sequencing information is imperative because it allows us to evaluate the adequacy of existing systems for the return of genomic findings.

Prior studies have assessed individuals' knowledge, risk perception, and information recall in the setting of genetic testing. Disclosure of genetic test results can lead to more accurate risk perception and increased knowledge.[8] Moreover, a study of multiplex genetic susceptibility testing concluded that patients commonly recall test results correctly and do not interpret test results in overly deterministic ways.[9] Yet disclosure may also be associated with misinterpretation and confusion. For example, individuals who receive "negative" results can underestimate their risk to pass a condition onto a child,[10] and individuals who are informed that they have variants of unknown significance or intermediate-risk alleles may have difficulty understanding and interpreting their results.[10–12] Individuals can also maintain pretesting risk perceptions, even when they understand the implications of genomic test results. For example, a study of *APOE* testing for Alzheimer disease risk showed that a subset of research participants accurately recalled their testing-based risk but still believed that their risk was either higher or lower than the risk provided by the study team.[13]

Measuring how individuals understand and process disclosed genomic sequencing information is challenging for several reasons. First, sequencing results vary in terms of actionability, predictive value, and potential impact.[14] Measurement of understanding and information processing may be complicated by the fact that individuals often receive multiple results simultaneously and that the implications of each result may differ. In addition, there are different ways to assess accurate understanding. For example, should the gold standard for understanding be concordance between the person who disclosed the findings and the person whose DNA was sequenced? Should we measure the degree of understanding by asking individuals to report the health implications of testing and compare their answers to relevant data in expertly curated genomic databases? Is it enough that individuals accurately recall the information that was given to them, or should they also appreciate the personal relevance of the information? What weight should be given to subjective understanding (i.e., how well the individual believes he/she understands the information)?[15]

Projects in the CSER Consortium are measuring understanding, information processing, and affective response in a variety of ways. There are a few validated knowledge measures that are being adapted by some CSER Consortium projects, including a knowledge of sequencing scale developed by Kaphingst[16] in conjunction with ClinSeq colleagues.[9] Some sites have adapted a previously published measure of subjective understanding developed by Joffe et al.[15] Given the fact that emotion plays an extremely important role in information processing and decision making,[17,18] a number of sites are also using measures that include an assessment of affective outcomes such as anticipated regret and tolerance of uncertainty. The ClinSeq group is developing a novel measure of uncertainty, specific to genomic sequencing, that also includes an assessment of affective outcomes. Given the lack of standardization and complexities in defining and measuring postdisclosure understanding, however, the majority of projects have developed novel scales and are using qualitative methods (e.g., discourse analysis of disclosure visits, postdisclosure patient interviews) to explore the different ways individuals process and make meaning of information. These methods may help lay the groundwork for future measure development.

Psychological responses to the return of results

Research consistently shows that individuals generally experience minimal adverse psychological sequelae after receipt of genetic risk information. Even among individuals receiving results indicating increased disease susceptibility, most studies have shown either no change or a decrease in negative emotions such as anxiety and depression as compared with prereturn levels. These patterns are seen both in testing for conditions with no preventive options, such as Huntington disease, and in testing for conditions with preventive options, such as melanoma.[19,20] Similarly, most individuals have benign emotional responses to single-nucleotide polymorphism analyses, providing genetic risk information about multiple conditions simultaneously.[21] A possible exception to this general principle may occur among individuals with elevated psychological distress at baseline (representing a potentially more vulnerable group) and among certain individuals affected by disease as compared with healthy individuals.[22–24]

Although findings from prior work in the setting of single-gene and single-nucleotide polymorphism testing are generally reassuring, the effects of returning genomic sequencing results, particularly in a clinical context, are unexplored. Responses to the return of sequence results may differ for several reasons. First, genomic sequencing can provide huge quantities of unexpected risk information about a vast array of diseases. It may also provide information that is qualitatively different from susceptibility information, such as findings related to pharmacogenomics or ancestry. In other contexts, the disclosure of unexpected information has produced anxiety in some populations.[25]

Second, uncertainty pervades genomic sequencing information. Sequencing has the potential to introduce uncertainty not only about the probability of illness through the disclosure of known risk variants but also about whether specific

variants are actually associated with disease risk at all (variants of uncertain significance).[26] Unlike previous sources of genetic risk information, sequencing further introduces the complexity of uncertainty in disentangling gene–gene interactions and gene–environment interactions that lead to disease risk. Helping patients to understand these sources of uncertainty will be critical to managing expectations about sequencing information. To this end, ClinSeq investigators have developed and validated a novel scale of perceptions of uncertainty related to genomic sequencing that includes three subscales: medical, affective, and trustworthiness. Convergent and divergent validity data using measures of resilience and ambiguity aversion support hypothesized relationships. Publication of the scale is underway. Prior research suggests that providers may interpret uncertainty differently than do patients.[27] Thus, effective communication will entail assessing perceptions of uncertainty to maximize understanding and minimize the negative impacts of uncertain information. **Supplementary Table S2** online details some of the specific questions related to psychological outcomes that the CSER Consortium is positioned to address.

Numerous well-validated scales that assess emotional states and traits, including depression and anxiety, have been used in research on genetic testing.[17] The majority of Consortium studies are using scales such as the 9-item Patient Health Questionnaire[28] to measure depression, the 7-item General Anxiety Disorder scale[29] to measure anxiety, or the 14-item Hospital and Depression Scale[30] to measure both. In addition, well-established scales to measure more encompassing constructs such as quality of life and happiness may be more appropriate for specific studies.

Identifying sensitive instruments that focus specifically on psychological responses to genetic information has been more challenging. Although a number of scales have been developed and tailored for *BRCA* testing,[31] Lynch syndrome testing,[32] and *APOE* genotyping for Alzheimer disease risk,[33] the Consortium has focused on two that can be adapted more easily to the disclosure of sequencing results. The first is the Psychological Adaptation to Genetic Information Scale,[34] a 26-item instrument that assesses frequency of intrusive thoughts, ability to discuss results with others, self-worth, certainty about information, and perceived control over consequences of genetic information. The second is the Multidimensional Impact of Cancer Risk Assessment scale,[35] a 25-item instrument assessing distress, uncertainty, and positive responses to genetic test results for cancer risk. Although both scales enhance the ability to examine the impact of specific information and more nuanced outcomes, each has limitations. Disadvantages of the Psychological Adaptation to Genetic Information Scale are limited use to date and an initial focus on single-gene testing, whereas disadvantages of the Multidimensional Impact of Cancer Risk Assessment include limited use outside of testing for cancer risk, focus on single-gene testing, and questionable internal consistency on subscales in some studies.[36,37] Examining the performance of well-adapted versions of each of these scales in the CSER Consortium will provide valuable guidance for future research.

Adaptation of these scales also raises important conceptual questions. First, what impact are researchers trying to assess? Is it the impact of information disclosure generally or of disclosure of specific results? Second, in the latter case, how should psychological impact be measured when multiple results, and/or results that have implications for both the individual and his/her family, are returned? The Psychological Adaptation to Genetic Information Scale and Multidimensional Impact of Cancer Risk Assessment were developed for testing specific genes and thus provide results that can be more easily categorized as "mutation positive," "mutation negative," and "indeterminate." More complex results may require broader conceptualization of potential responses. Third, when is the appropriate time to measure psychological responses? Data from targeted testing performed on largely self-selected populations have generally shown the emotional impact of test results to be transient.[38–40] In situations such as when sequencing is being used to inform cancer care, individuals may not be able to appreciate the implications of sequencing results until treatments have been completed. Conversely, the emotional impact of learning about increased risk for future illness may intensify as individuals approach the typical age of onset or show initial signs of disease.[41] Investigators will need to address these issues as they consider how to best adapt and administer questionnaires for their specific research questions, populations, and settings.

Finally, questions remain about whether mild "negative" psychological responses might be beneficial in some situations. Negative emotions can be powerful motivators for action when avenues exist to reduce risks, and efforts to minimize anxiety and distress may be counterproductive in situations in which patients would optimally engage with the threatening information and work toward reducing their risks.[42]

Behavioral impact

The CSER Consortium projects are also investigating the influence of genomic sequencing on various health behaviors. For instance, some projects are studying whether learning about previously unknown health risks through sequencing will motivate (or demotivate)[43] adults to reduce those risks through health-promoting lifestyle changes (e.g., diet, smoking cessation). The Consortium is also investigating effects on plans or intentions regarding future behaviors such as those involving childbearing or insurance purchases. In addition, there is a broad interest in information seeking (e.g., individuals' attempts to get additional information about sequencing and results) and sharing (e.g., their disclosure of results to other individuals). Understanding the effects of sequencing on behavioral outcomes is important because of their potential to guide clinical adoption of sequencing and to inform policies affecting public health and health services.

Most of the existing research has investigated these kinds of outcomes after targeted genetic testing. Many studies have

focused on changes in smoking behaviors following return of results showing increased genetic risk for pulmonary or other diseases.[44–46] Although some positive effects have been found for smoking cessation[46] and quit attempts, null results are common. More broadly, a recent Cochrane review of research on behavioral effects of providing genetic risk information identified effects on self-reported diet and intentions to change behavior, but little or no effects on actual behavior change for smoking and physical activity.[47,48] Notably, the included studies were generally of poor quality and underpowered to detect what are likely to be small effects. In addition, potential negative behavioral effects of providing genetic risk information have not been rigorously studied. It is therefore premature to draw conclusions about positive or negative effects of genetic risk information—especially information from genomic sequencing—on behavioral outcomes.

Motivated by the dearth of research and the expansive scope of genomic sequencing information, several Consortium projects are using qualitative and mixed methods to develop a rich understanding of participants' behavioral responses to sequencing. These approaches are well suited to discovering patterns of behaviors and their psychosocial correlates. In addition, Consortium projects are using quantitative research methods to study behavioral outcomes. Several institutions are using brief validated measures (e.g., the Rapid Assessment of Physical Activity)[49] as well as questions from large national studies such as the National Cancer Institute's Health Information National Trends Survey[50] or Cancer Care Outcomes Research and Surveillance Consortium[51] or the Centers for Disease Control and Prevention's National Health and Nutrition Examination Survey[52] and Behavioral Risk Factor Surveillance System.[53] Because suitable measures did not exist for some outcomes of interest (e.g., medication/supplement use, information sharing), many Consortium projects have developed novel measures.

Future research would benefit from guidance from theoretical models of health decision making or health behavior change.[54] For instance, theory will be useful in determining the circumstances under which sequencing may initiate a "teachable moment" capable of prompting behavior change.[55] The Information–Motivation–Behavioral Skills model states that enacting certain complex behaviors requires a combination of learning information about them, motivation to enact them, and the behavioral skills necessary for doing so.[56] Thus, although genomic results may be capable of motivating behavior change (e.g., as indicated by changes in intentions), that change may not occur if other crucial factors are missing (e.g., skills or resources required for behavior change). Research currently under way in the Consortium to assess barriers and facilitators of decision-making and behavior change after testing could provide additional information about the potential for sequencing to shape individuals' health behaviors.

Health-care resource utilization

A major question about the clinical implementation of genomic sequencing is its economic impact on patients and on the health-care system. Multiple issues arise when sequencing data are integrated into clinical care. First, the expected costs of data generation, interpretation, and reporting are substantial. Second, many institutions will need to develop or expand their infrastructures to deliver genomic-based care and to provide adequate follow-up for a range of incidental findings. Needed resources will be highly dependent on the clinical indication for testing and on policies and processes for return of incidental findings. Economic resources that should be considered include (i) institutional genomic review committees, if utilized; (ii) education and genetic counseling for patients; (iii) education for health-care providers; (iv) evaluation of patient preferences for incidental findings; and (v) the time required to return and discuss results with patients.

A more controversial issue is the downstream impact of genomic sequencing results on utilization of health-care resources. Some have argued that incidental findings, false-positive results, and results that are ambiguous could result in expensive and possibly unnecessary tests and consultations, and also lead to increased anxiety and other possible harms.[57]

Projects in the CSER Consortium are studying the economic impact of genomic sequencing in a variety of ways, primarily through measurement of health-care resource utilization (HRU). A number of the short- and intermediate-term HRU behaviors being measured in the CSER studies are shown in **Table 1**. These data can be collected using a variety of methods, including evaluation of electronic medical records, reimbursement claims, patient surveys, and patient diaries. Each method has strengths and weaknesses. If patients are all enrolled in the same health-care system with electronic medical records or claims data, evaluation will be more straightforward.[58] One challenge—as with all economic evaluations—is balancing the resources and burden of collecting these data with the value they provide. Another consideration is how to evaluate the longer-term HRU impact of changes in health status resulting from interventions initiated by findings from genomic sequencing. Typically, decision analysis models are used to estimate such broad and longer-term clinical and economic effects.

Four challenges specific to assessing whether genomic sequencing is a justified use of health-care resource utilization are (i) assessing the HRU of family members who may elect to pursue genomic testing based on the index patient's findings; (ii) assessing the tremendous diversity of potential health impacts and associated HRU changes resulting from return of incidental findings; (iii) incorporating inherent value that patients place on receiving findings, independent of their clinical relevance (personal utility); and (iv) identifying appropriate comparator groups in order to access the effectiveness of changes in care. At a minimum, investigators should consider collecting information about whether patients have informed family members of their results. Further efforts to identify uptake of genomic testing or other medical care services initiated by sharing of results may be warranted but will be resource intensive. Given the variety of incidental findings that could be returned, it will not be possible to model all potential impacts

of return of incidental findings. Approaches to assess the depth and breadth of this issue will need to be developed based on experience with ongoing research studies. Last, although the value that patients place on personal utility is not routinely considered in the development of clinical or reimbursement guidelines, personal utility is a patient-centered outcome that may drive much of genomic sequencing uptake and utilization.[59] Approaches to measure personal utility, understand its importance, and consider it in policy development are needed. As a preliminary step in exploring personal utility, at least one project will conduct a discrete-choice experiment that can provide estimates of the value patients place on different types of incidental findings.[60]

Decision satisfaction and regret

Another area of inquiry within the CSER Consortium includes an exploration of how individuals arrive at, and reflect upon, their decisions related to the receipt of genome sequencing results. Elements of the decision making process include, but are not limited to, understanding, engagement, risk perception, worries, uncertainty perceptions, response efficacy, and attitudes toward learning results. Various scales have been used for several decades to assess both the process and outcomes of medical decision making,[61] and several outcome scales have been used to assess the quality of decision making specifically in the context of genetic testing. To date, much of the research in genetic testing has focused on outcomes of decisions to undergo prenatal testing and testing for cancer risk.[62–65]

In most of the CSER Consortium projects, when decision making is studied, the focus is on decision outcomes, including informed choice, decision satisfaction and/or regret, and satisfaction with communication. Often, eligible individuals are asked to make decisions not only about having genomic testing performed but also about the types of incidental findings they wish to receive. Such decisions could result in learning useful, distressing, or unwanted information that has implications not only for the person tested but also for family members. Decision-quality assessments are key to understanding the overall value of sequencing results in both clinical and research contexts. A number of the projects in the CSER Consortium are using the Decision Regret Scale to evaluate postdecision outcomes.[66] It includes five items to rate the level of remorse or distress over a treatment-related decision. Decision regret has been shown to be negatively associated with satisfaction with the decision-making process and positively associated with poorer health outcomes and decisional conflict.[66]

Projects in the Consortium are also measuring satisfaction with physician–patient communication and with genetic counseling.[67] Although some projects have developed novel communication satisfaction measures, others are adapting items (e.g., from the Health Information National Trends Survey,[50] the Roter Interaction Analysis System)[68] or are using validated instruments such as the general communication subscale of the Quality of End-of-Life Communication measure.[69] Due to the diversity of communication systems and genomic decision support in the CSER projects, some projects also aim to assess individuals' satisfaction with the usability of computer systems employed in the course of genomic sequencing.[70,71] In addition, a number of sites are creating novel measures to evaluate individuals' satisfaction with testing and items that aim to capture whether genomic sequencing meets their expectations.

Assessments of decision quality are likely to be related to other process and outcome variables that are being measured by most of the CSER projects. For example, several projects are assessing the process and/or content of informed consent or return of results, as well as psychosocial outcomes of genomic sequencing, such as quality of life, anxiety, multidimensional impact, and coping. The quality of the decision is likely to be predictive of these types of outcome measures as well.

DISCUSSION

In addition to identifying core domains for coordination, our Working Group has discussed a number of methodological and conceptual issues that are likely to transcend individual projects. As noted previously, because the landscape of genomic sequencing is underexplored, complementary qualitative and quantitative research methods may be needed to capture the breadth and depth of people's experiences with sequencing. Such mixed-methods approaches are likely to yield the most comprehensive understanding of impact. Second, there may be significant differences, both in terms of impact and in terms of the assessment of impact, when sequencing is performed in a clinical versus a research context. For example, obligations related to the return of incidental findings or the requirements for informed consent might differ depending on whether sequencing is performed for clinical or research purposes. Third, practical, normative, and ethical factors may influence decisions about whether or not to elicit individuals' preferences for genomic test result disclosure, and decisions about preference elicitation are likely to influence impact. Finally, when assessing the impact of genomic sequencing on individuals, it is important to note that there are likely to be interactions between individual, health-care system, and social context characteristics, and outcomes. Accurate measurement of important covariates will be essential if we are to understand the ways in which the impact of sequencing varies across different populations and settings.

Although the CSER Consortium Outcomes and Measures working group has identified a number of domains as high priority for investigation, we readily acknowledge that rapid advances in genome science create a dynamic environment in which the implications of testing will evolve. Behavioral and social scientists, legal and ethics scholars, clinical investigators, and clinicians will need to coevolve with genome scientists in order to ensure that their work continually addresses core genomic questions. Continued efforts to coordinate sequencing-related research activities across sites and disciplines will aid in our understanding of individuals' experiences with genomic sequencing and help to establish best practices for sequencing programs of the future.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/gim

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DISCLOSURE

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Distress among women receiving uninformative *BRCA1/2* results: 12-month outcomes

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Abstract

***Objective*: Few data are available regarding the long-term psychological impact of uninformative *BRCA1/2* test results. This study examines change in distress from pretesting to 12-months post-disclosure, with medical, family history, and psychological variables, such as pretesting perceived risk of carrying a deleterious mutation prior to testing and primary and secondary appraisals, as predictors.**

***Methods*: Two hundred and nine women with uninformative *BRCA1/2* test results completed questionnaires at pretesting and 1-, 6-, and 12-month post-disclosure, including measures of anxiety and depression, cancer-specific and genetic testing distress. We used a mixed models approach to predict change in post-disclosure distress.**

***Results*: Distress declined from pretesting to 1-month post-disclosure, but remained stable thereafter. Primary appraisals predicted all types of distress at 1-month post-disclosure. Primary and secondary appraisals predicted genetic testing distress at 1-month as well as change over time. Receiving a variant of uncertain clinical significance and entering testing with a high expectation for carrying a deleterious mutation predicted genetic testing distress that persisted through the year after testing.**

***Conclusions*: As a whole, women receiving uninformative *BRCA1/2* test results are a resilient group. For some women, distress experienced in the month after testing does not dissipate. Variables, such as heightened pretesting perceived risk and cognitive appraisals, predict greater likelihood for sustained distress in this group and could be amenable to intervention.**

Keywords: cancer; oncology; BRCA1/2; uninformative; distress

Introduction

BRCA1/*BRCA2* (*BRCA1/2*) gene testing increasingly has become a part of routine clinical care for high-risk women. Mutations in *BRCA1/2* confer a 40–66% lifetime risk of developing breast cancer, with up to a 52% lifetime risk of developing a new, contralateral breast cancer, and a 13–46% risk of ovarian cancer [1,2]. *BRCA1/2* testing typically begins with a breast and/or ovarian cancer-affected individual (proband). Testing then is offered to family members if a risk-conferring mutation is detected. However, uninformative test results, in which a deleterious mutation is neither identified nor definitively ruled out, are possible and indeed, quite common [3,4]. Three distinct reasons for an uninformative result include (1) not detecting a mutation in an affected individual from a high-risk family after fully sequencing the *BRCA1/2* genes (*BRCA1/2* negative); (2) not detecting a mutation in an affected high-risk individual of Ashkenazi Jewish descent after targeted testing for three common founder mutations responsible for the majority of cases in this population (Ashkenazi Panel negative) [5–9]; and (3) detecting a genetic variant of uncertain clinical significance (VUCS).

Research examining distress among women seeking *BRCA1/2* testing has focused on three primary outcomes: situation-specific distress, including cancer-related distress [10] and less commonly, genetic testing-related distress [11], as well as global anxiety and depressive symptoms [12]. Distress generally is heightened pretesting. This dissipates somewhat after receipt of uninformative *BRCA1/2* test results, reaching levels that are higher than those of women receiving true

negative test results and comparable to or slightly lower than those of mutation carriers [3,13–16]. Although the impact of modest ongoing distress in this population is unclear, moderately heightened distress predicts subsequent rates of contralateral prophylactic mastectomy in mutation carriers [17] and 'over-adherence' to BSE and CBE among women with strong family histories of breast cancer [18].

In this report, we use Baum *et al.*'s [19] adaptation of the Transactional Model of Stress and Coping [20] as a guiding framework to examine predictors of distress. Briefly, the Transactional Model states that when people experience a stressor, they evaluate the relevance of the situation (primary appraisal) and their coping resources (secondary appraisal), implement coping strategies, and experience an emotional outcome. Baum and colleagues propose that these appraisals and overall adjustment to genetic testing is determined by (1) test result and related uncertainty, (2) personal and family history of disease, (3) risk reduction and disease management options, and (4) individual differences.

Test result and uncertainty

As noted, three types of uninformative *BRCA1/2* test results include *BRCA1/2* negative, Ashkenazi Panel negative, and a VUCS. The latter result indicates that a sequence alteration was detected, but whether this variation is deleterious or a benign polymorphism is unknown, making the associated risk highly variable and difficult to assess [21]. The increased uncertainty associated with receiving a VUCS may result in heightened distress, though previous studies utilizing very small samples have found no effect [3,22].

Personal and family history of disease

Residual risk estimates for women receiving uninformative *BRCA1/2* test results are highly variable and dependent primarily upon personal and family cancer history. Cancer-affected women report higher levels of cancer-related distress than unaffected women, as do those with higher pedigree-based risk [14] and those diagnosed more recently [15]. These clinical features also predict how likely women with uninformative *BRCA1/2* test results believe they are to carry a deleterious mutation [3], potentially impacting long-term outcomes. Research has not examined whether other risk-conferring variables impact distress [23].

Risk reduction and disease management options

Women at high risk for breast and/or ovarian cancer can manage their cancer risk through risk-reducing surgery [24] and enhanced screening [25]. While specific screening guidelines exist for mutation carriers, no established guidelines exist for women who receive uninformative *BRCA1/2* test results [26]. In addition to uncertainty regarding future risk-management decisions, the impact of having had prophylactic mastectomy prior to receiving uninformative *BRCA1/2* test results is unclear [14], potentially resulting in regret or relief from having to make further risk-management decisions [27].

Individual difference factors

Individual differences, such as sociodemographics, personality, and cognitive appraisals may predict distress. According to our guiding conceptual model, appraisals regarding the stressfulness of genetic testing (primary appraisals) and perceived coping ability (secondary appraisals) should predict adjustment. Carriers report stronger primary appraisals than other groups post-testing, as do those with higher trait anxiety [28], but we do not know whether appraisals predict emotional outcomes. Further, younger women, those who retain heightened post-testing perceived breast cancer risk, and those reporting discomfort when confronting uncertain information report greater post-testing distress [15].

Using a mixed model approach, we examined medical and psychological predictors of post-disclosure distress during the year after receiving uninformative *BRCA1/2* test results. We predicted that distress would be highest at pretesting, decrease considerably in the month post-disclosure, decreasing slightly thereafter [13–15]. Predictors of heightened and sustained distress would include (1) variables suggestive of higher residual cancer risk (family history of ovarian cancer, 2+ family members with breast cancer, no previous risk-reducing surgery); (2) variables associated with heightened uncertainty (not having made a cancer risk-management decision, receiving a VUCS result, higher perceived likelihood of carrying a mutation at pretesting); (3) demographics previously associated with heightened distress (younger age); and (4) psychological variables (stronger primary and weaker secondary appraisals, perceived risk of carrying a deleterious mutation).

Methods

Participants

Participants were 214 female probands who received uninformative *BRCA1/2* test results through the clinical research genetic counseling programs at one of three sites [Lombardi Comprehensive Cancer Center (Washington, DC), Ruttenberg Cancer Center (New York, NY), or

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Englewood Hospital (NJ)] from April 2001 to June 2003. Eligible probands had a personal history of either breast or ovarian cancer and a family cancer history resulting in approximately $\geqslant 10\%$ prior probability of having a *BRCA1/2* mutation. Five women with uninformative *BRCA1/2* test results were excluded due to missing data (final $N = 209$); 89% completed four assessments, 8% completed three assessments, and 3% completed two assessments. A strength of multilevel modeling is its ability to handle unbalanced data of this type [29].

Procedure

Measures were completed at pretesting and 1-, 6-, and 12-month post-disclosure. As a part of a larger intervention trial designed to encourage informed decision making among mutation carriers, trained research assistants determined eligibility. Eligible participants completed a structured interview and were offered an appointment with a genetic counselor.

All participants received standard genetic counseling (details described elsewhere [13]). Each participant received her test result at a genetic counseling disclosure session during which the counselor discussed test result implications and cancer risk-management options. The genetic counselor provided a qualitative estimate of residual risk for breast/ovarian cancer that was based on test results and the individual's personal/family history of breast and ovarian cancer, confirmed via medical records whenever possible. Given this was a high-risk population, surveillance recommendations were consistent with recommendations for high-risk individuals [26]. A summary letter outlined all guidelines and recommendations. Participants could discontinue their participation at any time.

Measures: predictor variables

Sociodemographics

Participants provided demographic information at pretesting including age, race, education, marital and employment status, and income. Men were not included due to our inclusion of risk-management decisions in our analyses.

Medical

Participants provided self-report information regarding personal and family cancer history. Participants also were asked, 'Have you made a final decision about how to manage your breast cancer risk?' Although some women may perceive themselves to have made a final decision, they may face future risk-management decisions. Consequently, decision status is a psychological indicator, not an objective behavioral endpoint.

Perceived likelihood of carrying a deleterious mutation

Participants rated their pretesting perceived risk for carrying a deleterious mutation on a 4-point scale (*not at all likely–very likely*) [30].

Appraisals

The 10-item genetic testing appraisal measure [28] assesses primary and secondary appraisals related to the receipt of genetic test results on a 4-point scale (*not at all–very*) at the 1-month assessment. Primary appraisals ($\alpha = 0.81$) assess the stressfulness of cancer risk, risk-reduction efforts, and family communication regarding genetic testing results. Secondary appraisals ($\alpha = 0.64$) assess confidence in dealing with these issues.

Genetic test result

Participants included probands for whom no mutation was detected after full sequencing of the *BRCA1* and *BRCA2* genes (*BRCA1/2 negative*), Ashkenazi Jewish women for whom mutation was not detected in targeted testing for the three Ashkenazi Jewish founder mutations (*Ashkenazi panel negative*) [5–7], and women receiving a VUCS result.

Measures: outcome variables

Anxiety and depression

We used 12 items of the Brief Symptom Inventory (BSI) [12] to assess anxiety and depressive symptoms at each timepoint ($\alpha = 0.89$–0.91). The original BSI uses a 5-point response scale; we used a modified 4-point scale (*not at all–extremely*), indicating the discomfort caused during the past 2 weeks. Scales were summed due to large correlations ($rs \geqslant 0.70$, $ps < 0.001$).

Cancer-specific distress

The 15-item Impact of Event Scale (IES) [10] was used to measure cancer-specific distress at each timepoint ($\alpha = 0.87$–0.90), indicating intrusive and avoidant thoughts/behaviors associated with a trauma/stressor (in this case, the experience of familial cancer). Items are scored on a 4-point scale (*not at all–often*), indicating how frequently each thought/behavior occurred during the past week.

Genetic testing distress

We used the Multidimensional Impact of Cancer Risk Assessment Questionnaire (MICRA) [11] to assess post-disclosure genetic testing distress. The MICRA contains 25 items on a 4-point scale (*not at all–often*) measuring specific responses to the receipt of genetic test results, including three factors (Distress, Uncertainty, Positive Experiences), combined as a total score. It has

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demonstrated adequate reliability previously [15,31,32]. However, in this study, the Positive Experiences factor did not converge with other factors at the 6-and 12-month timepoints, lowering reliability. Therefore, we excluded this factor in our analysis; remaining scale reliability was adequate (0.80–0.85).

Data analysis

We developed multilevel models using Hierarchical Linear Modeling for Windows (full maximum likelihood estimation) in order to analyze our hierarchically structured data (assessments nested within participants). Level 1 analyzes estimate each individual's unique initial status and rate of change for each outcome. Level 2 analyzes, modeled simultaneously, enable examination of between-person predictors of average initial status and rate of change (*fixed effects*) as well as variation around average initial status and rate of change (*random effects*). We examined post-disclosure trajectories of distress, with 'initial status' referring to the 1-month post-disclosure assessment (time was coded 0, 1, 2 corresponding to post-disclosure timepoints), controlling for scores at pretesting.

After examining descriptive statistics, including variable distributions, we tested multilevel models to investigate post-disclosure trajectories for each outcome. We specified an unconditional growth model (to examine the extent and direction of mean individual change over time) followed by a conditional growth model (in which predictors explain fixed and random variation [33]). Non-significant effects were dropped to yield parsimonious final models. All continuous predictors were grand mean centered to facilitate interpretation [29]. To protect against inflated Type I error, we used partial Bonferroni correction. Taking into account the three outcomes and mean intercorrelations = 0.50, the adjusted significance level was 0.029 [34]. Sample sizes are sufficient to find effect sizes of 0.04, 0.11, and 0.06 for anxiety and depression, cancer-specific and genetic testing distress, respectively, with a power of 99%.

Table 1. Descriptive statistics for predictors

Variable	*n*	*M*(SD)
Medical and family history		
Personal cancer history		
Breast	186	
Ovarian	15	
Breast/ovarian	8	
Family cancer history (first- and second-degree relatives)		
Breast ($\geqslant$2)	100	
Ovarian ($\geqslant$1)	37	
Genetic test result		
BRCA1/2 negative	89	
Ashkenazi panel negative	101	
VUCS	19	
Mastectomy history		
Bilateral treatment mastectomy	10	
Prophylactic mastectomy	27	
Oophorectomy history	40	
Made final risk-management decision	156	
Perceived likelihood of carrying mutation[a]		2.26(0.71)
Psychological		
Primary appraisals[a]		1.51(0.60)
Secondary appraisals[a]		3.64(0.46)

[a]Range = 1–4.

Table 2. Descriptive statistics for distress outcomes

Variable	Pretesting	Post-disclosure		
		1 month	6 month	12 month
Anxiety+depression[1]				
M	17.76_a	16.43_b	16.00_b	16.05_b
SD	6.08	5.90	5.42	5.24
N	207	209	194	190
Cancer-specific distress[2]				
M	18.63_a	13.09_b	12.26_b	11.80_b
SD	14.58	13.75	13.21	13.16
N	206	209	195	191
Genetic testing distress[3]				
M		8.30_a	6.13_b	5.14_b
SD		9.91	8.48	7.33
N		209	195	184

[1]Range = 12–48.
[2]Range = 0–75.
[3]Means are for two of three factors on the MICRA (range = 0–75). Means in the same row with different subscripts are significantly different ($p < 0.05$).

Results

Descriptive statistics

Descriptive statistics appear in Tables 1 and 2. Participants had been affected with only breast cancer (89%), only ovarian cancer (7%), or both (4%) and were the first member of their family to seek *BRCA1/2* testing. Average age at diagnosis was 46.3 (SD = 8.9, range 27–71). Diagnosis occurred, on average, just under 7 years before assessment ($M = 6.8$, SD = 7.9). Forty-three percent of participants received *BRCA1/2* negative results, 48% received Ashkenazi panel negative results, and 9% received VUCS results. Five percent had a bilateral mastectomy for treatment of breast cancer prior to enrolling in the study, 12% reported having a prophylactic mastectomy prior to enrolling, and another 1% reported having a prophylactic mastectomy between pretesting and 1-month post-disclosure. Nineteen percent had oophorectomy prior to enrollment, and one woman had the surgery between pretesting and 1-month post-disclosure. Having prophylactic surgery post-disclosure was not significantly associated with test result.

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Outcome measures were highly intercorrelated ($ps < 0.001$). Primary and secondary appraisals were negatively correlated ($r = -0.37$, $p < 0.001$): women who rated genetic testing as more stressful felt less confident as well. Item means indicated that participants had stronger primary and weaker secondary appraisals when considering how to deal with their cancer risk (1.80, 3.41, respectively) and their personal cancer risk-management decisions (1.64, 3.51, respectively) than for communicating with their family about their test result (1.30, 3.69, respectively) or dealing with the impact of the test result on their family (1.32, 3.68, respectively). Not surprisingly, women who had undergone a prior bilateral prophylactic mastectomy were most likely to report that they had reached a final breast cancer management decision ($p < 0.01$).

Anxiety and depression

Anxiety and depression declined significantly from pretesting to 1-month post-disclosure ($t(206) = 3.99$, $p < 0.001$), but not significantly thereafter (Table 2). This pattern described women who received *BRCA1/2* negative results and Ashkenazi panel negative results better than those who received VUCS results, whose anxiety and depression stayed stable pretesting through 6-month post-disclosure and then decreased from 6- to 12-month post-disclosure ($t(17) = 2.88$, $p = 0.01$). Women who received VUCS results had higher anxiety and depression at the 1- and 6-month post-disclosure assessments than other women ($ps = 0.01$–0.07).

The unconditional growth model revealed significant fixed, $\gamma = 16.36$, $t(208) = 41.50$, $p < 0.001$, and random, $\tau_{00} = 23.97$, $\chi^2(201) = 750.39$, $p < 0.001$, effects for initial status. Thus, anxiety and depression were significantly greater than zero at the 1-month post-disclosure assessment, and women varied significantly at 1 month. The fixed, $\gamma = -0.19$, $t(208) = -1.20$, $p = 0.23$, and random, $\tau_{11} = 0.24$, $\chi^2(201) = 204.49$, $p = 0.42$, effects for rate of change from 1- to 12-month post-disclosure were non-significant, indicating flat trajectories. Consequently, we tested rate of change as a fixed effect in the conditional growth model, allowing us to examine potential moderators of longitudinal change.

The final conditional growth model for anxiety and depression (Table 3) indicated that higher pretesting anxiety and depression predicted higher 1-month post-disclosure anxiety and depression $\gamma = 0.60$, $t(203) = 7.57$, $p < 0.001$, as did stronger primary appraisals, $\gamma = 1.65$, $t(203) = 3.53$, $p = 0.001$. The

Table 3. Final multilevel models predicting post-disclosure distress

Fixed effects	Anxiety and depression	Cancer-specific distress	Genetic testing distress	
	Coefficient(SE)	Coefficient(SE)	Coefficient(SE)	
Mean initial status (π_{0i})				
Intercept	6.85(1.38)***	13.05(0.63)***	12.25(1.79)***	
Pre-disclosure score	0.60(0.08)***	0.43(0.05)***		
White ethnicity	−1.08(1.06)*			
Age		−.21(0.06)***		
Married				
Treatment bilateral mastectomy				
Prophylactic mastectomy				
Uninformative *BRCA* negative (vs VUCS)			−4.65(1.88)*	
Ashkenazi panel negative (vs VUCS)			−4.46(1.85)*	
Perceived risk for positive result			1.25(0.51)*	
Primary appraisal	1.65(0.47)**	6.20(1.44)***	7.10(1.22)***	
Secondary appraisal			−6.40(1.17)***	
Mean rate of change (π_{1i})				
Intercept	0.52(0.57)	−0.47(0.40)	−1.60(0.27)***	
Pre-disclosure score × Time	−0.09(0.03)**			
Age × Time		0.10(0.04)*		
White ethnicity × Time	0.92(0.40)*			
Prophylactic mastectomy × Time				
Primary appraisal × Time			−1.71(0.68)*	
Secondary appraisal × Time			1.83(0.64)**	
Random effects	**Parameter**	**Variance**	**Variance**	**Variance**
Initial status	(τ_{00})	6.46***	39.35***	16.89***
Rate of change	(τ_{11})		6.03*	
Within person[a]	(σ^2)	10.12	51.08	29.93

[a]Test of significance undefined for this parameter.
*$p < 0.029$ (adjusted p-value); **$p < 0.01$; ***$p < 0.001$.

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effects of primary appraisals remained stable throughout the year following disclosure.

Pretesting anxiety and depression, $\gamma = -0.09$, $t(583) = -2.80$, $p = 0.01$, and ethnicity, $\gamma = 0.92$, $t(583) = 2.29$, $p = 0.02$, predicted post-disclosure rate of change. We probed these interactions by graphing trajectories of predicted scores. Women with lower pretesting anxiety and depression demonstrated an increase in these across time, though at each assessment, their scores were lower than the scores of women with higher pretesting anxiety and depression. Furthermore, although White women were slightly less distressed than non-White women at 1-month post-disclosure, their distress increased over the post-disclosure period until they were slightly more distressed than non-White participants by 12-month post-disclosure. The final model explained 67% of the between-person variance at 1-month post-disclosure.

Cancer-specific distress

Overall, cancer-specific distress declined significantly from pretesting to 1-month post-disclosure, $t(205) = 6.55$, $p < 0.001$, but did not change significantly thereafter (Table 2). This pattern described women who received all types of genetic test results, although this decline was only marginally significant for women who received VUCS results. The unconditional growth model revealed a significant fixed effect for initial status, $\gamma = 13.07$, $t(208) = 14.19$, $p < 0.001$; however, the fixed effect for rate of change was non-significant, $\gamma = -0.48$, $t(208) = -1.17$, $p = 0.24$. The random effects for both initial status, $\tau_{00} = 133.81$, $\chi^2(201) = 774.70$, $p < 0.001$, and rate of change, $\tau_{11} = 7.16$, $\chi^2(201) = 254.64$, $p = 0.006$, were significant.

The final conditional growth model (Table 3) revealed that higher pretesting cancer-specific distress predicted higher distress at 1-month post-disclosure, $\gamma = 0.43$, $t(205) = 9.89$, $p < 0.001$, while older age predicted lower cancer-specific distress at 1-month post-disclosure, $\gamma = -0.21$, $t(205) = -3.25$, $p = 0.002$. Stronger primary appraisals predicted higher distress at 1 month after testing, $\gamma = 6.20$, $t(205) = 5.86$, $p < 0.001$. The only variable to interact with time was age: younger women reported greater cancer-specific distress at 1-month post-disclosure, though their distress declined over time. By 12-month post-disclosure, there were no age differences. The final model explained 71% of the between-person variance at 1-month post-disclosure and 16% of the variance in between-person post-disclosure rate of change.

Genetic testing distress

Genetic testing distress was not measured at pretesting. Overall, mean post-disclosure genetic testing distress decreased from 1- to 6-month post-disclosure, $t(194) = 3.53$, $p = 0.001$; the decline between 6- and 12-month post-disclosure was not significant, $t(176) = 1.95$, $p = 0.053$ (Table 2). This pattern described women who received *BRCA1/2* negative results and Ashkenazi panel negative results (although the decline from 1- to 6-month post-disclosure was only marginally significant for the latter group), but not those who received VUCS results; genetic testing distress stayed stable from 1- to 6-month post-disclosure ($t(17) = 1.52$, $p = 0.15$) and then decreased somewhat from 6- to 12-month post-disclosure ($t(16) = 1.93$, $p = 0.07$). Women who received a VUCS had higher genetic testing distress than women in the other groups at all assessments ($ps \leqslant 0.001$–0.05).

The unconditional growth model revealed significant fixed effects for initial status, $\gamma = 8.11$, $t(208) = 12.45$, $p < 0.001$, and rate of change, $\gamma = -1.59$, $t(208) = -5.47$, $p < 0.001$. The random effect for initial status was significant, $\tau_{00} = 63.85$, $\chi^2(201) = 727.62$, $p < 0.001$, reflecting significant individual variation around the mean at 1-month post-disclosure. However, the random effect for rate of change was non-significant, $\tau_{11} = 2.26$, $\chi^2(201) = 231.83$, $p = 0.07$, suggesting no significant individual variation around the mean for rate of change over time. Rate of change was tested as a fixed effect in the conditional growth model.

The final conditional growth model revealed several significant effects (Table 3). First, women's genetic test results predicted genetic testing distress at 1-month post-disclosure: Women who received an uninformative *BRCA1/2* negative test result, $\gamma = -4.65$, $t(203) = -2.47$, $p = 0.01$, or an Ashkenazi panel negative test result, $\gamma = -4.46$, $t(203) = -2.41$, $p = 0.02$, reported significantly lower distress at 1-month post-disclosure than women receiving a VUCS, such that adjusted distress scores for those who received a VUCS result were approximately half a standard deviation higher than other women. Test results did not predict rate of change, indicating that these differences persisted throughout the year post-disclosure. Second, women who had higher pretesting perceived risk for carrying a deleterious mutation reported higher genetic testing distress at 1-month post-disclosure, $\gamma = 1.25$, $t(203) = 2.47$, $p = 0.02$; this effect did not vary over time. Third, women with stronger primary appraisal scores reported significantly higher genetic testing distress at 1-month post-disclosure, $\gamma = 7.10$, $t(203) = 5.84$, $p < 0.001$, and those with stronger secondary appraisal scores reported significantly lower genetic testing distress at 1-month post-disclosure, $\gamma = -6.40$, $t(203) = -5.46$, $p < 0.001$, each moderated by significant interactions with rate of change: Although women with stronger primary appraisal and weaker secondary appraisal scores at 1-month post-disclosure reported greater distress at that timepoint, their scores declined more steeply over

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time. However, at 12-month post-disclosure, their genetic testing distress remained higher than those with weaker primary appraisal and stronger secondary appraisal. Notably, having made a final cancer risk-management decision did not predict genetic testing distress, nor did any demographic, medical, or family history characteristics. The final model explained 61% of the between-person variance at 1-month post-disclosure.

Discussion

We examined the impact of receiving uninformative *BRCA1/2* test results on trajectories of psychological adjustment, as assessed by measures of cancer-related and genetic testing distress, as well as anxiety and depressive symptoms, in the year following testing. Generally, distress declined significantly from pretesting to 1-month post-disclosure, but remained stable thereafter. Rates of distress are comparable to those found in previous studies and, similar to these studies [13,14], our results suggest that as a group, our participants report modest distress. Likewise, post-testing decreases in distress appear to be clinically significant. For example, using IES criteria [10], 59 (28%) participants were in the medium category for symptomatology at pretesting, while 90 (43%) were in the high category. This decreased to 39 (19%) and 64 (31%), respectively, at 1 month and 40 (19%) and 50 (24%), respectively, at 12 months.

Our results also suggest that some women report elevated distress after testing that does not dissipate. Furthermore, certain testing-related variables predict greater likelihood for sustained genetic testing-related distress, underscoring the importance of identifying those who might be particularly vulnerable to ongoing, situation-specific distress and providing additional resources. Based on appraisal item means, participants reported greater difficulty dealing with their cancer risk and their personal cancer risk-management decisions than communicating with their family about their test result or dealing with the impact of the test result on their family, perhaps because uninformative results are shared less frequently with family members than mutation-positive results [35]. Decision support materials could be developed to address the risk-management strategies available to these women in order to facilitate the coping process. Most intervention research of this kind has focused on mutation carriers [35–37]. The fact that appraisals were most strongly predictive of genetic testing distress suggests that interventions should address issues specific to that form of distress as opposed to global distress.

A number of variables predicted distress in the month following disclosure. In particular, primary appraisals predicted all three types of distress at 1-month post-disclosure. Primary and secondary appraisals predicted genetic testing distress at 1 month as well as change over time. Appraisals have differentiated *BRCA1/2* carriers from those receiving other results [28], and our results suggest that they also predict adjustment among women with uninformative *BRCA1/2* test results. Although women who perceived greater stress surrounding genetic testing and who felt less confident about coping with genetic testing issues reported elevated genetic testing distress at 1 month after disclosure of their test results, their genetic testing distress also declined more steeply over time. These results might suggest that these women demonstrate improved adaptation and coping over time, though this must be examined further.

With the exception of age and primary appraisals, no variables predicted cancer-specific distress other than pretesting distress, as found previously [13,15,38,39]. This complements other studies that report limited post-testing differences in cancer-specific distress among affected women [13,31]. While this adds to questions about the sensitivity of measures of cancer-specific distress among affected women [31,40], it also suggests that providers need not be unduly concerned about offering cancer survivors *BRCA1/2* testing [38]. Varying trajectories emerged for anxiety and depression based on race, though our sample's ethnic homogeneity tempers this finding. In contrast, several variables predicted genetic testing distress, highlighting the importance of measuring relevant constructs. Women with VUCS results reported higher, and more sustained, genetic testing distress than women with other uninformative *BRCA1/2* test results immediately after testing. This finding conflicts with previous studies that found no such differences [22]. This may be due to the substantially larger sample size in our study, as well as measurement of genetic testing distress, which the previous study did not assess.

Pretesting perceived risk for carrying a deleterious mutation also predicted genetic testing distress; those who entered the study with higher, sustained perceived risk reported higher genetic testing distress. This result may suggest that, for various reasons, these women did not fully adjust their post-testing perceived risk [41]. Alternatively, these women may continue to interpret the uncertainty around their risk status in a more negative light; perhaps due to personal and family histories conferring greater risk, they may continue to believe that they carry an undetected mutation.

Several predictors did not reach significance, most notably, family cancer history, risk-management decision status, and most demographic and medical variables. Because women with a more extensive family history are counseled that they remain at elevated risk following an uninformative *BRCA1/2* test result, we expected to see higher

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levels of distress within this group, replicating previous studies [14]. This difference may be explained by our inclusion of only cancer-affected women and our high-risk sample; the previous report included affected and unaffected women. These factors may have affected other null findings.

This study has several limitations. First, all participants received free genetic services and likely differ from those receiving testing in true clinical settings. Likewise, not all women who receive testing in a clinical setting would have the strong family history or other criteria needed to meet our recruitment guidelines. These women may react differently to the receipt of an uninformative result, especially a VUCS result than our sample. A better understanding of the experience of this growing group of women is an important area of future study. Second, most participants were White, college educated, employed, and affluent. Although these demographics are similar to other studies of this type, they are not representative of more diverse groups who might begin to utilize these tests in coming years as they become more commonly integrated into clinical care [42] and used to inform treatment decisions [43]. It is increasingly important to seek representative samples [44,45]. Further, our sample consisted only of cancer-affected women who, on average, received their diagnosis several years prior. Unaffected women report lower levels of distress [14] and may have different predictors of distress, as might women who are newly diagnosed with cancer. Third, although our sample of women who received a VUCS result was larger than that of previous studies, these women still represent a small percentage of our sample. This, in combination with the varied interpretation of VUCS results, makes it difficult for us to learn more about this group and specifically, to further examine the patterns of distress. Fourth, our measure of perceived risk was measured by one item and only at pretesting, limiting our ability to further examine this potentially important construct. Finally, our measure of residual risk was relatively crude. Increasingly, patients who receive uninformative *BRCA1/2* test results are counseled regarding their residual risk using qualitative and quantitative approaches. It is possible that had we better characterized residual risk, we might have observed the predicted associations between residual risk and distress outcomes. Despite these limitations, this study contributes to the growing understanding of the impact of receiving uninformative *BRCA1/2* test results and highlights several clinical and theoretical issues requiring further exploration.

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Technologies for Genomic Medicine

The GMW, A Genetic Medical Workflow Engine

A RENCI TECHNICAL REPORT
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List of Technical Terms and Websites

1000 Genomes Project, www.1000genomes.org

AnnoBot (Annotation Bot), www.renci.org/TR-14-04

Apache™ ActiveMQ STOMP – JMS mapping (Simple/Streaming Text Orientated Messaging Protocol – Java Mapping Services), activemq.apache.org/stomp.html

ApacheTM SOAP MTOM (Simple Object Access Protocol Message Transmission Optimization Mechanism), cxf.apache.org/docs/mtom.html

Apache™ SVN (Subversion)® Repository, subversion.apache.org

CANVAS (CAroliNa Variant Annotation System), www.renci.org/TR-14-04

CASAVA (Consensus Assessment of Sequence and VAriation), www.illumina.com/software/genome_analyzer_software.ilmn

Chrome development tools, www.google.com/intl/en/chrome/browser

CLIA (Clinical Laboratory Improvements Amendments), www.fda.gov/medicaldevices/deviceregulationandguidance/ivdregulatoryassistance/ucm124105.htm

ClinVar (Clinical Variants Resource database), www.ncbi.nlm.nih.gov/clinvar

daemons, en.wikipedia.org/wiki/Daemon_%28computing%29

dbSNP (Single Nucleotide Polymorphism Database), www.ncbi.nlm.nih.gov/SNP

Eclipse IDE (Integrated Development Environment), www.eclipse.org

ELSI (Ethical, Legal, and Social Implications) Research Program, www.genome.gov/elsi

ESP (Exome Sequencing Project), evs.gs.washington.edu/EVS

Firefox FireBug 1.10.3, getfirebug.com

GMW (Genetic Medical Workflow) Engine

HGNC (HUGO Gene Nomenclature Committee), www.genenames.org

HGMD® (Human Gene Mutation Database), www.hgmd.cf.ac.uk/ac/index.php

iRODS (integrated Rule-Oriented Data System), www.irods.org/index.php/IRODS:Data_Grids,_Digital_Libraries,_Persistent_Archives,_and_Real-time_Data_Systems

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MaPSeq (Massively Parallel Sequencing) System, www.renci.org/TR-14-03

Microsoft IIS 7.0 (Internet Information Services), www.iis.net

Microsoft SQL Server 2008 R2, www.microsoft.com/en-us/sqlserver/product-info.aspx

Microsoft SQL Server Management Studio, www.microsoft.com/en-us/download/details.aspx?id=8961

MySQL (Structured Query Language), www.mysql.com

OSG (Open Science Grid), www.opensciencegrid.org

PHP 5.3 (Hypertext Preprocessor), www.php.net/manual/en/intro-whatis.php

PostgreSQL database, www.postgresql.org

PostgreSQL pgAdmin, www.pgadmin.org

pythonTM modules, www.python.org

REDCapTM (Research Electronic Data Capture) application, www.project-redcap.org

RefSeq (Reference Sequence Collection), www.ncbi.nlm.nih.gov/refseq

Sparx Enterprise Architect, www.sparxsystems.com

SQL Server, www.microsoft.com/en-us/sqlserver/default.aspx

TeraGrid, info.teragrid.org

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About RENCI

RENCI is an institute of the University of North Carolina at Chapel Hill that develops and deploys advanced technologies to enable research discoveries and practical innovations. The institute was launched in 2004 as a collaborative effort involving UNC Chapel Hill, Duke University, and North Carolina State University. For more information, see www.renci.org.

*Phillips Owen serves as technical lead on the GMW Engine; Kirk Wilhelmsen serves as Principle Investigator and Director of RENCI's Biomedical Research division, which is leading the development of the GMW Engine; all other team members are listed alphabetically.

Introduction

Genomic data are rapidly amassing as a result of recent advancements in next-generation genomic sequencing and other high-throughput "-omics" technologies (Mardis, 2008; Horvitz and Mitchell, 2010; Koboldt et al., 2010; Kahn, 2011). Yet, we are far from an era of routine genetic screening (Evans and Berg, 2014). In order to take full advantage of the wealth of genomic data available today, and thereby better serve patients, technological advances are required to enable the secure, cost-effective, efficient, and accurate processing of genome-wide data, from sample collection in the clinic to physician or researcher interpretation of results (Ahalt et al., 2014; the Global Alliance to Enable Responsible Sharing of Genomic and Clinical Data, 2013; Data and Informatics Working Group, National Institutes of Health BD2K Initiative, 2012).

Herein, we describe the Genetic Medical Workflow (GMW) Engine—an open source system that provides end-to-end capture, analysis, validation, and reporting of genome-wide data for use in research and routine clinical care.

The GMW Engine

The GMW Engine was developed initially to support a National Institutes of Health (NIH)–funded clinical research study, "North Carolina Clinical Genomic Evaluation by NextGen Exome Sequencing" (NCGENES; Foreman et al., 2013) at the University of North Carolina at Chapel Hill (UNC). NCGENES has both clinical and research arms and aims to explore the use of whole exome sequencing data in genomic medicine.

The initial development of the GMW Engine was prompted by an early recognition that in order to achieve the goals of NCGENES, a comprehensive solution was required for the management of numerous people, processes, samples, and information—a complex endeavor. Initially, RENCI evaluated existing open source or proprietary workflow management systems; however, none of the existing systems were deemed capable (without major modification) of managing all of the disparate groups and legacy data systems in place at UNC. A custom solution was needed to meet the following high-level criteria:

- Present a secure user interface (UI) to capture and display contextually relevant information to and from users representing greater than 20 unique study roles;
- Manage and orchestrate complex processes that span numerous UNC laboratories and research teams;
- Orchestrate initial, secondary, and tertiary data analysis pipelines on multiple UNC compute clusters;
- Automatically collect analysis results and situational awareness information from multiple and disparate UNC data systems; and
- Monitor and audit user and process performance, as well as overall system health.

All of these features were incorporated into the custom-built GMW Engine. The GMW Engine serves as a centralized workflow manager; it executes discrete, automated- or user-driven workflows, UIs, and tracking systems (Figure 1). Specifically, it activates and tracks workflows related to: patient/subject flow from the initial clinic visit to consultation regarding genomic findings to follow-up visits; genetic sample flow from collection to processing to sequencing; and data flow from analysis to annotation to reporting. The GMW Engine provides several services via this process: system integration; system management; quality control; auditing; signaling; and reporting.

To understand the GMW Engine and the operations of the different workflows, consider the Project Operations workflow. This is where operations specific to a research project take place, from the identification of potential subjects to enrollment and informed consent to collection of blood for the processing of genomic DNA. The Project Operations workflow also involves interactions between the clinician researcher (or ELSI researcher) and the patient/subject.

Each step of the Project Operations is securely tracked by the GMW Engine such that only authorized persons (e.g., the researcher, research nurse, information technology staff) can view the status of the project at any given time. Automated tracking also allows for auditing and signaling to ensure compliance with all privacy, security, and ELSI requirements. It should be noted that the Project Operations workflow is comprised of more than one workflow, each of which is orchestrated by the GMW Engine. For example, the Initial Subject Enrollment sub-workflow (described under Use Case #2) is just one of several sub-workflows that are managed under the Project Operations workflow.

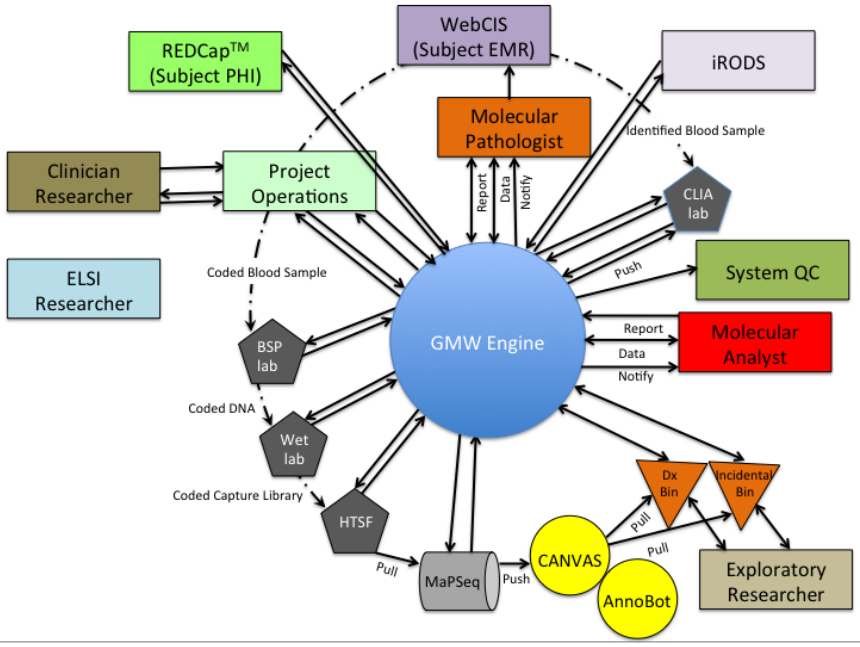

Figure 1. A schematic showing the workflows managed by the GMW Engine, with arrows depicting the flow of information. AnnoBot = Annotation Bot; BSP lab = BioSpecimen Processing laboratory; CANVAS = CAroliNa Variant Annotation Store; CLIA lab = a laboratory certified to meet U.S. Congressional Clinical Laboratory Improvements Amendments; Dx = diagnostic; ELSI Researcher = Ethical, Legal, and Social Implications Researcher; EMR = Electronic Medical Record; iRODS = integrated Rule-Oriented Data System; MaPSeq = Massively Parallel Sequencing system; PHI = Protected Health Information; QC = Quality Control; WebCIS = Web-based Clinical Information System; Wet lab = basic science laboratory.

Completion of the Project Operations workflow automatically leads, via the GMW Engine, to the processing of the coded blood sample by the BioSpecimen Processing (BSP) laboratory, where a new BSP Laboratory Information Management System (LIMS)–based workflow is initiated to track the initial processing of samples (i.e., DNA isolation). The BSP LIMS–based workflow is fully integrated with the GMW Engine, in order to keep the systems synchronized. Of note, all subject Protected Health Information (PHI) is securely stored using the open source REDCap™, which is also integrated with the GMW Engine. The PHI data are derived from the Web-based Clinical Information System (WebCIS), which is UNC Health Care's homegrown Electronic Medical Record (EMR) system. (We note that UNC Health Care is transitioning to the commercial Epic EMR system, so further modification of the GMW Engine is expected. The system is designed for flexibility, so modifications require minimal effort.)

After the BSP workflow has been successfully executed and coded DNA has been obtained, the samples are sent to a basic science laboratory in UNC's Genetic Medical Building, where a new workflow is initiated. There, samples undergo further processing (i.e., DNA amplification and other steps in preparation for sequencing). Completion of secondary sample processing leads to the execution of the High-Throughput Sequencing Facility (HTSF) workflow, where the raw genomic sequencing data are generated. Both the Genetic Medical Building and HTSF workflows are managed using the BSP LIMS, although all workflow steps are tracked by the GMW Engine for auditing purposes and to allow only authorized users to view the status of any given workflow.

Completion of the HTSF workflow leads to the execution of the MaPSeq system, which is designed to perform multiple levels of genomic data analysis on a massively parallel computational cluster (Reilly et al., 2014). Specifically, MaPSeq is an open source, plugin-based, service-oriented application developed by RENCI in collaboration with UNC's Information Technology Services, Research Computing Division, the UNC High-Throughput Sequencing Center, and Lineberger Comprehensive Cancer Center. MaPSeq provides a framework for facilitating the construction, deployment, and activation of project-specific, downstream, sequence analysis pipelines. The analysis pipelines invoke project-defined computation on the output from the raw HTSF data, such as genomic sequence alignment and variant calling. MaPSeq is designed to opportunistically take advantage of available institution-wide and cloud-based computational resources, including OSG, TeraGrid, and computational clusters available at RENCI and UNC's Department of Computer Science. MaPSeq was developed initially to support a genomics research project within the Lineberger Comprehensive Cancer Center at UNC, but it is now used to support numerous high-throughput sequencing projects at UNC, including NCGENES.

The MaPSeq workflow pushes data into CANVAS[1], which works together with AnnoBot as open source,

[1] CANVAS (CAroliNa Variant Annotation System) was originally termed VarDB (Variant DataBase)

homegrown technologies to enable the capture, storage, and updating of annotations to provide critical clinical interpretations of genomic data and metadata to attribute provenance or "ownership" and record the history of a given data set (e.g., type of sample, laboratory processing steps, analysis steps, validity and reliability estimates, etc.) (Bizon et al., 2014). CANVAS is a relational PostgreSQL database that stores up-to-date annotation and related metadata on genomic variants. As variant data from GMW Engine–supported research projects are pushed into CANVAS, they are matched against reference variant data from RefSeq and annotated accordingly. Additional annotation and associated metadata on variants are pulled into CANVAS by AnnoBot. AnnoBot is comprised of a set of python™ modules, as well as software driver code, designed to automatically monitor targeted databases for updates, extract new or revised annotation, and add that annotation to the variant data in CANVAS. The databases that are currently monitored by AnnoBot include dbSNP, the 1000 Genomes Project, ESP, HGNC, HGMD®, and ClinVar. CANVAS and AnnoBot together provide interpretations of genomic variant data that can be used to evaluate the diagnostic capability of identified genomic variants.

For NCGENES, CANVAS uses a Clinical Binning schema (ClinBin) to compute on the annotated variant data in order to determine which of two database Bins the identified patient/subject variants should get pushed into: the Diagnostic (Dx) Bin or the Incidental Bin. The Dx Bin includes variants that were targeted for a given patient/subject on the basis of a defined phenotype and have established clinical validity and utility (Shoenbill et al., 2014); in contrast, the Incidental Bin includes incidental findings[2], or variants that were identified during the sequencing effort but were not targeted as part of the diagnosis. (See Foreman et al., 2013 for a more detailed description of the binning process.) Note that only the targeted diagnostic findings are used for clinical care; incidental findings are used for research purposes only, unless they are classified as "medically actionable" under guidelines put forth by the American College of Medical Genetics and Genomics (Foreman et al., 2013; Green et al., 2013).

Table 1 shows the current number of genes/loci associated with the different diagnostic classes currently explored by NCGENES. Note that the data in both the Dx and Incidental Bins can be used for exploratory research (as opposed to the initial hypothesis-driven research), in which case the researcher re-analyzes the data post hoc to data-mine for unrecognized, potential associations between phenotype and genotype. Note also that the Incidental Bin is further subdivided on the basis of the degree of clinical validity and utility of

Table 1. Number of targeted genes associated with different diagnostic classes in the NCGENES study.

Number genes/loci	Diagnostic Class
31	Arrhythmia
15	Autoinflamation
82	Cancer
75	Cardiomyopathy
449	CNS
420	Dysmorphology
59	Immunodeficiency
521	Intellectual Disability and Autism
46	Leukodystrophy
69	Microcephaly
109	Mitochondrial
15	Myasthenia
99	Myopathy
315	Nueromuscular Disorders
80	Neuropathy
5	Polyposis
18	Progeria
214	Retina
46	Rhabdomyolysis
103	Seizure
162	Skeletal Dysplasia
45	Spastic Paraplegia
91	Storage Disorders
12	Thoracic Aneurysm/ Dissection

As required by the 1988 U.S. Congressional CLIA, patient (as opposed to research) samples are processed in a CLIA-certified laboratory to ensure analytical validity (Shoenbill et al., 2014) and to meet the quality standards put forth by the Centers for Medicare & Medicaid Services and the Food & Drug Administration. After processing in MaPSeq, variant data that are derived from a patient sample are reviewed by a Molecular Analyst, who determines which

[2]"Incidental findings" refer to genomic variants that are identified as a result of a genetic screening test but are unrelated to genes targeted by the testing. The ethical use of incidental findings is a topic of much debate (Evans and Berg, 2014).

of the identified mutation(s) is clinically significant. Those results get passed to a Molecular Pathologist, who performs a secondary sequence analysis of the genetic sample in order to ensure that the mutation(s) truly exists (i.e., to verify the genetic finding[s]). The Molecular Pathologist's final report is then sent to WebCIS for incorporation into the patient's EMR. Each step in these workflows is executed and tracked by the GMW Engine.

iRODS (Moore and Marciano, 2005; Rajasekar et al., 2010a,b; Schmitt et al. 2013) is used by the GMW Engine for secure data transfer and indexing among the disparate data analysis systems that are managed by the GMW Engine. iRODS is an open source, policy-based solution to access, share, integrate, publish, preserve, and manage data and associated metadata among remote data sources and diverse user communities. iRODS was developed by the Data Intensive Cyber Environments groups at UNC and the University of California at San Diego, with contributions from RENCI and other groups through the iRODS Consortium. iRODS was architected and designed to allow different adopter groups, with differing institutional goals and security concerns, to develop and deploy policies for data sharing that are specific to organizational needs. The GMW Engine relies on iRODS for secure, policy-based data transfer.

Finally, background daemons perform a continuous Quality Control (QC) check on the GMW Engine and the various systems and processes it relies on. The daemons use process connectors to query systems in order to track patients/subjects/samples/data and send error notification signals or alerts to Administrators and the staff member(s) who is responsible for the item of interest at that particular stage of processing. QC reports are also periodically generated for auditing.

Examples of GMW Engine Functionality

Although the GMW Engine was developed initially for NCGENES, it has since been modified and expanded for use in several additional research studies (see Impact section), and development continues as new user needs and tools become available. The workflows that are invoked by the GMW Engine are specific for each project and tailored to achieve the aims of that project. Each workflow depicted in Figure 1 is typically comprised of a comprehensive set of specific tasks organized in a decision tree or a linked subset of workflows organized in a similar manner.

We present two use cases for the GMW Engine: (1) the overall GMW Engine workflow processes and UIs engaged by NCGENES; and (2) the Initial Subject Enrollment and Genomic Sequencing workflows invoked by NCGENES.

Use Case #1: GMW Engine Workflow Processes and UIs for NCGENES

Figure 2 depicts the GMW Engine workflow processes that are engaged by NCGENES and specific to that project. (Not shown are the underlying REDCap™, iRODS, and System QC systems. Also not shown are the ELSI Researcher and Exploratory Researcher.) The process begins with step (1), when the Clinician Researcher activates the Project Operations workflow, which includes the Initial Subject Enrollment workflow discussed below. The numerical steps can then be traced to show the flow of patients, subjects, samples, and data. The final step, as outlined here, is step (20), in which the clinical report from the Molecular Pathologist is loaded into WebCIS for incorporation into the patient's EMR.

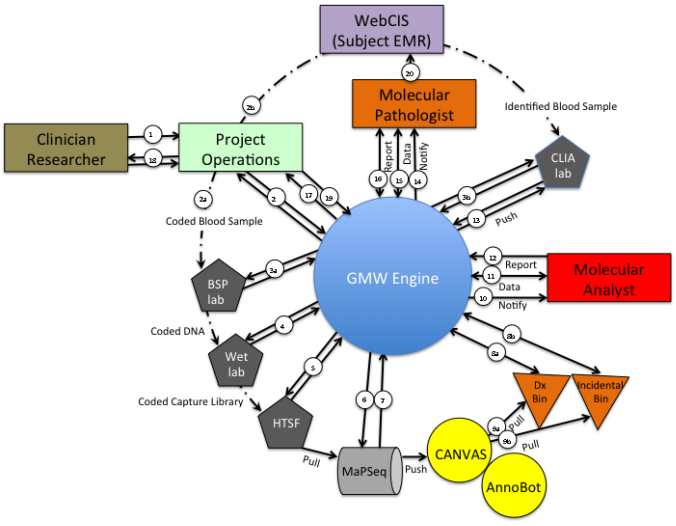

Figure 2. The main GMW Engine workflows engaged by NCGENES, with the flow of information marked as numerical steps. AnnoBot = Annotation Bot; BSP lab = BioSpecimen Processing laboratory; CANVAS = CAroliNa Variant Annotation Store; CLIA lab = a laboratory certified to meet U.S. Congressional Clinical Laboratory Improvements Amendments; Dx = diagnostic; EMR = Electronic Medical Record; MaPSeq = Massively Parallel Sequencing system; WebCIS = Web-based Clinical Information System; Wet lab = basic science laboratory.

A unique feature of NCGENES is its UIs. RENCI worked with NCGENES investigators to develop comprehensive UIs that are currently being used to support the NCGENES research project and will be evaluated for use as general Genomic Clinical Decision Support tools. Two example UIs are shown in Figures 3 and 4. The UI shown in Figure 3 displays study status and details for an individual patient or subject (identified in the figure as NCG_00256) and includes information related to diagnostic and incidental genomic findings, completed NCGENES workflows, current status (in terms of study completion), and whether the subject is in compliance with the study protocol. This UI provides information that is easy to read and interpret and can be used by any member of the study team, from Study Coordinator to Clinician Researcher to System Administrator.

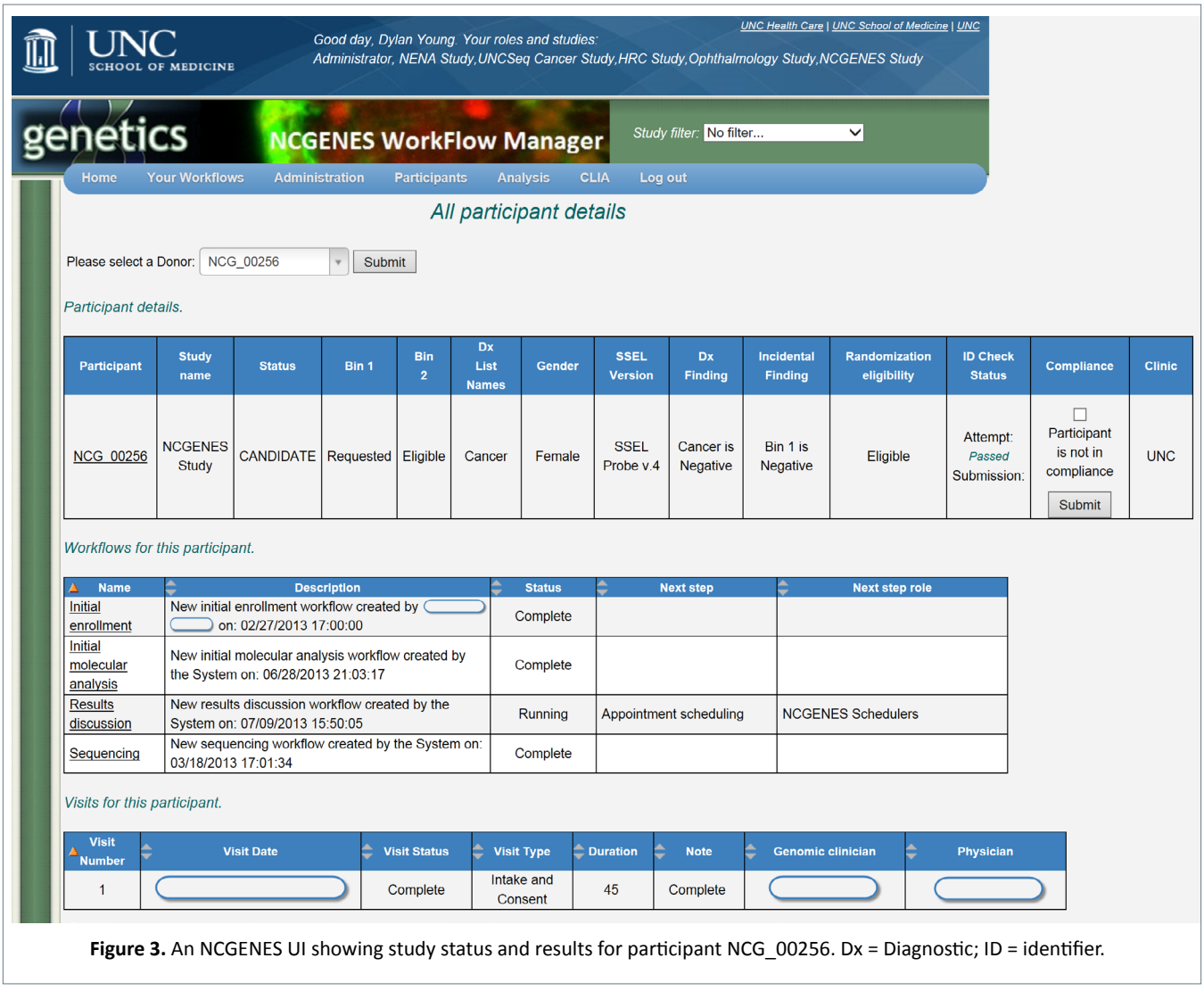

Figure 3. An NCGENES UI showing study status and results for participant NCG_00256. Dx = Diagnostic; ID = identifier.

In contrast, the UI shown in Figure 4 provides more comprehensive, detailed information than that shown in Figure 3. This UI was designed for use by the Molecular Analyst; it provides all of the information required to interpret the genomic sequencing results and reach a conclusion regarding an individual patient or subject. For example, information is provided on the effect of the variant on protein structure and function, the variant's accession number (if available), QC metrics, annotation derived from other sources, and molecular transcript information. Many of the UI fields contain hyperlinks to additional data sources, including the annotation sources that are monitored by AnnoBot and pushed back into CANVAS. The Molecular Analyst UI requires advanced training in the interpretation of fields and thus would not be used by a Study Coordinator, System Administrator, or any member of the study team other than the Molecular Analyst.

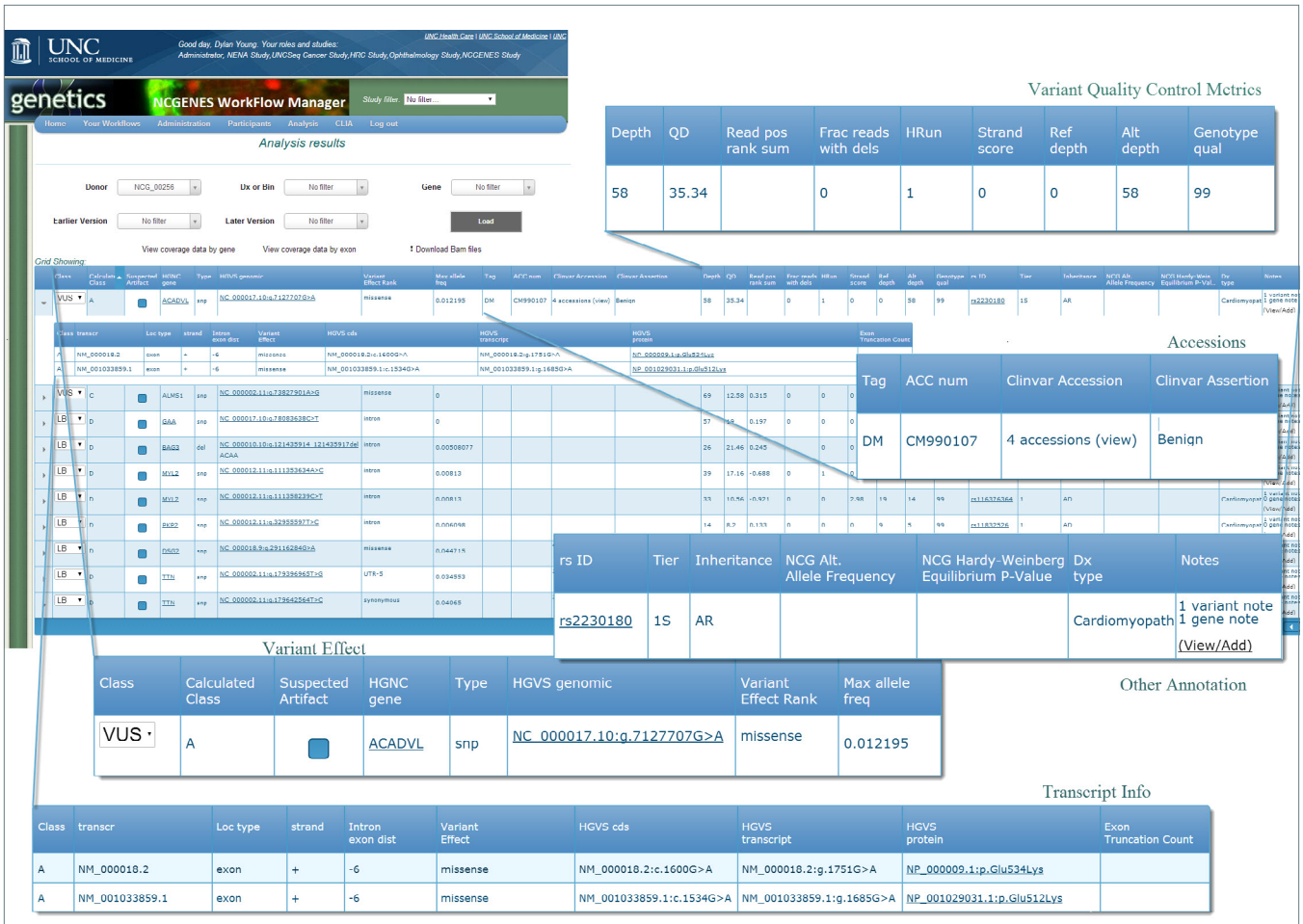

Figure 4. An NCGENES UI showing detailed results for review by the Molecular Analyst. Note that this UI is intentionally more comprehensive and detailed than the UI shown in Figure 3 because it is designed to provide all of the information required by the Molecular Analyst to analyze the results for a given patient or subject. The blow-ups show the types of information available through this UI.

Use Case #2: Workflow Schematics for NCGENES

As discussed, each of the workflows depicted in Figures 1 and 2 typically involves numerous steps and processes and often includes sub-workflows. One such sub-workflow, under Project Operations, is the Initial Subject Enrollment workflow (Figure 5). Note that each and every step in this seemingly "simple" workflow is specified and tracked by the GMW Engine. This level of detail provides for a comprehensive, secure process to facilitate genomic research.

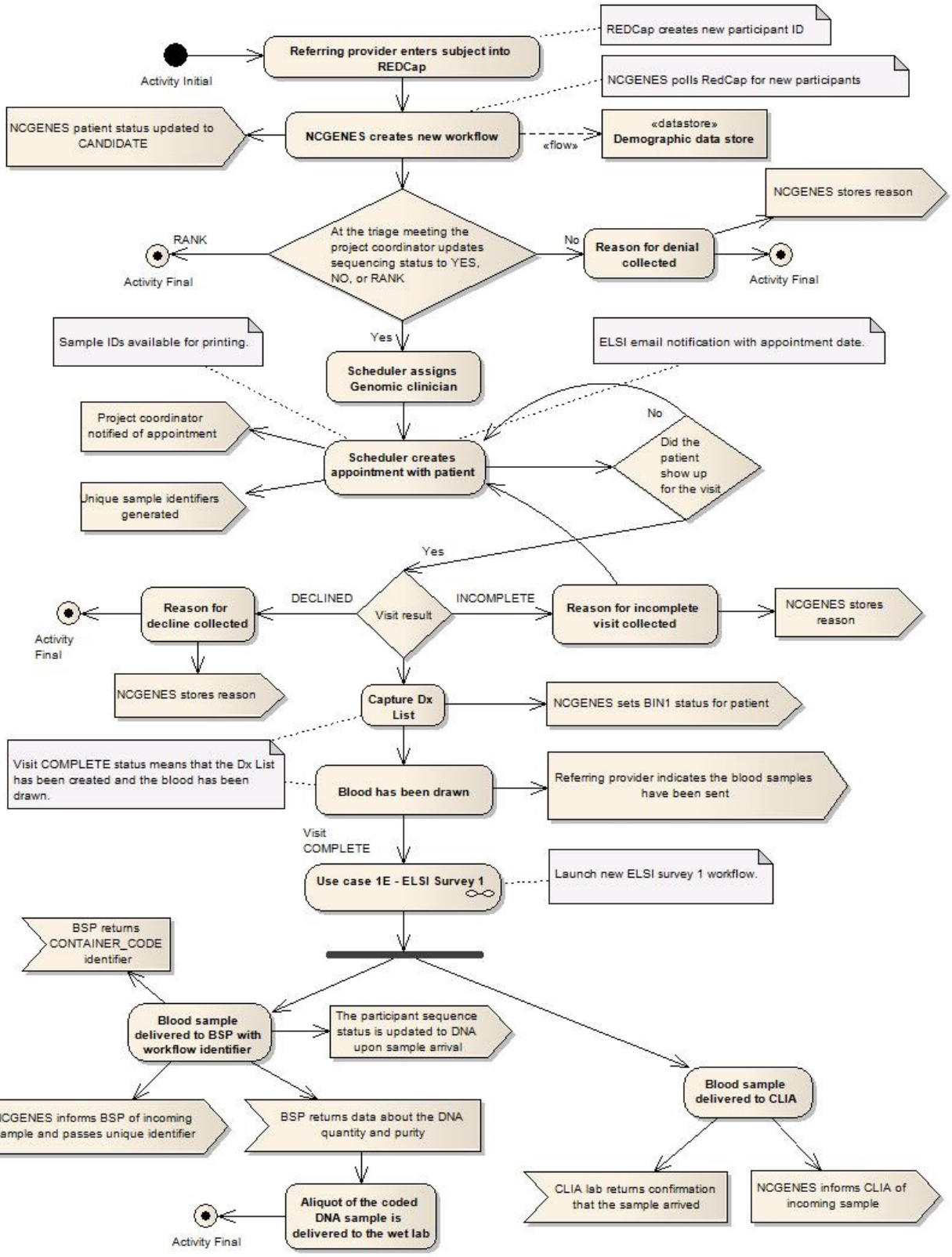

Figure 5. The Initial Subject Enrollment sub-workflow invoked during the execution of the Project Operations workflow. Note the complexity of the sub-workflow. The GMW Engine tracks each step of this sub-workflow and any others that are engaged by a given research project. BSP = BioSpecimen Processing laboratory; CLIA lab = a laboratory certified to meet U.S. Congressional Clinical Laboratory Improvements Amendments; Dx = diagnostic; IDs = identifiers; iRODS = integrated Rule-Oriented Data System; NCGENES = North Carolina Clinical Genomic Evaluation by NextGen Exome Sequencing; wet lab = basic science laboratory.

An important workflow is the Genomic Sequencing workflow (Figure 6). Note that this workflow contains its own sub-workflows, including the sequence analysis workflow used by MaPSeq and the binning workflow invoked by CANVAS. Of mention, communication and data transfer between the MaPSeq and CANVAS workflow pipelines are managed by iRODS. In particular, the MaPSeq workflow is registered with iRODS and uses iRODS to request a table in CANVAS, as needed. The GMW Engine is integrated with iRODS, MaPSeq, and CANVAS and manages the request by using metadata tags in iRODS to automatically look up the appropriate data files in MaPSeq and load those files into CANVAS.

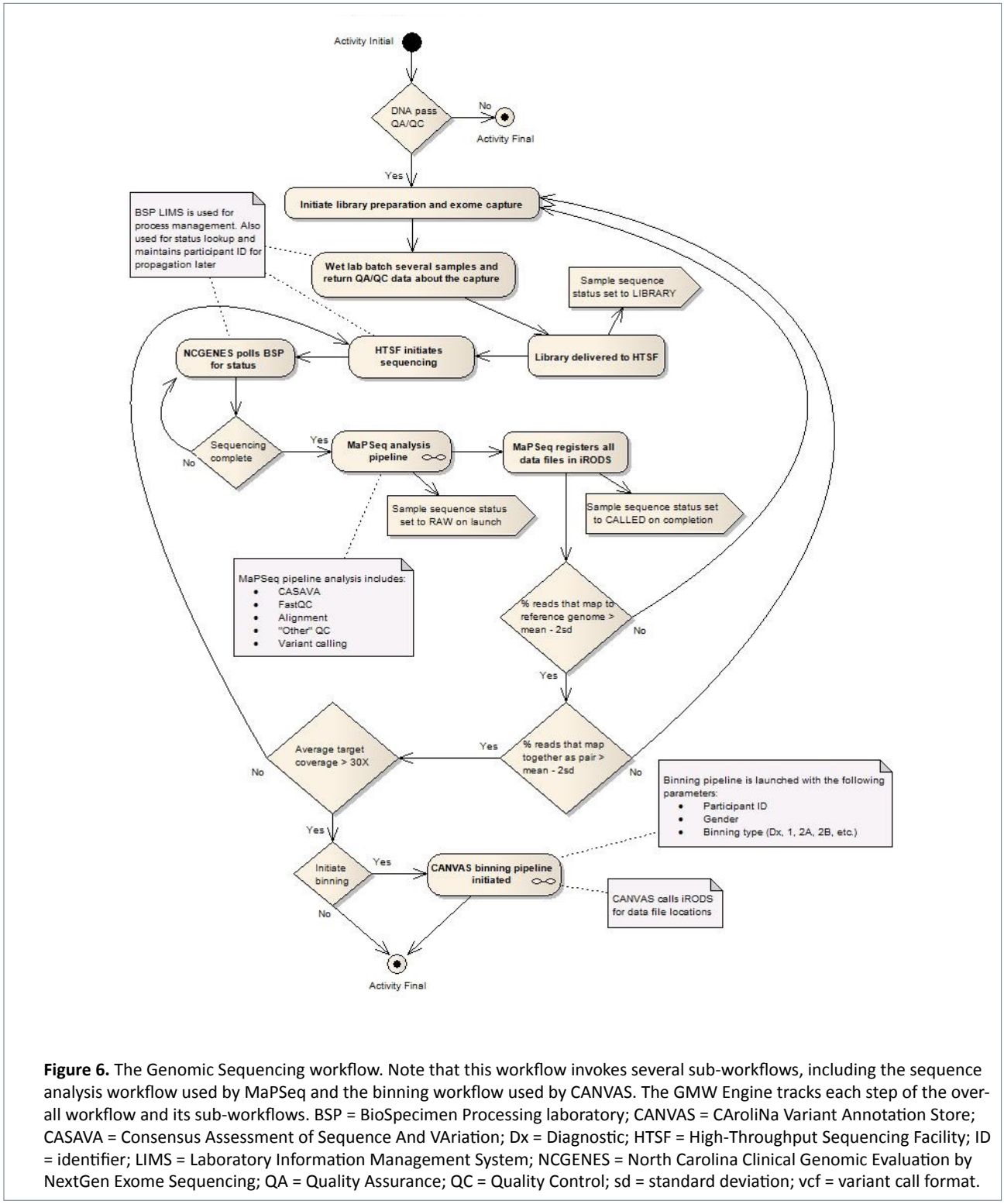

Figure 6. The Genomic Sequencing workflow. Note that this workflow invokes several sub-workflows, including the sequence analysis workflow used by MaPSeq and the binning workflow used by CANVAS. The GMW Engine tracks each step of the overall workflow and its sub-workflows. BSP = BioSpecimen Processing laboratory; CANVAS = CAroliNa Variant Annotation Store; CASAVA = Consensus Assessment of Sequence And VAriation; Dx = Diagnostic; HTSF = High-Throughput Sequencing Facility; ID = identifier; LIMS = Laboratory Information Management System; NCGENES = North Carolina Clinical Genomic Evaluation by NextGen Exome Sequencing; QA = Quality Assurance; QC = Quality Control; sd = standard deviation; vcf = variant call format.

Conclusion

The GMW Engine is an open source architecture that seamlessly coordinates numerous workflows, sub-workflows, samples, data, and people to provide an end-to-end approach to genomics, from initial clinic visit to reporting of genomic findings, thus enabling the secure and efficient use of whole-genome data in genomic research today and in genomic medicine in the near future.

Key Features:

- Architecture is open source.
- Numerous open source technologies are incorporated.
- UIs can be tailored to meet any user's needs Engine is modifiable, extendable, and scalable.
- Workflows are customizable.
- Workflows can be modified while running.
- Multiple workflows are capable of running simultaneously.

Underlying Software and Technologies:

Technology Stack:

- Apache™ SOAP MTOM
- Apache™ ActiveMQ STOMP – JMS mapping
- iRODS
- Microsoft IIS 7.0
- Microsoft SQL Server 2008 R2
- PHP 5.3
- JQuery 1.7.1
- JQWidgets
- Several database connectors, including SQL Server, MySQL, Oracle, and PostgreSQL
- Multiple UI plugins, including a calendar, barcodes, etc.

Development Environment:

- Apache™ SVN® Repository
- Chrome development tools
- Eclipse IDE
- Firefox FireBug 1.10.3
- Microsoft SQL Server Management Studio
- PostgreSQL pgAdmin
- Sparx Enterprise Architect

Impact:

- Currently supports variant annotation for the following research programs: (1) National Human Genome Research Institute–funded NCGENES, "North Carolina Clinical Genomic Evaluation by NextGen Exome Sequencing" (Dr. James Evans, PI), which is conducting whole exome sequencing of >2,000 patient samples drawn from multiple disease categories; (2) National Institute of Child Health and Development–funded NC Nexus, "North Carolina Newborn Exome Sequencing and Newborn Screening Disorders" (Dr. Cynthia Powell, PI), which aims to conduct whole exome sequencing on 400 patient samples; (3) UNCSeq, which applies tumor sequencing technology for >2,000 patient samples in order to identify mutations that are amenable to targeted treatments; and (4) National Institute on Drug Abuse–funded NIDASeq, "Deep Sequencing Studies for Cannabis and Stimulant Dependence" (Dr. Kirk Wilhelmsen, PI), which is conducting whole genome sequencing of ~5,500 patient samples.
- Also supports the NIH-funded Clinical Genome Resource (ClinGen) initiative (Dr. Jonathan Berg, Site PI), which involves a national effort to develop consensus annotation for the NIH Clinical Variant (ClinVar) database.
- Aggregates and stores ~6,000 additional genomes derived from public databases and used for analysis in ongoing genomic research studies; these are obtained from the 1000 Genomes project, The Cancer Genome Atlas project, the national Exome Sequencing Project, and Complete Genomics.

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PROFESSIONAL ISSUES

Genetic Information, Non-Discrimination, and Privacy Protections in Genetic Counseling Practice

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Abstract The passage of the Genetic Information Non Discrimination Act (GINA) was hailed as a pivotal achievement that was expected to calm the fears of both patients and research participants about the potential misuse of genetic information. However, 6 years later, patient and provider awareness of legal protections at both the federal and state level remains discouragingly low, thereby, limiting their potential effectiveness. The increasing demand for genetic testing will expand the number of individuals and families who could benefit from obtaining accurate information about the privacy and anti-discriminatory protections that GINA and other laws extend. In this paper we describe legal protections that are applicable to individuals seeking genetic counseling, review the literature on patient and provider fears of genetic discrimination and examine their awareness and understandings of existing laws, and summarize how genetic counselors currently discuss genetic discrimination. We then present three genetic counseling cases to illustrate issues of genetic discrimination and provide relevant information on applicable legal protections. Genetic counselors have an unprecedented opportunity, as well as the professional responsibility, to disseminate accurate knowledge about existing legal protections to their patients. They can strengthen their effectiveness in this role by achieving a greater knowledge of current protections including being able to identify specific steps that can help protect genetic information.

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Introduction

Significant advances in genomic technology are rapidly expanding the number and scope of genetic tests available both for diagnosing existing disorders and for predicting treatable ones before the onset of symptoms. Public awareness of genetic testing options has been most recently heightened by news stories reporting Angelina Jolie's decision to have prophylactic surgery after her testing revealed a *BRCA1* gene mutation (Jolie 2013) and by the recent, controversial Supreme Court ruling on gene patents (AMP et al. v. Myriad Genetics, Inc et al. 2013). There is growing interest in and demand for genetic testing as treatment options expand, the cost of using newer sequencing technologies declines, the insurance coverage for testing widens, and the population for whom testing is recommended broadens.

Ironically, as genetic testing becomes an increasingly powerful diagnostic and prognostic tool, health care providers and their patients remain wary of the potential of genetic testing to trigger discrimination. Limited awareness of the true scope of legal protections afforded by legislation including the Genetic Information Nondiscrimination Act (GINA) persists and is still fueling fears of genetic discrimination by both patients and their health care providers nearly 6 years after the law's passage (Huntsman Cancer Institute Survey 2013). Genetic counselors can play an influential role in increasing awareness about these legal protections, both because they are more knowledgeable about them than most other health care providers and because their patients can derive direct benefits from this knowledge. Correcting patients' common misconceptions about this topic is, in and of itself, an admirable goal

but genetic counselors could further expand their influence by learning how their patients can take specific steps to help prevent discrimination. Yet counselors may find it difficult to attain an adequate understanding of the legal protections, and their limitations, because the combination of state and federal laws have created a patchwork of protections that vary between individuals and their family members depending upon their circumstances. Though these laws continue to have lingering gaps, patients and their health care providers, including genetic counselors, could benefit from a greater knowledge of the breadth of protections that are currently in place.

Patient fear of genetic discrimination has been reported with the application of genetic technologies to patient care (Lapham et al. 1996; Hall et al. 2005; Allain et al. 2012). Despite widespread apprehension that genetic information will inevitably be misused, there is limited, convincing, empirical evidence that discrimination on the basis of genetic information has occurred (Hall et al. 2005; Pollitz et al. 2007). Whether the lack of evidence stems from under reporting, confusion about what constitutes illegal discrimination, or if it is a true reflection of the situation, is unclear (Sharpe and Carter 2006). The discrepancy between the magnitude of patient concern over potential misuse on one hand and the limited evidence of its occurrence on the other hand, may leave genetic counselors uncertain as to which and how much information they should provide to their patients about the potential for discrimination (Pamarti 2011). This uncertainty can be encapsulated by the following questions: How can a genetic counselor best summarize the legal protections and their caveats and yet acknowledge the limited evidence of discriminatory practices in a time-sensitive manner and without causing patient distress? Which resources can a genetic counselor recommend to a patient who expresses concerns about discrimination? What actions can a patient take if he or she experiences discrimination?

The goal of this paper is to illustrate elements of the legal protections against genetic discrimination that are applicable to issues that arise during a counseling session. We first summarize research findings about fears of genetic discrimination among health professionals and the public, review their understandings of the laws banning discrimination, and describe the current practice of discussing the possibility of discrimination during a counseling session. We then present three genetic counseling cases to highlight ways that issues of genetic discrimination can arise during a session and provide the relevant background information on the applicable legal protections.[1]

[1] This article presents general information about the law in order to educate genetic counselors about legal protections regarding genetic discrimination. It is not legal advice. Professional legal advice should always be sought before any legal action is taken. Application of the law may vary across situations because it is dependent on individually specific circumstances and on the applicable state and federal law.

Genetic Discrimination and Genetic Counseling Practice

Fears of Genetic Discrimination

As genetic technologies have become integrated into clinical care, patients and health care providers have consistently raised alarms about how certain actors – most notably insurers and employers – could potentially use genetic information (Pollitz et al. 2007; Bombard et al. 2012). In 2000, reasoning that health care professionals could be expected to be more knowledgeable than patients about the validity of the potential threat, Matloff et al. conducted a survey of cancer genetic professionals and found that 26 % would use an alias for genetic testing because of their concern about discrimination (Matloff et al. 2000). By contrast, in a 2013, post-GINA version of the study, these percentages had plummeted almost tenfold; from 26 to 3.2 % (Matloff et al. 2014).

It remains to be seen if and to what extent patients' fears of discrimination might also be alleviated by increased awareness of existing laws. In a post-GINA study, many patients still favored anonymous testing out of fear of discrimination related to life insurance (42.7 %), health insurance (30 %), or employment (29.1 %)(Ader et al. 2009). Fears of discrimination have been reported most commonly when the symptoms of a genetic condition begin in adulthood but they appear to have little influence on genetic testing decisions made in prenatal and pediatric settings. Possible explanations for this difference could be that children are typically symptomatic when tested, and, if they have health insurance, they are usually covered under their parents' policies or by the state. Furthermore, their employability is not usually a pressing concern (Hall and Rich 2000). Prior to federal legal protections, evidence that fears of discrimination were scaring patients away from clinical genetic testing and from participating in genetic research (Hall and Rich 2000; Hadley et al. 2003), led to efforts that, in 2008, resulted in the passage of GINA.

Broadly speaking, GINA prohibits employers and health insurance companies from discriminating against an individual based on his or her genetic information. Importantly, these entities are not allowed to collect genetic information in order to use it to raise premium rates, deny coverage, or make adverse employment determinations. Health insurance companies are permitted to request limited genetic information when it involves their decision about whether or not to pay for a medical procedure (GINA 2008).

Overall, GINA has greatly improved protections for many individuals in the US not only by prohibiting some forms of genetic discrimination but, also, although this facet remains less well-recognized, by transferring to patients a much greater control over who has access to their genetic information. Despite these significant gains, the prudent genetic counselor will paint a balanced picture of the current legal landscape – acknowledging both the gaps in the law as well as the

uncertainty about how often genetic discrimination occurs. But counselors should also be careful not to undersell the law's substantial benefits.

Genetic Counselor, Public, and Physician Knowledge of GINA

During a session, genetic counselors attempt to provide a balanced portrayal of both the benefits and the gaps of existing protections; however, the crazy quilt of laws is complicated and requires general knowledge about the law as well as its specific provisions. Genetic counselors are quite well informed about GINA's general protections. A recent survey by Pamarti reported that 99.3 % knew that GINA protects against health insurance discrimination; however, many fewer were knowledgeable about specific details of the law (Pamarti 2011). For example, only 44.2 % of the 257 counselors in this survey knew that GINA does not apply to symptomatic individuals and only 33.8 % knew about the implications for direct-to-consumer genetic testing (Pamarti 2011). Thus, although genetic counselors are aware that GINA offers protection, they may not fully appreciate some of the potential applications to specific situations that they may encounter in their practice. Additionally, because the aforementioned survey only measured counselor knowledge about GINA's anti-discrimination provisions, it did not assess what they knew, or didn't know, about the act's privacy protections; a less well-recognized facet of the law that has direct applications to individuals with a family history of a genetic condition.

Genetic counselors may have some knowledge gaps about GINA's specific protections but a much higher percentage of genetic counselors are aware of the law's existence and its general provisions as compared to people in the general population (AMA 2013). In the previously described survey, genetic counselors estimated that only about 15 % of their patients were aware of GINA prior to their discussion of it during the counseling session (Pamarti 2011). This limited public awareness is corroborated by other surveys that directly measured public knowledge of either the existence of GINA or the existence of laws protecting the privacy of genetic information. In 2006, **prior** to the passage of GINA, in a general population survey administered by Cogent Research, 18 % of 1,000 respondents believed that there were laws to protect the privacy of genetic information (Cogent 2010). Astoundingly, their 2010 survey, conducted **after** the passage of GINA, showed that even **fewer** (16 %) believed that protective laws existed. Likewise, in 2011, an online survey of the general public found that only 8.8 % of 295 respondents had ever heard of GINA (Huang et al. 2013). Similarly, in striking contrast to the public's increasing knowledge about genomic advances, knowledge about the social implications of genetic testing, such as the potential impact on the ability to obtain health insurance, has lagged far behind (Haga et al. 2013).

Even within a population for whom GINA would be expected to be highly relevant, many remain unaware of it. In one study, fewer than half of the asymptomatic individuals who had an expanded allele for Huntington Disease (HD) were familiar with the law, a far fewer number than the three quarters of them who were familiar with the Health Insurance Portability and Accountability Act (HIPAA) (Dorsey et al. 2013). An Australian study of those at risk for HD found a similar lack of awareness about legislation that prevents employers and health insurers from accessing and using genetic information in that country (Goh et al. 2013).

Family physicians appear to have a level of knowledge about GINA that lies between that of genetic counselors and the general public. In a 2010 study of family practitioners, 54.4 % said they were unaware of GINA, 35.2 % knew about GINA, but had no knowledge about any specific features, and 10.3 % had basic knowledge of GINA and its specific protections (Laedtke et al. 2012).

Given the relatively high levels of knowledge about GINA among genetic counselors and the relatively low levels among some physicians and the general public, genetic counselors could serve as a valuable source of information about the implications of both the privacy and nondiscrimination protections of the law.

Discussing Genetic Discrimination

Genetic counselors have an unparalleled opportunity and ability to disseminate accurate knowledge of existing protections of genetic information to their patients. Despite this opportunity, Pamarti found that fewer than half of the 257 counselors surveyed reported discussing GINA during a session (Pamarti 2011). In this sample, counselors only discussed the law if a patient specifically inquired about discrimination (Pamarti 2011). The same study showed that, perhaps not surprisingly, cancer genetic counselors reported discussing the possibility of genetic discrimination with their patients more often than counselors in other specialties; 68 % as compared to 28 % in pediatric and 11 % in prenatal (Pamarti 2011).

Given the amount and complexity of genetic information that is typically conveyed during a session, suggesting that balanced information about legal protections and their limitations also merits inclusion may seem unrealistic. It may also be viewed as an unnecessary diversion given the lack of empirical evidence of discrimination. A concise discussion about the existence and scope of legal protections need not be a major focus of the session, but the failure to describe a realistic picture of the current legal landscape surrounding genetic information can cause future harm to patients and their families. There are several organizations and websites to which patients can be referred that provide more detailed information about GINA and the gaps in the law (Resources). Referring patients to these sources can help

genetic counselors balance the time constraints of a session with their responsibility to present accurate information. It is important to realize that even when patients do not ask questions about genetic discrimination, they may still have concerns. Simply discussing basic information about GINA has been reported to lower patient fears about potential discrimination (Allain et al. 2012). Therefore, combining a brief overview of the current legal protections of genetic testing with a referral to resources that describe the gaps and the limitations of the law could be an efficient method that, at the very least, introduces patients to the existence of the law and its general provisions. Depending upon their circumstances, some patients may need more comprehensive information.

Genetic Discrimination Post-GINA

There continues to be anecdotal stories of genetic discrimination but data on the use or misuse of genetic information in employment and insurance are lacking and few additional empirical reports of genetic discrimination have been published in the 6 years since GINA became law. It is not clear if this sparse amount of data is due to lack of genetic discrimination overall or lack of collected evidence. Additionally, there are likely many more violations of GINA's privacy provisions, in contrast to its anti-discrimination protections, in part because the public and provider awareness of these aspects is even lower. There have been several studies exploring the existence of genetic discrimination in life insurance, but due to limited methodological rigor and the few number of subjects studied, the validity of the conclusions remains uncertain (Joly et al. 2013). Despite lack of empirical evidence that discrimination is occurring, fear of genetic discrimination remains a barrier to the uptake of genetic testing, even in a post-GINA world (Allain et al. 2012). Therefore, the discussion between counselors and patients about the legal protections that exist remains both necessary and beneficial.

Case Studies

The following cases illustrate some common questions and fears that genetic counseling patients may have regarding the potential for discrimination and summarizes the relevant legal background. *We use these cases to highlight particular features of the legal protections; however similar real life situations may have different outcomes if an individual's insurance or employment falls under legal exceptions, since the determination of whether state or federal laws apply depends on individual circumstances*.

Case study 1

A 38-year-old woman calls a genetic counselor because her mother, maternal aunt, and maternal grandmother all had breast cancer. She would like to schedule an appointment for risk assessment and to discuss options for genetic testing but is worried about the possibility of genetic discrimination if information about her family history is entered into her medical record. What information does the genetic counselor need to address this concern?

Legal Protections

Most genetic counselors know that GINA regulates how some employers and health insurance companies can use genetic information. They may not, however, fully appreciate how broadly GINA defines some crucial terms. "Genetic information," as defined by GINA, includes not just genetic test results, but also family medical history, use of genetic services – such as genetic counseling –, and participation in genetic research (GINA 2008). Therefore, those employers and health insurance companies regulated by GINA are banned from using the woman's family medical history or the fact that she had a consultation with a genetic counselor to do the following: raise her premium rates, deny her health insurance, make adverse employment decisions against her, or otherwise discriminate against her.

GINA's definition of "family member" is also very broad, and includes first, second, third, and fourth degree relatives – all the way back to great, great-grandparents, and includes first cousins once-removed (CFR 2013). An individual's genetic information, therefore, includes manifested conditions in any of these relatives.

In this case, the counselor could reassure the woman that employers and health insurers regulated by GINA would be banned from discriminating against her because of her relatives' diagnoses of breast cancer. Additionally, her session with a genetic counselor would also be classified as "genetic information", so the appointment itself – regardless of whether she decides to have genetic testing or not – is also protected information that cannot be used to discriminate. There are situations, especially when medical records are requested, in which an employer or health insurance company can obtain genetic information, including family history. These circumstances will discussed further later, but, in all situations, even if a covered entity **learns** of genetic information, it cannot **use** this information to discriminate.

Case Study 2

A couple consults with a genetic counselor because the woman, who is 15 weeks pregnant, had fragile X testing. Her results showed that she is a carrier and has a pre-mutation of

78 CGG repeats in the *FMR1* gene. The couple is worried that her employer or health insurer may be able to use the results of the test to discriminate against her even though she has no signs of premature ovarian insufficiency (POI); a condition associated with carrier status. They are also concerned about potential discrimination against the fetus, should they decide to have prenatal testing and find out the fetus has inherited the expansion. What information does the genetic counselor need to address these concerns?

Legal Protections

GINA includes a specific provision to emphasize that the genetic information of a fetus is considered part of the genetic information of the pregnant woman (GINA 2008). Therefore, in this situation, any genetic information discovered during prenatal testing would be considered the mother's genetic information under GINA. After the baby is born, any testing done during pregnancy would also still be considered his or her own genetic information.

The association of expanded repeats in the *FMR1* gene with an increased risk for POI as well as the fragile X tremor and ataxia syndrome (FXTAS) highlights one of the legal thresholds or limitations of GINA. Although the law protects against discrimination on the basis of genetic information, this protection does not extend to "manifested conditions". The genetic information is protected under the law, even if symptoms begin, but the symptoms themselves are not protected. For example, in this case, the woman's carrier status is protected genetic information. However, if she begins to have symptoms of either premature menopause or FXTAS, GINA would no longer protect her from being discriminated against because of these symptoms. Even after her symptoms develop, however, covered employers or health insurance companies could not cite her carrier status as the reason for an adverse decision. As genomic sequencing becomes more commonly performed, this category of individuals, those who are asymptomatic but who are at risk for multiple phenotypes, could become more prevalent as the pleiotrophic effects of genomic variants become increasingly recognized (Kocarnik and Fullerton 2014).

To determine whether a covered employer or health insurer could use, for example, the woman's premature menopause symptoms to legally discriminate, it is necessary to look to other laws. The Patient Protection and Affordable Care Act (ACA) currently makes it illegal for health insurers to deny health insurance or raise premiums based on a pre-existing condition (Patient Protection and Affordable Care Act of 2010) for adults. Genetic information is explicitly not considered a pre-existing condition under GINA (GINA 2008). Therefore, protections under GINA and the ACA meet at the point when a person manifests symptoms that could reasonably lead to diagnosis (Fig. 1). A health insurer would be prohibited from using the woman's carrier status to discriminate under GINA, but also would be prohibited from using her symptoms to discriminate under the ACA.

Legal protections in the employment arena are less comprehensive as compared to those applicable to health care. The Americans with Disabilities Act (ADA) protects against discrimination on the basis of a disability. In order for medical symptoms to be protected under this law, they must meet specific criteria. A "disability" is defined as "a physical or mental impairment that substantially limits one or more major life activities, a record of such an impairment, or being regarded as having such an impairment" (ADA 1990; ADAAA 2008). Some symptoms and conditions will not fall under the definition of disability if they do not create a substantial limitation for the individual. Therefore, for some conditions, a gap remains between the legal protections of GINA and the ADA against employment discrimination (Rothstein 2008).

The symptoms of FXTAS and POI could potentially fall under the ADA, although this depends upon how the symptoms affect the individual. For example, an ataxia may substantially limit the major life activity of walking and POI may substantially limit the major life activity of reproduction. However, in the early stages, the symptoms may not reach the level of a substantial limitation and therefore not yet rise to the level of a disability under the ADA. It is possible that, in this circumstance, the woman's employer could legally fire her based on early symptoms, although legal counter-arguments could be made under the ADA, especially under the 'regarded as' portion of the definition (Rothstein 2008).

Case study 3

After his 49-year-old father died of liver failure, a 20-year-old man becomes convinced that the cause was undiagnosed hemochromatosis and wonders about his own risk of this condition. He asks his primary care physician about the option of genetic testing and the physician orders *HFE* gene testing. The results showed that the man is homozygous for the deleterious Cys282Tyr mutation.

Since requesting the test, the man has read about the possibility of discrimination based on the results of genetic testing. He is now concerned because he is applying for a new job and he doesn't want a prospective employer or health insurance company to discriminate against him based on the results of his genetic test. He has told his physician that he doesn't want to know his results until he gets a new job. The primary care provider calls a genetic counselor for advice. What information does the genetic counselor need to address these concerns?

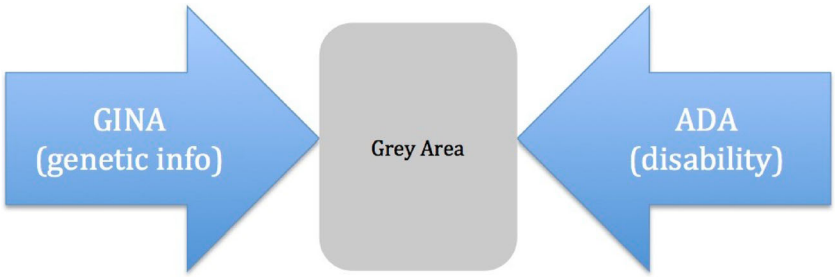

Fig. 1 Application of Gina and the ADA in the continuum from asymptomatic genetic information to manifested disease

Legal Protections

Although heralded as "the first civil rights bill of the new century" (CGF 2008), GINA extends the definition of anti-discrimination far beyond society's colloquial meaning of the concept. GINA bans covered health insurers and employers, not just from using genetic information to harm an individual – in most instances it also **prevents** these actors from **collecting** genetic information in the first place (GINA 2008). Genetic information includes the results of a genetic test, such as the *HFE* gene testing in this case. It is important to note that the definition of genetic testing itself extends beyond single gene, highly penetrant disorders – even though these are the examples that are most often used. A genetic test is defined as "an analysis of human DNA, RNA, chromosomes, proteins, or metabolites that detects genotypes, mutations, or chromosomal changes" (GINA 2008). This definition would extend to many other situations such as learning about carrier status for an autosomal recessive disorder. Additionally, the definition does not depend upon when the genetic test was done or who performed it, so direct-to-consumer genetic tests, tests ordered by physicians and other healthcare professionals, and tests completed prior to the passage of GINA, in 2008, all would be protectable under the law.

The term "collection" in GINA encompasses requesting, requiring, or purchasing an individual's genetic information – including any family medical history. Although covered health insurance companies are generally prohibited from collecting genetic information, in reality, as described above, they often gain access to genetic information through requests for medical records. GINA requires that all requests for medical records state that no genetic information should be included in the request, unless it is directly related to a payment determination (GINA 2008). The law then places the onus on the healthcare professional to redact out all genetic information from the copy of the medical record to be submitted to the insurer. Redaction does not mean removal of the information from the original medical record. Rather, it is the removal or masking of information from the copy of the medical record that is being transmitted to the requesting insurance company or employer. Redaction probably occurs infrequently due to the voluminous amounts of genetic information, including family medical history, sprinkled throughout medical records.

Patients could collaborate with healthcare professionals to attempt to limit the amount and type of genetic information inadvertently given to health insurers by focusing on redacting information that is of particular concern to the individual. Ideally, it has been suggested genetic information could be kept in a separate section of the medical record that is not provided to health insurers or employers that are covered by GINA and requires a separate consent to obtain (Prince 2012).

It is important to appreciate GINA's expansive definition of genetic discrimination for two reasons. First, the broader conceptualization of what counts as discrimination under the law means that genetic discrimination for which an individual could bring a complaint likely occurs at a much higher rate than is currently acknowledged. Imagine the difference in the response to a survey question asking individuals whether or not they have ever been denied insurance, fired from a job, or otherwise adversely affected based on their genetic information versus one that asks if a health insurance representative or employer has ever requested genetic test results or, even more likely, family history information from them. The second question is much more likely to garner a positive response; however, both instances are equally illegal and actionable under GINA. Both health professionals and the general public generally remain unaware that it is illegal for some entities to request genetic information, including family medical history and, furthermore, that in those instances, individuals can legitimately decline these requests thereby protecting the privacy of this information.

Secondly, the prophylactic ban on collection of genetic information by covered health insurers and employers places the patient in an unusual position of power. Lawsuits are incredibly time consuming, costly, and – especially in the case of employment and health insurance – very difficult for a plaintiff to win. In part, this is because it is relatively easy for an employer or health insurance company to invent reasons for a denial that mask the true, underlying reason of genetic discrimination. In one example, an insurance company denied a woman health insurance coverage because it was stated that her weight was slightly too low and she took birth control. The insurance denial also mentioned that she had had a prophylactic surgery – indicating that the stated reasons of low weight and being on birth control could have been proxies for genetic discrimination. The recent changes banning health

insurance denials based on pre-existing conditions under the ACA now make this type of proxy reasoning unlikely to be effective in the health insurance arena.

Proxy genetic discrimination remains a risk in the employment cases– especially if an individual is considered to be an "at-will" employee. In this type of employment, an employee can be fired for "any reason or no reason" – as long as it is not a discriminatory reason (Guz 2000). This rule makes employment cases very difficult for employees to win since a savvy employer can easily hide a discriminatory intent for the adverse decision. In the case described above, it would be difficult for the man to know if he had been victim of genetic discrimination if he was not hired for a job for which he applied. Employers do not generally tell a person why he or she was not hired, so discriminatory intent can be very difficult to prove.

The enforcement mechanisms for health vs. employment claims under GINA are different. Individuals who believe that they have been discriminated against in employment can file a complaint with the Equal Employment Opportunity Commission (EEOC). Once individuals have exhausted the EEOC's administrative process, they can litigate in federal court. In some cases, the EEOC will litigate a complaint that has been filed on behalf of the individual. For example, the EEOC recently settled with Founders Pavilion, a nursing and rehabilitation center, and the company agreed to pay $370,000 because they collected family history as part of a medical exam for new hires (EEOC 2014).

It is much more difficult to uncover the number and types of complaints about genetic discrimination in health insurance because GINA's enforcement provisions are tied to state-specific procedures. If an individual feels that a health insurance company has violated his or her rights, he or she can file a complaint with the state department of insurance. Every state has a different agency and mechanism for these complaints, making it difficult to gather comprehensive data. The current evidence of genetic discrimination in health insurance remains anecdotal, just as it was prior to GINA. However, given the broad definition of genetic information and the ban on collecting genetic information, genetic discrimination – as defined by GINA – likely occurs much more often than people realize or report.

Realistically, patients may opt not to enforce their legal rights because of the hassles and cost of appeals and litigation. An individual may decide that this process is too costly, both financially and emotionally, as compared to a monthly premium rate increase. Avoiding legal action is an understandable decision for many individuals – and unfortunately in some cases, a necessity, when the cost of litigation is prohibitive. Therefore, knowledge that GINA bans the collection of genetic information is an important and powerful tool for individuals that enable them to help prevent genetic discrimination from occurring in the first place.

In contrast to genetic status, the nature of the bias in most forms of discrimination is generally readily apparent – one can often tell an individual's race and gender, and sometimes even a person's religion or disability, simply by looking at them. However, genetic information, in the absence of manifested symptoms, is never obvious just from looking at an individual. Therefore, if an individual can prevent an employer or health insurance company from obtaining information about his or her genetic status, he or she can prevent the possibility of subsequent genetic discrimination based on that information.

One of the most practical steps individuals can take is simply to refuse to answer general questions about their genetic information – including family medical history – that is asked by a covered health insurance representative or employer. Sometimes, even though questions about family medical history are not asked on the application, a representative from the company may ask these questions over the phone if they have not been properly trained on the law. Similarly, a covered employer may ask about family medical history or other genetic information during medical examinations or in other situations. The questions in both of these instances would likely be illegal; however they are still routinely being asked. The man concerned about his genetic test result for hemochromatosis could simply refuse to answer requests by a health insurance company or by his potential employer if either is a "covered entity". This action stops the company from gaining access to his genetic information and therefore prevents genetic discrimination before it can occur.

Some Exceptions to GINA's Ban on Collection of Genetic Information

It is important to note that there are several exceptions that allow companies to collect genetic information. As stated above, health insurance companies are permitted to request genetic information if it involves their decision about whether or not to pay for a medical procedure. For example, if the cost of the genetic testing for hemochromatosis was billed to his health insurance, the company can ask the man for family medical history, such as the father's liver disease, to show that testing was medically necessary. Similarly, if a woman's BRCA sequencing is negative and her genetic counselor recommends BART testing, the health insurance company could request the initial test results. In these cases, the insurer can only ask for the minimum amount of information necessary to make their determination. Additionally, these insurers are not permitted to use the collected genetic information to discriminate.

The employment setting represents a less protected environment than that of health insurance and there are several additional exceptions to the prohibition on collecting genetic information. These exceptions include inadvertent acquisition, a voluntary disclosure by the individual as requested by

a "wellness" program, any publicly available information, disclosure via a family and medical leave request, information requested for law enforcement purposes, and requests made as a part of a company's toxic substances monitoring. For the most part, individuals can most easily prevent an employer from gaining access to genetic information in each of the first three exceptions by not discussing genetic information at work, refusing to answer questions about genetic information, including family history, during enrollment and participation in wellness programs, and by limiting the amount of genetic information publicly available, such as that posted on social media.

Genetic counselors can educate patients by explaining the circumstances under which they do not have to provide information about genetic tests and family medical history to covered health insurers and employers. With this knowledge, patients can take simple, specific steps to help prevent genetic discrimination before it occurs. In the case above, the man can decline to answer questions about his genetic information, including his family history, to potential employers, and, if he insists on posting information about his genetic status on social media sites and blogs, he should, at the very least, restrict the accessibility of others to these sites. These steps can help to protect him against discrimination based on genetic information in the employment and health insurance settings.

Case Study 3 Revisited

After hearing about the steps he can take to limit the amount and nature of the genetic information disclosed to a prospective employer, the man says, "Great because the job I really want is with a small start up company that has only 10 employees." What information does the genetic counselor need to address this statement?

Legal Protections

GINA prohibits only **certain kinds** of entities, namely **some** health insurers and **some** employers, from using genetic information to discriminate against individuals (GINA 2008). Many private health insurers in the US are included under GINA's umbrella and some that are excluded, such as the Federal Employee Health Benefits Plan, Tricare, Veteran's Health Benefits, and the Indian Health Service, have their own restrictions against use of genetic information. Since these health insurers are group plans, they do not take any medical information, including genetic information, into account when setting rates and eligibility. GINA does **not** extend to insurance companies that provide life, long-term care, or disability insurance **nor** does it apply to other entities such as education or licensing.

Many employers, including state, local, and some private employers, are included under GINA but the law does **not** apply to federal government employees and members of the military that have their own rules about what constitutes genetic discrimination (NHGRI 2014). Laws covering federal employees broadly ban employment discrimination based on genetic information but they do not include the privacy protections of GINA. Military rules are less protective and allow some use of genetic information in employment decisions, such as the military's prerogative to decide upon service placement based on genetic susceptibilities to disease (NHGRI 2014; Baruch and Hudson 2008). For example, some branches use the results of genetic testing to make specific assignments to avoid adverse events (Baruch and Hudson 2008). Members of the military can refer to their employment policies to determine whether their branch provides information regarding genetic information, discrimination, and employment.

In the private sector, employers with fewer than fifteen employees do not have to comply with GINA. This segment accounts for about 15 % of the US workforce leaving a substantial minority of workers without federal-level protections against genetic discrimination in employment (SBA 2011). Some of these workers are still protected against genetic discrimination at the state level and several states extend the employment protections to include businesses with fewer than fifteen employees (NCSL 2008). Therefore, these individuals have some state protections against genetic discrimination but these are typically not as broad as the federal level protections.

State Laws Covering Gaps in GINA

GINA creates a baseline of protection and, importantly, **does not pre-empt** stronger state laws. Therefore, individuals who work for a private employer with more than 15 employees may have the choice to file a complaint under either state or federal law. However, state laws are typically not as robust as GINA and the protections and the enforcement mechanisms against infractions vary greatly. For example, the size of employer that must comply with state statutes varies and is state law-dependent. Most notably, many state laws that "protect" against genetic discrimination in employment do not include the powerful prohibition that GINA has against the collection of genetic information. Some states do incorporate the broader protections by legislating that the entities that must comply with state law, must also comply with GINA.

As an example, if the man in this case was applying to a Californian employer with only ten employees, GINA would not apply, but Cal-GINA, a recently passed state law, may be applicable (Cal-GINA 2011). Cal-GINA applies to employers with five or more employees and, although it bans genetic discrimination, it does not prohibit those employers from

collecting genetic information. Therefore the man would not enjoy the broader privacy protections of GINA in this case, but he would still be protected from genetic discrimination. He can still take steps to limit a prospective employer's access to his genetic information, such as limiting public access via social media, but a small business employer could be allowed to ask about his genetic information directly. Importantly, however, even if he were asked for this information directly, in California, the small business employer would be banned from using that information to discriminate against him.

A comprehensive discussion with genetic counseling patients about their legal protections becomes even more difficult because of the familial nature of genetic information. If a patient works for a large employer who offers health benefits, it may be tempting for the genetic counselor to paint broad-brush strokes and briefly note that GINA protects him or her against genetic discrimination. However, there are two serious flaws in this approach. First, it is likely that an individual, especially a younger patient, will switch jobs, be covered under different insurance companies, and/or move across state lines during his or her lifetime. These changes could affect his or her current legal protections because of gaps in the law. Secondly, the genetic information of an individual could impact others in the family and it is likely that some of these relatives will have different legal protections based upon where they live or who employs them.

Genetic counselors can consult guides such as the Council for Responsible Genetics (CRG) to identify the protections afforded by a specific state. Determining whether or not specific state laws apply to employers that operate across state lines is often very complicated.

Case Study 2 Revisited

After the delivery of their son, the couple call the genetic counselor and explain that they want to obtain life insurance so that they can be assured that their children will be provided for should anything happen to them. They ask if the woman's risk for POI and FXTAS will be considered pre-existing conditions in their life insurance application. In addition, the man's father has recently developed symptoms of Alzheimer disease (AD). They have learned about the option of *APOE* testing and wondered about the implications if the man has this testing. What information does the genetic counselor need to address this statement?

Legal Protections

One of the most notorious gaps in GINA is that it does not apply to three types of insurance that individuals with genetic conditions may greatly desire; namely, life, long-term care, and disability insurances. Patients who discover they have a predisposition for cancer, AD, or other chronic illness are likely to seek insurance coverage to pay for nursing home care or to provide for their family when they pass away. At the same time, life, long-term care, and disability insurance companies are likely to seek information about an applicant's risk level in order to make the best economic decisions for the company. In the vast majority of cases, these insurances can legally use genetic information in coverage decisions and could even require that an individual take a genetic test before deciding whether or not to cover them (Schultz 2013).

Although it is true that GINA does not apply to these three types of insurances, some state laws regulate the use of genetic information in these arenas (NCSL 2008). All laws, however, are not created equally and it is important for patients and genetic counselors to refrain from equating the existence of a law with adequate protection. For the most part, states only **regulate the use** of genetic information in these insurances – **not ban the use**. For example, some state laws simply require that insurance companies show actuarial justification for charging different premium amounts or for denying coverage (NCSL 2008). Actuarial justification requires insurers to show that their premium rates are reasonable given their expected costs – a task that is fairly straightforward if an individual has a genetic pre-disposition to a health condition because of the implication that expected costs will be higher. Therefore the requirement of actuarial justification does not protect individuals in the same way that the public commonly conceptualizes the word "protection".

In other states, the laws regulating the use of genetic information in life, long-term care, and disability insurance simply requires "informed consent" from the enrollee when and if the insurance company requires a genetic test (NCSL 2008). These laws do **not** prevent insurers from gathering genetic information and making coverage decisions based upon the information. For example, New Jersey's law prohibits 'unfair' genetic discrimination in life insurance but this legally translates to requiring actuarial justification to use genetic information and obtaining "informed consent" from the individual prior to performing a genetic test (New Jersey Code 2008).

Finally, GINA broadly defines "genetic information" to include family medical history, use of genetic services, and participation in genetic research (GINA 2008). However, most state laws were passed prior to GINA and so define "genetic information" much more narrowly; namely, as genetic test results (NCSL 2008). California's law is an exception and includes family medical history in its expansive protection against discrimination in life, long-term care, and disability insurance (Cal-GINA 2011).

Unfortunately, the patchwork of state laws in life, long-term care, and disability insurance provides little concrete protection for individuals in these arenas. As new state laws continue to be passed, patients and genetic counselors must look carefully at the protections and should not assume that the laws are as comprehensive as GINA.

Options for Access to Supplemental Insurance

The lack of comprehensive protection at the state level, unfortunately, creates a difficult decision for individuals who are considering testing and yet are concerned about the possibility of genetic discrimination. One often-advised option is to secure coverage prior to having genetic testing although this approach has limitations as described in the next section. Additionally, if an individual is denied life, long-term care, or disability insurance, he or she should check the relevant state law to see if it is possible to appeal the decision (CRG resource). Although state laws vary, individuals have won appeals for denials based on genetic information, especially in states with more protective coverage, like California. Finally, policy makers at the federal and state level are increasingly considering legislation to improve access to these insurances for individuals with genetic conditions. Individual experiences and stories can be invaluable information to share with policy makers to increase their understanding about how current industry practices are affecting the public.

In the case study above, life, long-term care, and disability insurance companies may be able to deny the couple coverage based on genetic information – depending on which state they live in. In this situation, the genetic counselor could advise the couple to find out more about their state law by referring to a credible resource (CRG, NCSL, or consulting with an attorney specializing in insurance law). It could be more difficult for the man to secure insurance due to his family history of AD because his case appears to present a stronger actuarial justification for increased costs. The man could consider getting insurance coverage prior to genetic testing, although in many states, the insurers would be allowed to ask about the family history, ask about genetic test results, and in some situations, require him to have genetic testing before making coverage determinations.

Fraudulent Information

When completing insurance applications or otherwise providing information to insurance companies, it is important that individuals be warned against committing fraud or lying on their applications. Unfortunately, it is not uncommon for patients to be incorrectly advised that, as long as a genetic test result is not in the medical records, they can state to an insurance company that they have not been tested. This ill-advised tactic can create substantial problems for them in the future. For example, if a long-term care insurer discovers that an individual committed fraud on an application, they can likely revoke the coverage and past reimbursements. If this discovery occurs after an individual has been in a nursing home for a number of years, it can result in a considerable financial obligation that the patient will then owe to the facility since the insurance is very likely to retroactively revoke past reimbursement payments.

Similarly, although GINA shields individuals from disclosing genetic information to health insurers and employers in most cases, it does not sanction fraud. If, in violation of GINA, a covered entity asks for genetic information, the appropriate response would be not to lie about testing results or family history, but rather to choose not to answer these questions.

Table 1 Protections of the collection and use of Genetic Information (GI) by entity

A. Health Insurance (HI)
Private HI
• GINA usually applies
• Collection of GI is banned except for payment decisions
• Use of GI to discriminate is banned
Group HI through military, federal or state government
• GINA does not apply
• Collection of GI may be allowed
• No medical information, including GI used for rates and eligibility
B. Employment
Private employer
15 or more employees
• GINA applies
• Collection of GI generally banned with few exceptions
• Use of GI to discriminate is banned
Employed in a state with an applicable law
• Both GINA and state law may apply
• Determine best jurisdiction to file complaint
Fewer than 15 employees
• GINA does not apply, but state law may apply
State or local government employer
• GINA applies
• Collection of GI generally banned with few exceptions
• Use of GI to discriminate is banned
Military or federal government employer
• GINA generally does not apply
• Collection of GI is not banned
• Use of GI to discriminate under some circumstances (military)
• Federal employees, see Executive Order 13145; file complaints via Equal Opportunity Officer;
Military employees, see employee manual
C. Life, Long-term Care, or Disability Insurance (Supplemental Insurance)
• GINA does not apply
• State law may provide some protections
• Possible appeal of denials if no fraudulent information given

Conclusion

It is a promising sign that there have been increasing numbers of both state and federal laws passed to protect individuals from genetic discrimination. The resulting patchwork of legislation, however, creates important gaps relevant for genetic counselors and their patients (Table 1). The limited awareness of these laws, by both the public and health care professionals, greatly restricts their potential effectiveness. It is crucial that patients have access to credible information about the existing laws, as there may be actions they can take to help protect their genetic information and lower their risk of genetic discrimination, thereby preventing future harm to themselves and their families. Genetic counselors have both the unprecedented opportunity and the professional responsibility to disseminate accurate knowledge of existing legal protections to their patients. By acquiring additional knowledge of how these protections might apply to their practice, genetic counselors could help ease some unfounded concerns about possible discrimination and enlighten patients about actions they can take to help protect their genetic information, wherever possible.

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Further Reading

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Equal Employment Opportunity Commission (EEOC) – Where individuals must file a complaint of employment discrimination.

Gina Help: www.ginahelp.org - Website offering information about GINA and the gaps in the law.

Cancer Legal Resource Center – Organization has a free national telephone assistance line where patients can ask questions about cancer and genetic-related legal issues.

National Human Genome Research Institute - https://www.genome.gov/10002328.

Patient Advocate Foundation – Organization offers assistance with insurance appeals as well as general resources for patients.

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Article

MaPSeq, A Service-Oriented Architecture for Genomics Research within an Academic Biomedical Research Institution

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Abstract: Genomics research presents technical, computational, and analytical challenges that are well recognized. Less recognized are the complex sociological, psychological, cultural, and political challenges that arise when genomics research takes place within a large, decentralized academic institution. In this paper, we describe a Service-Oriented Architecture (SOA)—MaPSeq—that was conceptualized and designed to meet the diverse and evolving computational workflow needs of genomics researchers at our large, hospital-affiliated, academic research institution. We present the institutional challenges that motivated the design of MaPSeq before describing the architecture and functionality of MaPSeq. We then discuss SOA solutions and conclude that approaches such as MaPSeq enable efficient and effective computational workflow execution for genomics research and for any type of academic biomedical research that requires complex, computationally-intense workflows.

Keywords: service-oriented architecture; genomics; massively parallel sequencing; computational workflow; academic biomedical research; decentralized organization; distributed decision-making

1. Introduction

Genomics research presents well-recognized technical, computational, and analytical challenges [1–4]. For example, while the technology for massively parallel genomic sequencing has progressed to the point where large amounts of data can be generated at a rapid pace and for a reasonable cost, the analytical burden presented by this massive amount of data can quickly overwhelm the genomic analyst. Indeed, the analysis and interpretation of genetic findings is generally considered the rate-limiting step in the translation of genomic sequencing data into clinical practice and patient care [4].

Less recognized challenges to research in genomics and any biomedical field are the sociological, psychological, cultural, and political barriers, many of which arise from the organizational structure within which the research takes place. Indeed, research organizations tend to fall somewhere on a continuum between completely centralized and completely decentralized [5–8]. Each of these extremes has advantages and disadvantages. Centralized organizations traditionally function within a simple organizational design, with singular decision-making, top-level operational control, a consolidated budget, strong/clear communication channels, uniform culture and politics, and a high degree of efficiency, but at the expense of flexibility. Decentralized organizations, in contrast, generally operate within a complex organizational design, with distributed decision-making, local operational control, regionalized budgets, numerous weak or broken communication channels, inconsistent (and sometimes conflicting) culture and politics, and a high degree of flexibility, but at the expense of efficiency. The conceptualization, design, development, and implementation of information technology (IT) solutions for research in genomics and any biomedical field must therefore involve careful consideration of not only the needs of the user base, but also the organizational structure within which the research takes place.

Herein, we present a Service-Oriented Architecture (SOA) application—termed MaPSeq—that was conceptualized and designed to address the organizational challenges of computation-intensive biomedical research within a decentralized academic institution. In this article, we first describe the challenges that contributed to the conceptualization and design of MaPSeq. We then provide an overview of the technical architecture and capabilities of MaPSeq. Finally, we provide a discussion of service-oriented solutions such as MaPSeq.

2. Challenges Driving the Conceptualization and SOA Design of MaPSeq

The design of MaPSeq was motivated by challenges that arose during the implementation of a genomic sequencing project titled “North Carolina Clinical Genomic Evaluation by NextGen Exome Sequencing” (NCGENES). This project, which is funded by the National Human Genome Resource Institute, aims to conduct whole exome sequencing of 500 patient samples drawn from multiple disease categories. NCGENES is a complex project, with both research and clinical arms. Soon after the project

was initiated, the research and clinical teams realized that there were numerous barriers and roadblocks that needed to be overcome in order to achieve the analytical goals of the project. (See Table 1 for overview.)

Table 1. An overview of the challenges that contributed to the architectural design of MaPSeq.

Challenge	Description	MaPSeq SOA Solution	Benefits
Challenge 1	Diverse and evolving computational workflow needs; expanding complexity of workflows	Different services designed to address different needs	Flexibility; scalability; extensibility
Challenge 2	Silos of distributed, uncoordinated compute resources; network idiosyncrasies	Opportunistic use of distributed compute resources without need for a cloud-based software stack	Interoperability; extensibility; generalizability
Challenge 3	Political and cultural resistance to change; human roadblocks in the automation of workflow pipelines	Reusable automated attributes to gradually replace human workflow processes	Achievability; accessibility; functionality

2.1. Challenge 1

Academic institutions face the challenge of balancing the needs of large, funded, research projects that typically support the development of an informatics infrastructure with the needs of smaller, often unfunded, research projects that cannot afford significant development costs. Furthermore, few research projects are sufficiently funded to support future development needs. Our institution faced these challenges when trying to balance the needs of the NCGENES investigative team with those of other investigative teams and anticipate future needs. The scale, general applicability, and complexity of massively parallel sequencing favored the development of an SOA approach to support both current and future needs related to genomic and non-genomic computationally-intense serial workflows.

2.2. Challenge 2

As is typical for an academic institution, our genomics infrastructure developed in an *ad hoc* manner, with multiple investigative teams working independently across the university campus. The result was a burgeoning, uncoordinated cluster of distributed compute resources. Compounding this challenge were the numerous network idiosyncrasies that prevented administrators within one network from accessing compute resources within a different network; thus, access privileges to campus compute resources were determined locally and required on-site (rather than remote) access.

2.3. Challenge 3

Decision-making at large academic institutions tends to be decentralized, with numerous decision makers enforcing different (and often conflicting) policies and procedures. This organizational structure inevitably leads to political and cultural conflicts and resistance to change, particularly when "external" IT teams attempt to change the processes in place among "central" investigative teams. Political and cultural resistance to the NCGENES project was encountered early on as the investigative team identified many barriers to the automation of human user-controlled workflow processes. While the

existing human user-run workflows met the needs of small genomic sequencing projects and user groups, these workflows were inefficient for the computationally-demanding, whole-exome sequencing needs of NCGENES. Moreover, the use of a human contact as the point of access to an existing workflow created a roadblock to the execution of NCGENES, reduced the efficiency of genomic analysis, and threatened the security of sensitive patient data.

3. Existing Solutions

Numerous Workflow Management Systems and workflow pipelines for genomic analysis exist, including COSMOS [9], Ergatis [10], i2b2 [11], LONI [12], NG6 [13], NGSANE [14], Orione [15], RUbioSeq [16], SeqInCloud [17], STATegra EMS [18], TREVA [19], and Pegasus [20]. Our team evaluated each of these systems for their ability to overcome the challenges described above. We found that existing solutions could address some, but not all, of the roadblocks and barriers that were hindering progress on the NCGENES project and that a new solution was needed. While all of the existing workflow systems and pipelines have proven to be effective, each has limitations [21]. MaPSeq is not unique in this regard, but it is responsive to the key features of a decentralized research organization. Specifically, as an SOA, MaPSeq allows for integration with multiple clients and distributed systems, whether local, open source, or commercial, and provides tailored, reusable, automated service solutions that address the varying and evolving needs and preferences of decentralized decision-makers. MaPSeq is scalable and can support both small- and large-scale projects and thus is responsive to the computational needs of all investigators. MaPSeq is efficient and allows for seamless, opportunistic use of distributed compute resources. Finally, the service-oriented, automated approach requires little coordination or communication among individual user groups and thus avoids local nuances in politics and culture.

4. MaPSeq Technical Architecture and Capabilities

4.1. Overview of MaPSeq Architecture

MaPSeq was designed as an open source, plugin-based SOA solution [22–24] that provides modifiable services to make opportunistic use of multiple institutional and cloud-based compute resources in order to efficiently complete the multitude of steps involved in the analysis of large-scale, genomic sequencing data (see Figure 1). The plugin framework of MaPSeq is based on the Open Services Gateway initiative (OSGi). This framework was chosen because of its modular agile architecture and the ability to remotely manage workflow pipelines in an on-demand manner and within a sandboxed environment. Moreover, the investigative team had relevant prior experience with the Open Science Grid Engagement Program, which aims to facilitate collaborative research through advanced distributed computing technologies.

MaPSeq and, its sister technology, the Grid Access Triage Engine (GATE), are built on top of Apache™ Karaf, which is an OSGi-based lightweight container for application deployment. MapSeq works together with GATE to provide extensible capabilities for the analysis of genomic sequencing data, including: pipeline execution and management; meta-scheduling of workflow jobs; opportunistic

compute-node utilization and management; secure messaging and data transfer; and client access via web services.

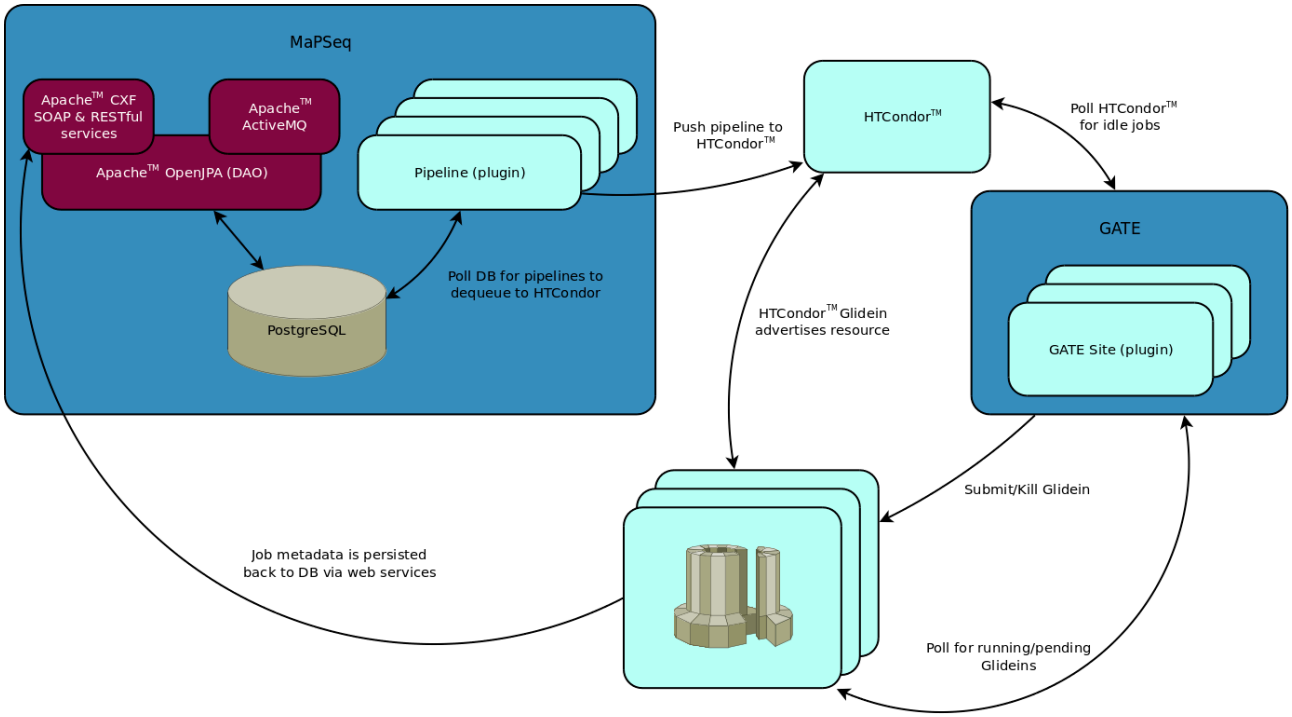

Figure 1. An overview of the MaPSeq architecture.

4.2. MaPSeq Pipelines

MaPSeq pipelines (Figure 1) are OSGi-based plugins comprised of a number of bundles and/or services. At a minimum, a MaPSeq pipeline consists of: (1) a Java Message Service destination that exposes a mechanism whereby a user can trigger a pipeline; (2) a workflow designed as a Directed Acyclic Graph (DAG) and consisting of a collection of programmatic tasks; (3) an executor that dequeues the workflows at a customizable frequency (e.g., two workflows every five minutes, ten workflows every three minutes, *etc*.); and (4) a metadata file that describes all of the aforementioned features and tracks their status. Complex pipelines can be broken into numerous smaller sub-pipelines to enable symbolic check-pointing or fault tolerance. For example, a genomic analysis pipeline can be logically split into two sub-pipelines: an alignment sub-pipeline and a variant calling sub-pipeline. This approach enables a researcher to, for example, modify a step in the variant calling sub-pipeline and re-run that sub-pipeline without the need to re-run the alignment sub-pipeline, thereby reducing the runtime burden. Additionally, this approach allows the sub-pipelines to be reused in other pipelines, thus fostering software re-usability. Of note, all pipelines are project-specific and defined by the needs of the project and research team such that pipeline development is tailored to a specific application.

4.3. HTCondor™

HTCondor (Figure 1) serves as a central manager and provides meta-scheduling for MaPSeq via the DAG Manager (DAGMan). MaPSeq workflows are comprised of numerous modules that form the vertices of a DAG. The DAGs can be exported for submission to HTCondor using DAGMan. MaPSeq

provides a suite of modules that wrap third-party libraries (e.g., GATK, Picard, *etc*.) for execution on the grid and that include a number of lifecycle events. These lifecycle events check for valid inputs and outputs, successful execution, and provenance of job metadata, thus ensuring consistency and rapid detection of errors. HTCondor manages serial execution of MaPSeq modules, as well as job-to-machine resource negotiation or "matchmaking". The matchmaking process identifies job requirements (e.g., four cores and 4 GB memory required), as defined by the job metadata, and pairs those requirements with available machine attributes (e.g., eight cores and 32 GB memory available). After a MaPSeq module is executed, that module, or job wrapper, persists the job metadata over web services into a PostgreSQL database. HTCondor Glideins are used to provision compute resources for the execution of jobs, as described below.

4.4. GATE

GATE (Figure 1) is a homegrown OSGi-based system that serves as a sister technology for MaPSeq. Whereas MaPSeq uses plugins to execute workflow pipelines, GATE uses plugins to access compute resources. GATE continuously monitors a local HTCondor instance for idle jobs and profiles compute resources for availability. If an idle job is detected, then GATE uses plugins to submit an HTCondor Glidein to the most appropriate compute resource, which then joins the local HTCondor pool. GATE defers matchmaking to the HTCondor Negotiator, which uses daemons to perform the matchmaking. GATE grows and shrinks the number of Glideins by assessing the number of running and idle local jobs against the number of running and idle Glidein jobs on the compute resource grid. After a Glidein is activated, it registers back to the HTCondor Central Manager as an available resource. This approach enables jobs to be both site-specific and site-agnostic.

4.5. Security, Interfaces, and Administration

Of significance, both MaPSeq and GATE use Secure SHell (SSH) technology, running with daemons, for authentication and data transfer. This level of security is particularly important for applications such as genomics that involve the movement of sensitive patient data.

Clients can interface with MaPSeq using Apache™ CXF (Figure 1), which is an industry-standard web service. Both Simple Object Access Protocol (SOAP) and Representational State Transfer (RESTful) services are supported by Apache CXF. Pipeline invocations are triggered via a JavaScript Object Notation (JSON)-formatted message to an Apache™ ActiveMQ destination. The JSON message contains the mapping between a MaPSeq-managed sample file instance and a workflow run instance. A pipeline-specific "message listener" then determines if the message is legitimate for subsequent processing. For genomic sequencing data, this process may involve verification that an object layer in the data file specifies that the data file contains raw sequencing data and sufficient metadata. A rich set of MaPSeq reports can be generated and sent to a client via email, for review and detection of potential problems (see example in Figure 2).

Apache Karaf is unique among containers in that it embeds an SSH daemon to enable a client to administratively manage pipeline deployment within a sandboxed environment. MaPSeq pipelines can be added, removed, or altered without having to stop the container, thereby provisioning a continuous,

uninterrupted environment to execute new pipelines while existing pipelines are running. This accessibility allows for a pipeline developer to independently iterate on pipeline improvements.

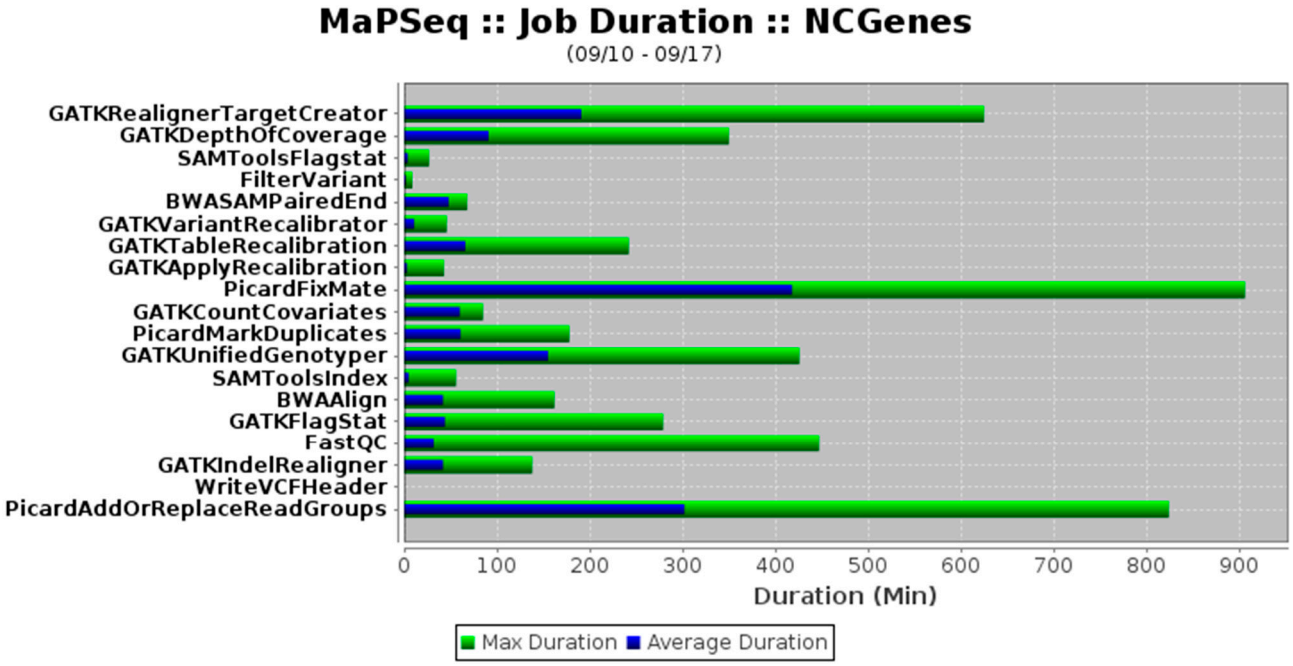

Figure 2. An example of a MaPSeq output log showing the duration of a job (total and average minutes (min) over a one-week time period) by specific task.

5. Discussion

Genomics research within an academic environment presents numerous challenges. In addition to the computational and technical challenges inherent in genomics research [1–4], there are complex sociological, psychological, cultural, and political challenges that affect operations within academic institutions and indeed many other types of organizations [25–29]. Moreover, academic biomedical research institutions tend to be decentralized in their organizational structure. Whereas centralized organizations tend to function within a simple organizational design, with singular decision-making, top-level operational control, a consolidated budget, strong/clear communication channels, uniform culture and politics, and a high degree of operational efficiency, decentralized organizations, in contrast, operate within a complex organizational design, with distributed decision-making, localized operations and budgets, weak communication channels, nuances in culture and politics across academic units, and minimal operational efficiency [5–8].

MaPSeq provides a reusable, service-oriented solution that addresses the diverse and evolving computational needs of decentralized decision-makers and scales to support both small- and large-scale projects. The automated approach requires little coordination or communication among individual user groups and thus avoids human roadblocks that may otherwise decrease efficiency. By leveraging the OSGi framework and Apache Karaf, MaPSeq allows for quick development iterations on MaPSeq pipeline plugins; pipelines can be created, altered, deployed, triggered, and removed without having to stop and restart the container. Finally, the use of HTCondor as a meta-scheduler and the addition of GATE as a sister technology allow MaPSeq to extend compute cluster capacity and make opportunistic use of distributed compute resources across the university campus.

In an environment of legacy systems, distributed and uncoordinated decision-making and compute resources, diverse and evolving user needs, and political and cultural resistance to change, centralized technical solutions will not promote efficient and effective biomedical research. SOA solutions provide the flexibility, scalability, extensibility, accessibility, interoperability, generalizability, achievability, and functionality required to attain efficient and effective, transformative biomedical research within a decentralized organization.

Limitations

Like any scientific workflow pipeline, MaPSeq is not without limitations [21]. First, while the underlying technology is open source and freely available, there is a considerable learning curve involved in implementation of the technology. Second, GATE is a homegrown solution and requires institution-specific adaptation before it can be adopted for use. Third, the MaPSeq solution must be continuously assessed against the evolving needs of relevant stakeholders, including users, patients, investigators, institutional administrators, and policy makers.

6. Conclusions

SOA solutions such as MaPSeq are well suited to overcome the many challenges to biomedical research that are inherent in a decentralized academic institution. MaPSeq has transformed genomics research at our institution and currently supports several large genomics research projects, as well as a few small ones. While MaPSeq was originally termed as an acronym for "Massively Parallel Sequencing" and designed to support genomics research, we note that the general architecture and approach can be adapted for other complex or computationally-intense workflows.

Finally, we note that MaPSeq (version 5.0) is available through a University of North Carolina Open Source Public License (version 1.1, ©2004). The only prerequisites are Java 1.7+, Apache™ Maven 3, and a network connection (full technical specifications and installation/operational instructions can be found at [30], with an accompanying RENCI technical report at reference [31]).

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Author Contributions

Jason Reilly designed and implemented MaPSeq with assistance from Phillips Owen as a replacement of earlier work by Charles Schmitt and based on prior work by John McGee, Kirk Wilhelmsen oversaw the implementation of MapSeq. Stanley Ahalt provided general guidance and facilities support for the development and implementation of MaPSeq.

Conflicts of Interest

The authors declare no conflict of interest.

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Cognitive and Emotional Factors Predicting Decisional Conflict among High-Risk Breast Cancer Survivors Who Receive Uninformative *BRCA1/2* Results

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Abstract

Objective—To investigate high-risk breast cancer survivors' risk reduction decision making and decisional conflict after an uninformative *BRCA1/2* test.

Design—Prospective, longitudinal study of 182 probands undergoing *BRCA1/2* testing, with assessments 1-, 6-, and 12-months post-disclosure.

Measures—Primary predictors were health beliefs and emotional responses to testing assessed 1-month post-disclosure. Main outcomes included women's perception of whether they had made a final risk management decision (*decision status*) and decisional conflict related to this issue.

Results—There were four patterns of decision making, depending on how long it took women to make a final decision and the stability of their decision status across assessments. *Late decision makers* and *non-decision makers* reported the highest decisional conflict; however, substantial numbers of women—even *early* and *intermediate decision makers*—reported elevated decisional conflict. Analyses predicting decisional conflict 1- and 12-months post-disclosure found that, after accounting for controls and decision status, health beliefs and emotional factors predicted decisional conflict at different timepoints, with health beliefs more important one month after test disclosure and health beliefs more important one year later.

Conclusion—Many of these women may benefit from decision making assistance.

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Keywords

breast cancer; genetic testing; decisional conflict; decision making; BRCA

Women with a strong family history of breast and/or ovarian cancer may choose to undergo genetic testing to determine whether they have a deleterious mutation in the *BRCA1* or *BRCA2* gene. Because the probability of identifying a mutation is highest if testing begins with an affected woman, the first person in a family to undergo *BRCA1/2* testing (the *proband*) is typically a woman who has had breast or ovarian cancer. If a mutation is detected, the proband is at elevated risk for a new breast cancer and ovarian cancer (Metcalfe et al., 2004; Easton, Ford, & Bishop, 1995; Breast Cancer Linkage Consortium, 1999), and other members of the family can be tested for the identified mutation. Yet, the majority of probands receive an uninformative test result (Vink, van Asperen, Devilee, Breuning, & Bakker, 2004). That is, although a deleterious mutation was not detected, hereditary risk cannot be ruled out due to the possibility of an undetected mutation in *BRCA1* or *BRCA2* or a mutation in another cancer susceptibility gene. Counselors typically provide these women with a qualitative estimate of their residual risk of carrying a mutation and of developing a second cancer. These risk estimates, which are based on various characteristics of a woman's family pedigree, are highly heterogeneous and entail a great deal of uncertainty. The uncertainty of this situation greatly complicates individual decision making about breast cancer risk management in this population.

It is not currently clear how receiving an uninformative *BRCA1/2* test result influences the difficulty of women's risk management decisions. To our knowledge, no research has examined women's psychological experience of risk management decision making after an uninformative test result. One relevant indicator of the psychological experience of medical decision making is *decisional conflict*, or the extent to which a person feels uncertain, unclear about personal values, uninformed, and unsupported in decision making (Janis & Mann, 1977; O'Connor, 1995). Higher decisional conflict scores have been associated with decision regret (e.g., Brehaut et al., 2003), likelihood of blaming a physician for adverse effects of cancer screening (Gattelari & Ward, 2004), and other adverse decision outcomes (see O'Connor, 1995, 2005). Women with higher decisional conflict may be more likely to vacillate between choices or to delay important decisions (O'Connor, 2005). To the extent this occurs, it could have serious consequences for women who are at high risk for new breast cancers, as is usually the case for women who receive uninformative *BRCA1/2* test results. Research addressing these issues would determine whether some of these women would benefit from additional decision support to reduce their uncertainty and distress and to help ensure that they engage in risk management activities that are appropriate for their degree of risk.

In light of the foregoing, our goal for this research was to investigate women's psychological experience of decision making following receipt of an uninformative *BRCA1/2* test result. We examined both their perception that they had made a final decision (*decision status*) and their decisional conflict. Decision status and decisional conflict were assessed 1-, 6-, and 12 months after disclosure of the uninformative genetic test result. First we sought to describe observed patterns of decision making across these three assessments. Second, we examined potential predictors of decisional conflict at 1- and 12-months post-disclosure in early to investigate correlates of elevated decisional conflict soon after test disclosure and one year later and to identify women at highest risk for poor decision making outcomes.

Potential Predictors of Decisional Conflict

The most commonly studied predictors of health decision making are health beliefs, which have a central place in leading social-cognitive theories of health protective behavior (see Weinstein, 1993). Perceived risk is one health belief that has received a great deal of research attention. For instance, research has shown that perceived risk for breast cancer is associated with health behaviors such as uptake of genetic counseling (Culver et al., 2001), use of mammography (e.g., Lerman, Rimer, Trock, Balshem, & Engstrom, 1990, McCaul, Branstetter, Schroeder, & Glasgow, 1996), and overuse of breast self-examination (Epstein et al, 1997).

Other commonly studied health beliefs are perceived benefits of and barriers to decision options (Weinstein, 1993). Research has generally found that perceived benefits are positively associated with screening behaviors such as mammography and pap tests whereas perceived barriers are negatively associated with them (e.g., Aiken, West, Woodward, & Reno, 1994; Rakowski et al., 1997; Russell, Champion, & Skinner, 2006). It may be that women are less conflicted about risk management decision making, in general, when they perceive that an option is associated with more benefits and fewer barriers. In the present study this seemed particularly likely to occur with respect to risk-reducing mastectomy (i.e., removal of non-diseased breast tissue as a prophylactic measure). Although risk-reducing mastectomy is not routinely recommended to women who receive uninformative results, it is an option that many of these women might consider. In fact, rates of risk-reducing mastectomy as high as 24% have been reported in the first year following an uninformative *BRCA1/2* test result in a sample of newly diagnosed breast cancer patients (Schwartz et al., 2004). We also examined perceived benefits and barriers to mammography, because this is the risk management option most frequently selected by women who receive an uninformative test result.

In addition, a characteristic of this population that is likely to have implications for their decision making is the fact that receiving an uninformative test result elicits various emotional responses. Evidence shows that women who receive an uninformative result experience distress that is not diminished by test disclosure, nor does it dissipate in the subsequent months. That is, their pre-testing levels of distress persist (Bish et al., 2002; O'Neill et al., in press; Schwartz et al., 2002; van Dijk et al., 2006). Follow-up assessments have documented elevated distress lasting as long as one year (O'Neill et al., in press), and some studies have shown comparable levels of distress among women who receive an uninformative test result and those found to carry a deleterious mutation (Schwartz et al., 2002; van Dijk et al., 2006). Although distress in both groups is generally modest on average, emotional reactions to an uninformative result vary across women (e.g., Hallowell et al., 2002). For instance, in a recent study of women who received an uninformative result we found significant individual variation in generalized distress, cancer-specific distress, and distress related to various aspects of genetic testing, with some women reporting highly elevated levels of distress (O'Neill et al., in press).

Importantly, there is growing evidence that emotions such as distress influence health decision making, and this evidence has revealed a way to advance understanding of health decision making beyond its current emphasis of health beliefs. Several theoretical approaches are relevant. For instance, Peters and her colleagues have suggested that emotions are a source of information that guides decision making, in addition to influencing decisions in other ways (Diefenbach et al., 2008; Peters, McCaul, Stefanek, & Nelson, 2006; Peters, Västfjäll, Gärling, & Slovic, 2006). Similarly, in Lazarus's cognitive-motivational-relational theory, emotions are said to denote core themes that describe the appraised relationship between a person and a potential stressor (Lazarus, 1999), an approach that is

relevant for understanding responses to an uninformative *BRCA1/2* test. For instance, anxiety reflects the appraisal that one is facing "uncertain, existential threat" (Lazarus, 1999, p. 96; also Lazarus, 1993). In this theory, as in Lazarus and Folkman's (1984) well-known transactional model of stress and coping, subjective responses to a potentially stressful event are viewed as an important determinant of whether it will evoke a stress response and emotions are viewed as an important determinant of how individuals will cope with a stressor.

Another emotion theory, developed by Consedine and colleagues, discusses emotion in evolutionary terms. They posit that discrete emotions evolved to direct motivational, cognitive, behavioral, and physiological responses to environmental conditions that have implications for survival (Consedine & Moskowitz, 2007). In this model different emotions have different implications for decision making. For instance, anxiety may hinder or facilitate risk management decision making depending on its focus. If it is focused on the result itself (e.g., genetic testing distress), anxiety may be reduced by avoiding the decision. However, if it is focused on adverse effects of not acting or selecting a particular option (e.g., anxiety regarding risk for breast cancer), anxiety may be reduced by reaching a decision and acting on it. Consistent with this general perspective is a study in which breast cancer-specific distress predicted overuse of breast self-exams, whereas generalized distress did not (Erblich, Bovbjerg, & Valdimarsdottir, 2000).

Of course, there is also the potential to experience positive emotions after genetic testing (e.g., Low, Bower, Kwan, & Seldon, 2008; Kinney et al., 2005). Positive emotions are rarely investigated with respect to decision making. To begin the process of understanding how positive experiences might influence decision making among women who receive uninformative genetic test results, we examined whether these were associated with decision making in addition to investigating the role of various types of emotional distress.

In sum, distress of various kinds is elevated among some women who receive uninformative genetic test results, and theory and research suggest that these emotional responses will have implications for risk management decision making. Moreover, some women report positive experiences after genetic testing and theory states that they, too, should influence decision making. Consequently, in addition to describing women's decisional conflict in the year following test disclosure and their patterns of decision making during that time (i.e., whether they felt they had made a final risk management decision), we investigated predictors of decisional conflict, focusing on decisional conflict 1- and 12-months post-disclosure (although we incorporated the 6-month post-disclosure outcomes for analyses focused on the trajectory of change in decisional conflict). These analyses allowed us to examine predictors of decisional conflict soon after test disclosure as well as later, after women had more time to consider their situation and their risk management options. Guided by social-cognitive theories of health protective behavior and theories positing a role of emotions in health decision making, predictors included frequently studied health beliefs (perceived risk for developing another breast cancer, benefits of and barriers to mammography and risk-reducing mastectomy) and generalized, cancer-specific, and genetic testing-related emotions (generalized anxiety, depressive symptoms, cancer-specific intrusion and avoidance, and genetic testing distress and positive experiences), all assessed at the 1-month post-disclosure assessment. We also investigated whether different patterns of decision making had implications for decisional conflict. Analyses predicting 1-month post-disclosure decisional conflict investigated concurrent relations, whereas analyses predicting 12-month post-disclosure decisional conflict investigated prospective relations.

Method

Participants

Potential participants were adult, English-speaking women with a history of breast cancer who were probands being tested for *BRCA1/2* mutations at Lombardi Comprehensive Cancer Center, Ruttenberg Cancer Center, or Englewood Hospital between April, 2001 and July, 2004. All had received an uninformative test result after either full sequencing of *BRCA1* and *BRCA2* or targeted testing for the three Ashkenazi Jewish founder mutations. They had to have at least a 10% probability of carrying a mutation prior to testing. For these analyses they also had to have at least one breast intact at the beginning of the study. Women were excluded if they were adopted ($n = 4$), missing decision status data at any assessment ($n = 64$), or missing decisional conflict data at all assessments ($n = 3$). Note that decision status data, which were needed to code women's pattern of decision making, were missing because some women missed all or part of a particular assessment; thus, women missing decision status data at a particular study assessment were also missing decisional conflict at that assessment. Compared to the 182 women in the final sample, the 71 women who were dropped were younger (Ms = 52.60 and 49.06 years old, respectively; $p = .02$); reported more benefits of mammography (Ms = 3.03 and 3.22; $p = .01$) and risk-reduction mastectomy (Ms = 2.53 and 2.76; $p = .05$); reported more barriers to mammography (Ms = 1.54 and 1.81; $p = .001$); were more likely to undergo bilateral mastectomy during the study (3% and 9%, $p = .03$); and more likely to have full *BRCA1/2* sequencing (37% and 54%; $p = .02$). They did not differ on other demographic or medical characteristics, other study variables or, when available, on decision status or decisional conflict.

Procedure

Participants were self-referred to the genetic counseling programs at each site. After providing consent, they received extensive pre- and post-test genetic counseling that was standardized across sites and monitored for fidelity. Pre-test counseling included discussion of the process of *BRCA1/2* testing, their risk for mutations, cancer risks associated with *BRCA1/2* mutations, interpretation of test results (including uninformative results), risk management, and potential benefits and risks of testing (see Schwartz et al., 2002, for a more detailed description). Post-disclosure counseling included disclosure of test results, discussion of implications of the specific test result received, and risk management recommendations. Women who received uninformative results were also given a qualitative estimate of their residual risk for breast and ovarian cancer based on their specific test result and their personal and family history. Because this was a high-risk population (≥ 10% probability of carrying a mutation), surveillance recommendations were consistent with recommendations for high-risk individuals (Burke et al., 1997), except that ovarian cancer screening and prophylactic oophorectomy were not discussed unless there was a family history of ovarian cancer. Women received a letter summarizing recommendations. Study measures were completed during telephone interviews conducted by trained research assistants prior to testing (Pretest) and 1, 6, and 12 months after disclosure of test results. Study procedures were approved by the Internal Review Boards at the study sites.

Measures

Decisional conflict was measured at 1-, 6-, and 12-months post-disclosure with the Decisional Conflict Scale (O'Connor, 1995), which assesses uncertainty about a decision (3 items), feeling uninformed (3 items), feeling unsupported in decision making (3 items), feeling unclear about values (3 items), and the perceived quality of the decision (4 items). Items such as "It's clear what choice is best for me" are rated on a scale from 1 (*Strongly agree*) to 5 (*Strongly disagree*). Women who had not made a final decision at a particular assessment were not asked questions about the perceived quality of their decision. In order

to create comparable scales for women who had made a final decision and those who had not, only the 12 items from the first four subscales were used. Items were averaged so that higher scores indicated higher decisional conflict. Cronbach's αs ranged from .85 to .93.

Decision Status was measured at the 1-, 6-, and 12-months post-disclosure with the question, "Have you made a final decision about how to manage your breast cancer risk?"

Depressive symptoms and generalized anxiety were measured 1-month post-disclosure with 12 items from the Brief Symptom Inventory (six items each for depressive symptoms and generalized anxiety; Derogatis & Melisaratos, 1983). Women were presented with a list of symptoms (e.g., "nervousness or shakiness inside") and rated how much discomfort each symptom had caused them in the past two weeks on a scale from 1 (*not at all*) to 4 (*extremely*). Items were summed; higher scores indicated greater symptomatology (α = .85 both subscales).

Cancer-specific intrusion and avoidance were measured 1-month post-disclosure with the Impact of Event Scale (Horowitz, Wilner, & Alvarez, 1979), which is commonly used to assess distress associated with a stressor (in this study, the experience of cancer in women's family). It includes seven items for intrusive thoughts and feelings (e.g., "I thought about it when I didn't mean to") and eight items for avoidance (e.g., "I stayed away from reminders of it"). Responses are made on a 4-point scale (0 = *not at all*, 1 = *rarely*, 3 = *sometimes*, 5 = *often*) to indicate how frequently each symptom occurred in the prior seven days. Items were summed to yield scales in which higher scores indicate greater intrusion (α = .84) or avoidance (α = .80).

Emotional responses to genetic testing were measured 1-month post-disclosure with the Multidimensional Impact of Cancer Risk Assessment Questionnaire (Cella et al., 2002). Three subscales are used to assess responses to the receipt of genetic test results (e.g., "feeling upset about your test result" and "feeling relieved about your test result"), including distress (six items), positive experiences (four items), and uncertainty (nine items), Responses are made on a 4-point scale (0 = *not at all*, 1 = *rarely*, 3 = *sometimes*, 5 = *often*), and items are summed to indicate higher distress (α = .72), positive experiences (α = .74), or uncertainty. In this study we did not use the uncertainty subscale because of conceptual overlap with decisional conflict.

Perceived risk for developing another breast cancer was assessed with a single question, "On a scale from 0 to 100, where 0 means that you definitely won't get breast cancer again and 100 means that you definitely will get breast cancer again, how likely would you say you are to develop breast cancer again?" This question has been recommended as an assessment of perceived risk (e.g., Fischoff, 1999) and is widely used (Bowen et al., 2004; Taylor et al., 2002).

Perceived benefits of and barriers to mammography were measured 1-month post-disclosure with a 15-item scale developed for this study. Seven items described potential benefits (e.g., early detection) and eight described potential barriers (e.g., radiation exposure). Women rated the importance of each item on a scale from 1 (*not at all important)* to 4 (*very important*). Ratings were averaged to create scales, with higher scores indicating greater importance of benefits or barriers. As might be expected given the diverse nature of these items, internal reliability was low for benefits (α = .65), although it was adequate for barriers (α = .78).

Perceived benefits of and barriers to risk-reducing mastectomy were measured at 1-month post-disclosure with a 16-item scale developed for this study. Seven items described potential benefits (e.g., reduced worry about breast cancer) and nine items described

potential barriers (e.g., risks of major surgery). Women rated the importance of each on a scale from 1 (*not at all important)* to 4 (*very important*). Ratings were averaged to create scales, with higher scores indicating greater importance of benefits (α = .85) and barriers (α = .78).

Sociodemographic characteristics were self-reported at Pretest and included age, marital status (married/other), race/ethnicity (White/non-White), educational attainment (high school or less/some college or more), annual household income, and having at least one child (yes/no).

Medical and genetic testing information was self-reported and included date of diagnosis, personal medical history, current treatment status (chemotherapy or radiotherapy), and personal and family history of cancer. For these analyses, risk for carrying a genetic mutation was calculated using Myriad Tables (Spring 2006 table; see Frank et al., 2002). Genetic test type was also recorded (Jewish panel negative versus full *BRCA1/2* sequencing negative).

Data Analyses

First, descriptive statistics were computed and a small number of missing values were mean or mode replaced (for continuous and categorical variables, respectively). Income was missing for 22 women and had limited variability (78% of women reported income in the highest category); therefore, income was not examined in these analyses. Next we examined women's reports of whether they had reached a final decision about how to manage their breast cancer risk at each study assessment (their decision status). Distinct patterns of decision making across the 1-, 6-, and 12-month post-disclosure assessments were investigated with descriptive analyses, and paired-sample *t*-tests and descriptive statistics were used to examine associations between patterns of decision making and changes in decisional conflict over time. Finally, we conducted hierarchical multiple regression analyses predicting 1- and 12-month post-disclosure decisional conflict. We focused on these two assessments (omitting analyses predicting 6-month post-disclosure decisional conflict) for several reasons. First, the differences between the 1- and 12-month assessments were expected to be larger and more easily interpretable than differences between the 1- and 6-month or the 6- and 12-month assessments. Second, examining predictors of 1- and 12-month post-disclosure decisional conflict allowed us to contrast concurrent and prospective relations observed for these two distinct timepoints. These regression models enabled us to examine the unique contribution of health beliefs and emotion variables as predictors of decisional conflict after controlling for other relevant variables, as described below.

Results

Women in the sample were, on average, 52 years old (*SD* = 10 years). Most were married (72%), White (96%), had completed at least some college (96%), and had moderate to high annual household income (median > $75,000). They had been diagnosed with breast cancer nearly six years earlier, on average (*M* = 5.96, *SD* = 7.80). Thirty-seven percent had undergone full *BRCA1/2* sequencing and the rest had undergone Jewish panel testing. Mean decisional conflict for the full sample at the 1-, 6-, and 12-month post-disclosure assessments was 2.00 (*SD* = 63), 1.94 (*SD* = 63), and 1.86 (*SD* = 60), respectively. Paired sample *t*-tests comparing sample means indicated no change from 1- to 6-months post-disclosure, *t* = 1,35, *p* = .18 and a trend toward a reduction in decisional conflict from 6- to 12-months post-disclosure, *t* = 1.74, *p* = .08. The reduction in decisional conflict from 1- to 12-months post-disclosure was significant, *t* = 2.88, *p* = .004.

Decision Status and Patterns of Decision Making

The percentage of women who reported having made a final decision was 66% at 1-month post-disclosure, 84% at 6-months post-disclosure, and 87% at 12-months post-disclosure. Several patterns were apparent. Fifty-nine percent of women had made a final decision across all three assessments (*early decision makers*). The second most common pattern was for women to say they had not made a final decision at 1-month post-disclosure, and then to say they had made one at 6- and 12-months (18%; *intermediate decision makers*). Next, 6% of women had not made a decision at 1- and 6-months post-disclosure but had made one at 12-months (*late decision makers*). The remaining 19% either transitioned from saying they had made a final decision to saying they had not, demonstrated a complex pattern that changed from assessment to assessment, or had not made a final decision at any assessment (*non-decision makers*).

These patterns had implications for decisional conflict (see Figure 1). Paired-sample *t*-tests revealed a significant decline in decisional conflict for early decision makers between 6-and 12-months post-disclosure, $t = 2.30$, $p = .02$, and a significant decline in decisional conflict between 1- and 12-months post-disclosure for intermediate decision makers, $t = 5.04$, $p < .001$, and late decision makers, $t = 2.68$, $p = .03$. In contrast, non-decision makers demonstrated a marginally significant increase in decisional conflict from 1- to 12-months post-disclosure, $t = -1.77$, $p = .09$.

Next we examined the percentage of women in each group who had high decisional conflict using a cutoff of 2 based on evidence that decisional conflict scores greater than 2 have been associated with adverse decision making outcomes (O'Connor, 1995, 2005). Among early decision makers, the percentages of women with decisional conflict scores greater than 2 at 1-, 6-, and 12-months post-disclosure were 24%, 31%, and 22%, respectively. Among intermediate decision makers, these percentages were 73%, 42%, and 21%; among late decision makers, they were 91%, 55%, and 64%; and among non-decision makers, they were 54%, 79%, and 67%.

Predicting 1-Month Post-Disclosure Decisional Conflict

Prior to testing this model, we evaluated the need to control medical and demographic factors by examining bivariate associations between 1-month post-disclosure decisional conflict and potential control variables. No medical or demographic variables were significantly associated with decisional conflict at this timepoint. Therefore, we conducted a hierarchical multiple regression in which 1-month post-disclosure decisional conflict was regressed on 1-month post-disclosure decision status (a dummy coded variable comparing women who had made a final decision at this timepoint to those who had not; Step 1), health belief variables (Step 2), and emotion variables (Step 3). The results of this analysis are shown in Table 1. The full model was significant, $F(12,169) = 8.08$, $p < .001$, and predicted 36% of the variance in the outcome. In Step 1, women who had made a final decision one month after test disclosure reported significantly lower concurrent decisional conflict than those who had not, $t = -8.30$, $p < .001$. Decision status predicted 28% of the variance in 1-month post-disclosure decisional conflict. In Step 2, health beliefs accounted for an additional 7% of the variance in this outcome. Of the five health belief variables, two predicted a significant proportion of variance: women with higher perceived risk, $t = 3.51$, $p = .001$, and those who perceived more benefits of risk-reducing mastectomy, $t = 2.17$, $p = .03$, had greater 1-month post-disclosure decisional conflict. In contrast, none of the emotion variables emerged as significant predictors in Step 3

Predicting Decisional Conflict One Year After Test Disclosure

Before testing a model investigating predictors of 12-month post-disclosure decisional conflict, we evaluated the need to control medical and demographic factors by examining their bivariate associations with this outcome, both before and after partialling out 1-month post-disclosure decisional conflict. We found that higher risk for carrying a genetic mutation (according to women's Myriad scores; Frank et al., 2002) was associated with lower 12-month post-disclosure decisional conflict after controlling the effect of 1-month post-disclosure decisional conflict ($p = .04$). Therefore we conducted a hierarchical multiple regression in which 12-month post-disclosure decisional conflict was regressed on risk for carrying a genetic mutation and 1-month post-disclosure decisional conflict (Step 1), three dummy coded variables testing the effects of the four decision status patterns (with non-decision makers as the comparison group; Step 2), health belief variables (Step 3), and emotion variables (Step 4). Because 1-month post-disclosure decisional conflict was controlled, all findings refer to residualized change in decisional conflict from 1- to 12-months post-disclosure.

The results of this analysis are shown in Table 2. The full model was significant, $F(16,165) = 7.97$, $p < .001$, and predicted 44% of the variance in 12-month post-disclosure decisional conflict. In Step 1, having higher decisional conflict one month after test disclosure predicted higher decisional conflict at the 12-month post-disclosure assessment, $t = 7.52$, $p < .001$. Further, women at high risk for carrying a genetic mutation reported lower 12-month post-disclosure decisional conflict, $t = -2.03$, $p = .04$. Together these variables accounted for 25% of the variance in 12-month post-disclosure decisional conflict. In Step 2, early decision makers reported lower 12-month post-disclosure decisional conflict than did non-decision makers, $t = -4.82$, $p < .001$. The same was true for intermediate decision makers, $t = -4.99$, $p < .001$, and late decision makers, $t = -1.97$, $p = .05$. Together, these variables accounted for an additional 11% of the variance. In Step 3, health beliefs were not significant predictors of 12-month post-disclosure decisional conflict, either individually (ps from .14 to .81) or as a group (p for step = .37). However, several emotion variables emerged as significant predictors in Step 4. Specifically, more positive genetic testing experiences 1 month after test disclosure was associated with lower 12-month post-disclosure decisional conflict, $t = -2.36$, $p = .02$, as was having higher generalized anxiety one month after test disclosure, $t = -2.87$, $p = .01$. Having higher depressive symptoms one month after test disclosure was associated with higher 12-month post-disclosure decisional conflict, $t = 2.78$, $p = .01$. Unique variance associated with other emotion variables did not predict a significant amount of variance in 12-month post-disclosure decisional conflict (ps ranged from .30 to .65). Together, emotional factors accounted for an additional 5% of the variance in this outcome beyond other variables in the model. In addition, after controlling for emotional factors in this step, perceived benefits of risk reduction mastectomy emerged as a significant predictor of 12-month post-disclosure decisional conflict, $t = 1.98$, $p = .049$.

Discussion

Women who receive an uninformative *BRCA1/2* test result must make risk management decisions without the benefit of knowing whether their cancer was due to a genetic mutation that also increases their risk for developing a new breast cancer. The results of the present study suggest that risk management decision making is a complex process for many of these women. We focused on decision making during the year following test disclosure because it is an ideal time to intervene to ensure that women engage in appropriate and effective risk management strategies. Nineteen percent of our sample demonstrated a pattern of decision making that suggested they had a difficult time reaching what they perceived to be a final risk management decision during that time. An additional 6% did not make what they felt was a final decision until a full one year after test disclosure. Of course, high risk breast

cancer survivors may be presented with new information at any time that could cause them to revisit their risk management decision (e.g., a suspicious screening test or the diagnosis of a second breast cancer).

An important issue revealed by our findings is that having made a final decision—even one that was seemingly stable—did not necessarily protect women from decisional conflict. That is, a substantial proportion of women who appeared to have made an early decision (i.e., "early decision makers") nonetheless remained at risk for poor decision outcomes, as indicated by their high decisional conflict scores. Clearly, even women who have made what they feel is a final risk management decision can experience lingering dissatisfaction with or lack of confidence in their decision. Notably, decisional conflict was highest among women who appeared to have struggled with decision making (i.e., "late decision makers" and "non-decision makers"), suggesting a need for further research to understand why women fall into these groups.

However strong, the association between decision status and decisional conflict was not perfect. To gain a more complete picture of decisional conflict after receipt of an uninformative BRCA1/2 test result, we investigated several sets of predictors of decisional conflict. The first set of predictors we investigated were health beliefs drawn from leading social-cognitive theories, namely, women's beliefs about their risk for developing another breast cancer and their beliefs about the benefits of and barriers to two potential risk management strategies: risk-reducing mastectomy and mammography. The role played by these health beliefs depended on the time point being examined. One month after test disclosure, women reported higher decisional conflict if they had higher perceived risk for developing another breast cancer or if they perceived more benefits of risk-reducing mastectomy. One year later the association between perceived benefits of risk-reducing mastectomy and decisional conflict was still apparent (albeit only after controlling for emotional factors). However, there was no prospective association between perceived risk and decisional conflict a year after test disclosure. Nonetheless, it should be noted that perceived risk could have an indirect effect on later decisional conflict through several channels. For instance, women with higher perceived risk were less likely to have made a final risk management decision at each assessment, less likely to be an early decision maker, and more likely to be a non-decision maker. One goal of genetic counseling is to ensure accurate risk perceptions. Therefore, women who have undergone genetic counseling should hold risk perceptions that are relatively accurate and it may not be appropriate to attempt to lower them. Rather, it may be that women with high perceived risk would benefit from decision aids. In our own research we have found that an interactive decision aid was particularly beneficial among *BRCA1/2* carriers who were having the most difficulty reaching a management decision in the month following receipt of test results (Schwartz et al., in press).

The positive association between perceived benefits of risk-reducing mastectomy and elevated decisional conflict suggests that women who were considering risk-reducing surgery found decision making to be more difficult than those who were not considering it. In support of this interpretation, post hoc analyses (not shown) revealed that women who perceived more benefits of risk-reducing mastectomy were more likely to say they were considering the surgery. This finding is not surprising; the decision about whether or not to undergo risk-reducing mastectomy is a difficult one. It may be even more difficult for women with uninformative test results, for whom actual risk is difficult to quantify. We note that objective risk was not associated with decisional conflict or perceived benefits of and barriers to risk-reducing mastectomy. Thus, it was not the case that decisional conflict was primarily elevated among those at the highest risk for breast cancer.

Although investigation of health beliefs has proven useful in understanding health protective decision making, social-cognitive theories exclude other potentially important classes of variables, including emotions. In light of this fact, we extended our investigation of health beliefs by also investigating associations between decisional conflict and women's emotional responses to *BRCA1/2* testing one month after test disclosure. As noted earlier, we and others have found enduring elevated distress among some women who receive uninformative test results (e.g., Bish et al., 2002; O'Neill et al., in press; Schwartz et al., 2002; van Dijk et al., 2006), and this distress may influence women's risk reduction decision making. In the present study emotions did not predict concurrent decisional conflict after accounting for women's decision status and their health beliefs. However, we found prospective associations between early emotional responses and later decisional conflict. Women who reported greater generalized anxiety and more positive genetic testing experiences one month after test disclosure reported lower decisional conflict one year later, whereas women who reported more depressive symptoms one month after test disclosure reported higher decisional conflict one year later. These findings suggest that depressive symptoms shortly after test disclosure could be used to identify women who need assistance with decision making. Notably, depression has been associated with cognitive styles such as pessimism (Corcoran et al., 2006) and underestimation of performance (Fu et al., 2005). It has also been found to have an adverse influence on decision outcomes (Damasio, 1997).

We also found a negative prospective association between generalized anxiety and decisional conflict. Anxiety and depression tend to co-occur, yet they were not so highly correlated in this sample that multicollinearity was a likely explanation for our findings. Furthermore, when depression was dropped from the model (analysis not shown), the effect of generalized anxiety remained negative, although it was no longer significantly related to decisional conflict. This pattern of results suggests that it was the unique variance associated with anxiety, controlling for other variables in the model, that was prospectively related to lower decisional conflict. The nature of that unique variance is unclear; however, the tripartite model of anxiety and depression (Clark & Watson, 1991) suggests that it may be worthwhile to focus on general physiological tension and hyperarousal in future research, in that these are characteristics that differentiate anxiety from depression.

The salutary effect of positive genetic testing experiences observed in this study is, to our knowledge, the first evidence for an association between positive emotions and testing-related decision making. Lazarus (1993, 1999) noted that positive emotions signal that progress is being made toward important life goals, and Consedine and his colleagues (Consedine & Moskowitz, 2007) suggest that they may affect health outcomes and medical decision making through cognitive pathways as well as more effective problem solving and cognitive flexibility (Isen & Labroo, 2003). Thus, positive genetic testing experiences may indicate that adaptive self-regulatory processes have been engaged. In the present study, women who reported more positive genetic testing experiences were more likely to report having reached a final risk management decision at each study assessment. They were also more likely to be an "early decision maker" and less likely to be a "non-decision maker." Such associations are consistent with the idea that these women were motivated to act quickly to reduce their risk and that they may have engaged in more effective cognitive processing concerning their options and the implications of each.

Notably, situation-specific domains of distress, including cancer-specific intrusion and avoidance and genetic testing-related distress, were not predictive of decisional conflict. Clearly, more research is needed to clarify the associations between specific emotions and decision making in this population. Recall that Consedine and colleagues (Consedine & Moskowitz, 2007) theorize that discrete emotions direct motivational, cognitive, behavioral, and physiological responses to environmental conditions. Given our findings, future

research on the decision making of women who have received an uninformative *BRCA1/2* test result should include measures that assess specific emotional responses most likely to occur among these women. These measures should capture differences in the focus of emotional responses (e.g., anxiety about the potential for a breast cancer recurrence versus anxiety about selecting a risk-reduction option that could later be regretted) and assess both positive and negative emotional responses to the test result and potential risk reduction options. For instance, some women may experience relief following receipt of an uninformative test result, and their cognitions, motivational states, risk reduction behaviors, and physiological responses may differ from those of women who do not experience this emotion.

There are some limitations of this study that should be noted. First, generalizability of these findings may be limited by several characteristics of our sample. Because all participants received free genetic counseling, they may differ from women who receive counseling in a clinical setting. Further, most participants were White, employed, college educated, and affluent, reflecting the population currently most likely to use *BRCA1/2* testing. Our sample also included only women who had been diagnosed with breast cancer who were at high risk for carrying a genetic mutation. It is currently unclear how decision making processes may differ in unaffected women, lower risk women, or women with ovarian cancer. In addition, missing data led us to drop a group of women who had skipped some assessments and who tended to be younger and to endorse greater benefits and barriers to risk reduction options. Although statistically significant, the small size of the observed differences and the lack of differences on most key study variables suggest that biases introduced as a result of missing data were minimal. However, the characteristics of the women dropped for missing data may indicate a need for further research on the unique needs of younger women facing this decision. Second, several of our measures could have been improved. Perceived risk was assessed with a single item, albeit one that is commonly used in the literature and has demonstrated validity. We should note that we also collected data using another commonly used measure of perceived risk that asked women to rate how likely they were to have a recurrence on a scale from “not at all likely” to “definitely.” Results did not differ when this alternative variable was used in analyses. In addition, our measure of residual risk was relatively crude. It is possible that had we better characterized residual risk, we might have observed stronger associations between residual risk and decisional conflict. Finally, these data are correlational, and associations observed in these findings are not necessarily causal. Of course, in addition to emotions influencing decisional conflict, decisional conflict may elicit emotional responses. Yet, the longitudinal, prospective study design revealed associations that are consistent with a causal relations. Furthermore, the plausibility of these associations is supported by theory and research. Nonetheless, research will be needed to clarify the causal direction of these results. Such research is particularly important if these findings are to guide development of interventions.

Despite these limitations, our findings provide useful insight into decision making following receipt of an uninformative genetic test result and extend current knowledge in important ways, particularly with respect to the role of emotions in decision making among women who receive an uninformative *BRCA1/2* test result. The findings suggest that a substantial number of these women may benefit from assistance with risk management decision making. Genetic counselors are one potential source of such assistance. Moreover, there is growing evidence that decision aids can lower decisional conflict and improve decision outcomes (e.g., O’Connor et al., 2007; Schwartz et al., in press). The development of a decision aid for women who receive uninformative *BRCA1/2* test results may be warranted, particularly in light of the increasing availability and use of these tests. Any such development should attend to recent critiques of this area (Nelson et al., 2007). Specifically, extending the conclusions of Nelson et al., it may be particularly important to help women

manage the uncertainty associated with their uninformative test result and their future cancer risks.

These findings may also have implications for other groups undergoing genetic testing, for instance, unaffected women undergoing *BRCA1/2* testing or individuals being tested for hereditary colon cancer. As new tests become increasingly available to the public, more people will be faced with the need to manage their risk for cancer and other serious diseases in the face of complex information and uncertain risk. The present study highlights the importance of health beliefs in health decision making as well as the benefits of considering emotional factors in addition to more commonly studied cognitive factors associated with decisional outcomes. It also provides evidence supporting the need for supportive resources to facilitate decision making among individuals coping with the results of genetic testing.

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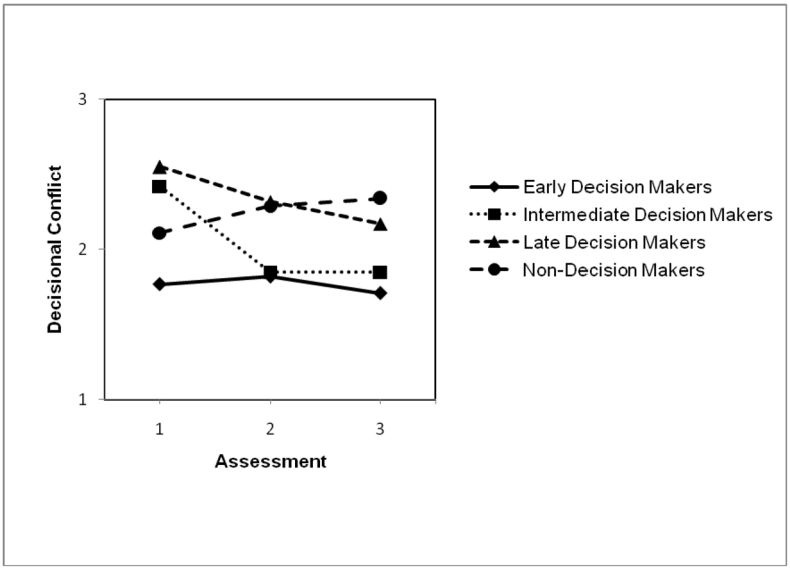

Figure 1.
Decisional conflict as a function of decision making pattern.

Table 1

Multiple Regression Predicting Decisional Conflict One-Month Post-Disclosure (N = 182)

	Step 1		Step 2		Step 3	
Variable	**B**	***SE***	**B**	***SE***	**B**	***SE***
Final Decision at Time 1	−.71	.09***	−.62	.09***	−.64	.09***
Perceived Risk For Developing Another Breast Cancer			.01	.002**	.01	.002**
Perceived Benefits of Mammography			.01	.09	.03	.09
Perceived Barriers to Mammography			−.02	.08	−.02	.09
Perceived Benefits of Risk-reducing Mastectomy			.11	.05*	.10	.05†
Perceived Barriers to Risk-reducing Mastectomy			.01	.07	−.01	.08
Genetic Testing Distress[a]					−.01	.10
Genetic Testing Positive Experiences					−.001	.01
Cancer-Specific Intrusion					.01	.01
Cancer-Specific Avoidance					−.01	.01
Depressive Symptoms					−.003	.02
Generalized Anxiety					.02	.02
Δ*F* for Step	68.83***		3.96**		.64	
R^2 for Step	.28		.07		.01	

† $p < .10$.

* $p < .05$.

** $p < .01$.

** $p < .001$

[a] Genetic testing distress was coded 1 = *any genetic testing distress*, 0 = *no genetic testing distress*.

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Table 2

Multiple Regression Predicting Decisional Conflict 12-Months Post-Disclosure (N = 182)

	Step 1		Step 2		Step 3		Step 4	
Variable	**B**	***SE***	**B**	***SE***	**B**	***SE***	**B**	***SE***
Time 1 Decisional Conflict	.47	.06***	.45	.06***	.46	.07***	.47	.07***
Risk for Carrying a Genetic Mutation	−.01	.003*	−.01	.003*	−.01	.003*	−.01	.003**
Early Decision Maker			−.49	.10***	−.49	.11***	−.45	.10***
Intermediate Decision Maker			−.62	.12***	−.64	.13***	−.63	.13***
Late Decision Maker			−.34	.17*	−.36	.18*	−.38	.18*
Perceived Risk For Developing Another Breast Cancer					−.002	.002	−.003	.002
Perceived Benefits of Mammography					.03	.08	.02	.08
Perceived Barriers to Mammography					−.02	.08	−.02	.08
Perceived Benefits of Risk-reducing Mastectomy					.07	.05	.10	.05*
Perceived Barriers to Risk-reducing Mastectomy					.08	.07	.04	.07
Genetic Testing Distress[a]							.09	.09
Genetic Testing Positive Experiences							−.02	.01*
Cancer-Specific Intrusion							.004	.01
Cancer-Specific Avoidance							−.004	.01
Depressive Symptoms							.05	.02**
Generalized Anxiety							−.05	.02**
ΔF for Step	30.10***		10.22***		1.09		2.62*	
R^2 for Step	.25		.11		.02		.05	

†$p < .10$.

*$p < .05$.

**$p < .01$.

***$p < .001$

[a]Genetic testing distress was coded 1 = *any genetic testing distress*, 0 = *no genetic testing distress*.

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Psychological adaptation and birth outcomes: The role of personal resources, stress, and sociocultural context in pregnancy

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Psychological Adaptation and Birth Outcomes: The Role of Personal Resources, Stress, and Sociocultural Context in Pregnancy

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Prenatal psychosocial predictors of infant birth weight and length of gestation were investigated in a prospective study of 120 Hispanic and 110 White pregnant women. Hypotheses specifying that personal resources (mastery, self-esteem, optimism), prenatal stress (state and pregnancy anxiety), and sociocultural factors (income, education, ethnicity) would have different effects on birth outcomes were tested using structural equation modeling. Results confirmed that women with stronger resources had higher birth weight babies ($\beta = .21$), whereas those reporting more stress had shorter gestations ($\beta = -.20$). Resources were also associated with lower stress ($\beta = -.67$), being married, being White, having higher income and education, and giving birth for the first time. There was no evidence that resources buffered the effects of stress. The importance of personal resources in pregnancy is highlighted along with implications for understanding the etiology of adverse birth outcomes.

Key words: pregnancy, stress, personal resources, adaptation, mastery

Pregnancy is a major life transition requiring adaptation of many kinds (Dunkel-Schetter, Gurung, Lobel, & Wadhwa, in press; Lederman, 1984; Lobel, 1998). A woman's ability to adapt to the changes and challenges of pregnancy affects her physical and mental health and appears to influence the health of her developing baby (Dunkel-Schetter & Lobel, 1998). Fully understanding psychological adaptation during pregnancy and its effects on birth outcomes requires consideration of the many factors that may affect prenatal adaptation. These factors include psychological resources and vulnerabilities and a woman's sociocultural milieu (Aneshensel, 1992; Pearlin, 1989; Taylor & Aspinwall, 1996; Taylor, Repetti, & Seeman, 1997).

Much health research on pregnancy is motivated by the serious social and medical ramifications of adverse birth outcomes, especially preterm delivery (PTD; birth before 37-weeks gestation) and low birth weight (LBW; birth weight ≤2,500 g). LBW infants may be small because of PTD or because of inadequate growth, technically referred to as *fetal growth restriction* (FGR; U.S. Department of Health and Human Services [USDHHS], 1985). PTD and LBW occur in a substantial percentage of live births in the United States (11% and 7%, respectively; Guyer, Strobino, Ventura, MacDorman, & Martin, 1996; National Center for Health Statistics, 1993) and are the major causes of perinatal, neonatal, and infant mortality and morbidity in the United States (Berkowitz & Papiernik, 1993; Paneth, 1995). Despite considerable research attention, the etiology of PTD and LBW remains little understood, and their incidence has tended to increase in the United States in recent years (Guyer et al., 1996; National Center for Health Statistics, 1993).

Stress in Pregnancy

There is growing evidence that women who experience more prenatal stress and anxiety have significantly higher rates of adverse birth outcomes (see Dunkel-Schetter, 1998; Lobel, 1994; Paarlberg, Vingerhoets, Passchier, Dekker, & Van Geijn, 1995). For instance, one prospective study provided evidence that high scores on a factor incorporating three phenomenological indicators of stress (state anxiety, perceived chronic stress, and life event distress) predicted

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lower infant birth weight and shortened gestation after controlling for medical risk, parity, and maternal substance use (Lobel, Dunkel-Schetter, & Scrimshaw, 1992; see also Copper et al., 1996; Hedegaard, Henriksen, Secher, Hatch, & Sabroe, 1996; Nordentoft et al., 1996; Wadhwa, Sandman, Porto, Dunkel-Schetter, & Garite, 1993). In general, previous research has suggested that the relationship between multidimensional stress measures and adverse birth outcomes is stronger for shortened gestation than for birth weight or fetal growth (Lobel, 1994).

State anxiety—an emotional response to environmental stressors (S. Cohen, Kessler, & Gordon, 1995; Lobel & Dunkel-Schetter, 1990)—has been the most commonly studied affective state in pregnancy and is associated, albeit weakly, with birth outcomes in some studies (Lobel, 1994). In addition, a contextually tied form of anxiety, pregnancy-related anxiety, has been developed in our research and is conceptualized as a woman's fears about her baby's health, her own health, and labor and delivery. Most prior studies of stress in pregnancy have not examined this form of anxiety, yet evidence suggests that it predicts shortened gestation (Wadhwa et al., 1993).

In addition to psychosocial stress, we investigated the possibility that maternal personal characteristics or resources influence birth outcomes. These resources include generalized beliefs about oneself (self-esteem), one's future (dispositional optimism), and one's perceived ability to control important outcomes (mastery or perceived control; Hobfoll, 1985). Such beliefs have been shown to promote adaptation and resilience in nonpregnant women by influencing processes such as stress appraisals, health-related behaviors, coping behaviors, and physiological and emotional responses to stressors (e.g., Aspinwall & Taylor, 1992; Bandura, Cioffi, Taylor, & Brouillard, 1988; Carver & Gaines, 1987; DeLongis, Folkman, & Lazarus, 1988; Folkman, Lazarus, Gruen, & DeLongis, 1986; Frankenhaeuser, 1982; Hobfoll & Leiberman, 1987; Marshall & Lang, 1990; Pearlin, Menaghan, Lieberman, & Mullen, 1981; Wiedenfeld et al., 1990; see also Epel, McEwen, & Ickovics, 1998; Park, 1998).

Although personal resources have received little attention in pregnancy research, existing evidence suggests that self-esteem and mastery may be associated with birth outcomes. In one study (Norbeck & Tilden, 1983), an index of "emotional disequilibrium" that included self-esteem, anxiety, and depression predicted infant complications after controlling for medical risk, life stress, demographic variables, and emotional support. However, it was not possible to distinguish the unique effects of self-esteem in these results. A second study (Goldenberg et al., 1991) found that both low self-esteem and low mastery predicted a higher likelihood of giving birth to a baby who was small for gestational age after controlling for known risk factors such as smoking, maternal education, height, weight, and age. However, a more recent study of 2,593 pregnant women by the same research team did not replicate the association between mastery and FGR (Copper et al., 1996). Differences in sample characteristics, outcomes studied, and the mastery measures are possible reasons for this inconsistency.

Unlike self-esteem and mastery, dispositional optimism (the generalized expectancy of positive outcomes) has not been examined in published research on birth outcomes; however, it has been associated with better adaptation to stressful life circumstances in terms of both physical and mental health outcomes in community and student samples (Scheier & Carver, 1992). Furthermore, dispositional optimism has been found to predict lower levels of anxiety, more positive states of mind, and reduced substance use during pregnancy (Park, Moore, Turner, & Adler, 1997).

The social or sociocultural level of analysis is particularly important in pregnancy research, as considerable evidence has shown (see Hobel, 1996; Hughes & Simpson, 1995; Kramer, 1987). Variables such as ethnic background and culture can influence the occurrence of events and activities in one's life; the way in which events are interpreted and coped with; access to social and personal resources; and the unique constellation of norms, demands, and opportunities in the immediate social environment (Revenson, 1990; Szapocznik & Kurtines, 1993; Taylor et al., 1997). For instance, cultural norms and values shared by many Hispanics may influence pregnancy and birth outcomes (Collins & Shay, 1994) through their effect on health-related behaviors (Myers, Kagawa-Singer, Kumanyika, Lex, & Markides, 1995), unique stressor exposure (e.g., acculturative stress; Berry, 1994), and coping strategies (Jung, 1995). Moreover, Latin cultures have been characterized as tending toward *fatalism,* or the belief that the world "is controlled by external natural and supernatural forces" (Arce & Torres-Matrullo, 1982, p. 231). To the extent that this is the case, one would expect Hispanics to be lower in optimism and mastery (e.g., Mirowsky & Ross, 1984).

In the United States, ethnic minority status and poverty are highly correlated (Williams, 1990). Not only can ethnicity influence pregnancy and birth outcomes through cultural norms and values, it may also exert an influence through its association with socioeconomic status (SES), especially income and education. Research has shown that lower SES groups experience a greater number of stressors and higher levels of psychological distress (Seguin, Potvin, St. Denis, & Loiselle, 1995). They are more likely to engage in adverse health-related behaviors (Adler et al., 1994), to live and work in riskier environments (Anderson & Armstead, 1995; Taylor et al., 1997), and to have fewer of the social resources that buffer stress during pregnancy (Seguin et al., 1995).

With respect to birth outcomes, both ethnic minority status and low SES have been linked to higher infant mortality and morbidity, shorter gestations, lower infant birth weight, and higher rates of FGR (Flack et al., 1995; Gould & LeRoy, 1988; Hughes & Simpson, 1995; Kramer, 1987; Lieberman, Ryan, Monson, & Schoenbaum, 1987; Nersesian, 1988; Newton & Hunt, 1984; Paneth, Wallenstein, Keily, & Susser, 1982; USDHHS, 1985). Potential mediators of these relationships remain unexplained and thus are important targets of research efforts. We examined stress and personal resources as possible mediators in this study.

The objective of this study was to examine the relationships between prenatal psychosocial stress, personal re-

sources, the sociocultural context, and infant birth weight and gestational age at birth. Three possible roles for personal resources were investigated. The first was their possible direct effect on birth outcomes. Second, the possibility was examined that personal resources indirectly protect against poorer birth outcomes by reducing appraised stress for all women, regardless of their psychosocial stress level. This hypothesis required that the effects of personal resources on birth outcomes be at least partially mediated by reduced stress. Third, we examined whether personal resources act as stress buffers (S. Cohen & Edwards, 1989; Taylor & Aspinwall, 1996), modifying the relationship between stress and birth outcomes.

We expected that psychosocial stress (state anxiety and pregnancy-related anxiety) would be associated with length of gestation but not with birth weight (see Lobel, 1994). Conversely, the literature on self-esteem, mastery, and birth outcomes reviewed here suggests an association between these personal resources and birth weight (see Goldenberg et al., 1991; Norbeck & Tilden, 1983). Therefore, we predicted that personal resources would have a direct effect on birth weight (i.e., intrauterine growth), most likely by influencing variables not measured here, for instance, health behaviors such as nutrition, substance use, and self-care. However, personal resources have also been associated with resilience in the face of stressors because of more positive appraisals of potential stressors and more adaptive coping efforts (Jerusalem, 1993; Major, Richards, Cooper, Cozzarelli, & Zubek, 1998). Consequently, we expected personal resources to indirectly influence length of gestation through stress reduction. Finally, we expected that Hispanics and individuals with low incomes and relatively little education would report more stress and fewer personal resources and would exhibit higher rates of adverse birth outcomes.

Method

Participants

The sample consisted of 230 pregnant women receiving prenatal care over a 3-year period (1993–1996) at a southern California medical center and an affiliated low-risk birthing center. These sites served an ethnically diverse and low-income population of women from the surrounding urban metropolitan area in southern California. Participants were recruited into the study during the late second or early third trimester (22–28 weeks) of pregnancy. Sixty-two percent of the women who were approached agreed to participate. Data were available for 145 (44%) of those who declined; they did not differ in age or marital status from the women who participated. However, they did differ in their likelihood of being Hispanic and in parity. Decliners were more likely to be Hispanic (68%) than nondecliners (48%) and had, on average, higher parity ($M = 1.55$, $SD = 0.55$) than nondecliners ($M = 0.82$, $SD = 1.00$).

The initial sample of women who agreed to participate included 276 women older than 18 years of age. All participants fluently spoke English or Spanish and were pregnant with singleton intrauterine pregnancies. Twenty-two of these women dropped out after the first assessment, reducing the sample size to 254 (8% attrition). Analyses indicated that participants who dropped out after the first assessment did not differ from those who remained in terms of age, income, education, parity, marital status, medical risk, or infant birth weight. They did differ in length of gestation, with women who dropped out giving birth nearly 1 week sooner ($M = 38.35$, $SD = 2.65$) than those who did not ($M = 39.26$, $SD = 1.54$, $p < .05$). Twelve of the remaining participants were excluded from analyses because they belonged to an ethnic group other than Hispanic or non-Hispanic White, and an additional 12 participants did not have complete birth outcome data because they delivered at sites other than the research sites. Thus, the final sample of 230 included women who self-identified as non-Hispanic White ($n = 110$) or Hispanic ($n = 120$) and had complete birth outcome data. The average age of the 230 participants was 25.73 years ($SD = 5.51$ years, range = 17–40 years). Half of the participants reported a household income of $20,000 or less per year. Average educational attainment was 12.1 years ($SD = 3.62$ years). Sixty percent of the participants were married, and 47% had never given birth (i.e., were nulliparous). Of the 120 Hispanics in the sample, 66% completed interviews and questionnaires in Spanish. Seventy-seven percent of the Hispanic women were born in Mexico, 18% were born in the United States, and the remainder were born in other Latin American countries. Foreign-born Hispanics had lived in the United States for a mean of 7 years ($SD = 6.80$ years).

Procedure

Bilingual staff members were trained in recruitment and interview procedures. Patients were recruited in English and Spanish during prenatal visits and were formally enrolled in the study after completing informed-consent procedures. Data were collected over two appointments scheduled approximately 2 weeks apart during the early third trimester of pregnancy (28–30 weeks). During each appointment, participants completed questionnaires and then met with a trained, bilingual interviewer for a 30- to 45-min structured interview conducted in English or Spanish.

Measures

Means and standard deviations for all measures are shown in Table 1. Instruments were chosen and developed with the goals of yielding equivalent meanings in Spanish and English and of being easily understood by women with little formal education. Instruments not already available in Spanish were created for this study by a professional translator using forward and backward translation procedures, followed by extensive pretesting.

Mastery. Mastery, the generalized belief that one's outcomes are under one's own control, was measured using the 7-item Mastery Scale (Pearlin & Schooler, 1978). Participants rated each item on a scale ranging from 1 (*strongly agree*) to 5 (*strongly disagree*). Reliability analysis revealed that the 2 positively worded items on the Spanish version of the scale exhibited low correlations with the total scale. These items were dropped from both English and Spanish versions of the scale. The mastery score for each participant was the mean of her responses to the 5 remaining items, which concerned not feeling able to control events, solve problems, or change important things; feeling helpless when dealing with problems; and feeling pushed around in life. Items were coded such that higher scores reflected greater mastery. This 5-item scale exhibited adequate internal reliability in both English ($\alpha = .81$) and Spanish ($\alpha = .74$).

Dispositional optimism. Dispositional optimism was assessed with the 8-item Life Orientation Test, a well-validated instrument (Scheier & Carver, 1985). Items were rated on a scale ranging from 1 (*strongly agree*) to 5 (*strongly disagree*). Reliability analysis

Table 1
Participant Characteristics by Ethnicity (N = 230)

Variable	Non-Hispanic Whites		Hispanics	
	M	*SD*	*M*	*SD*
Sociodemographic variables				
Age (in years)***	28.11	5.58	23.54	4.47
Marital status (%) ***				
Single	28		50	
Married	72		50	
Years of school***	14.38	2.10	10.00	3.44
Household income***[a]	4.86	3.00	2.25	1.94
<$20,000	25%		77%	
$20,000–$40,000	27%		13%	
$40,000–$60,000	16%		6%	
$60,000–$80,000	17%		2%	
>$80,000	15%		3%	
Birth-related outcomes				
Birth weight (in g)***	3,503.89	591.86	3,243.67	465.14
Low birth weight (≤2,500 g)	6%		5%	
Gestational age (in weeks)	39.22	1.59	39.26	1.46
Preterm delivery (<37 weeks)	8%		6%	
Parity	0.79	0.93	0.82	1.01
Nulliparous	47%		46%	
Psychosocial variables				
Mastery***	3.94	0.69	3.31	0.82
Optimism***	3.78	0.62	3.48	0.52
Self-esteem***	4.13	0.59	3.74	0.60
Pregnancy-related anxiety	1.69	0.32	1.75	0.44
State anxiety	1.85	0.59	1.91	0.52

Note. The sample included 110 non-Hispanic Whites and 120 Hispanics.
[a]Annual household income was measured using an ordinal scale ranging from 1 (*under $10,000*) to 10 (*over $90,000*), with each 1-unit increment corresponding to an increment of $10,000. A score of 4.86 represents a mean annual household income of between $30,000 and $50,000 for non-Hispanic White women. A score of 2.25 represents a mean annual household income of between $10,000 and $30,000 for Hispanic women.
***$p < .001$.

revealed good internal reliability for the English version of the scale ($\alpha = .81$) but poor reliability for the Spanish version ($\alpha = .38$). We examined item-total correlations and conducted a factor analysis in an attempt to identify a subset of items with adequate internal reliability in both languages. These were not successful. Accordingly, the full scale was used in both languages despite the poor internal reliability for the Spanish version.

Self-esteem. Self-esteem was measured with Rosenberg's (1965) 10-item scale. Items were rated on a scale ranging from 1 (*strongly agree*) to 5 (*strongly disagree*). Internal reliability for the English version of the self-esteem scale was similar to that found in past research ($\alpha = .89$), but reliability for the Spanish scale was substantially lower ($\alpha = .59$). Again, our attempt to find a subset of items with adequate internal reliability in both languages was unsuccessful. Accordingly, the full 10-item scale was used in both languages despite reduced internal reliability in the Spanish version of the scale.

State anxiety. State anxiety was measured by using a brief 10-item version of the State Anxiety Scale from Spielberger's (1983) State-Trait Anxiety Inventory. This version of the instrument was developed for brevity and has been found to have acceptable psychometric properties in past research (Spielberger, 1979). Items assessed the extent to which participants had experienced anxiety-related symptoms and emotions during "the last few days" by using a 4-point scale ranging from 1 (*not at all*) to 4 (*very much*). Internal reliabilities for the English ($\alpha = .90$) and Spanish ($\alpha = .83$) versions of the scale were acceptable.

Pregnancy-related anxiety. Pregnancy-related anxiety was measured with an expanded set of items based on those developed by Wadhwa et al. (1993). Ten items assessed the frequency with which (or the extent to which) participants worried or felt concerned about their health, their baby's health, labor and delivery, and caring for a baby (see the Appendix). Responses were made on a scale ranging from 1 (*never* or *not at all*) to 4 (*a lot of the time* or *very much*). We conducted an exploratory factor analysis with oblique rotation to investigate the factor structure of these items in English and Spanish. Examination of the eigenvalues revealed that the scores were best represented by a single factor in both languages. As such, a pregnancy-related anxiety score was computed by reversing scores where appropriate and calculating the mean of responses to all items. The internal reliability of the scale was acceptable in both English (Cronbach's $\alpha = .78$) and Spanish (Cronbach's $\alpha = .80$).

Birth outcomes. Birth outcome data were abstracted from medical charts after delivery. Two birth outcomes were studied: (a) gestational age at delivery (in weeks; estimated by using the last menstrual period and verified by ultrasound) and (b) infant birth weight (in grams). Birth weights in this sample ranged from 1,840 to 5,020 g, with a mean of 3,367.53 g ($SD = 543.89$ g). Gestational ages at birth ranged from 33.71 to 43.14 weeks, with a mean of

39.25 weeks (SD = 1.52 weeks). Six percent of the women in this sample gave birth to LBW babies, and 7% delivered prematurely. These rates were similar to California's rates, which in 1996 were 6% for LBW and 10% for PTD (Department of Health Services, State of California, 1998).

Sociocultural and sociodemographic variables. Sociocultural and sociodemographic variables, including age, marital status, ethnicity, country of birth, years lived in the United States, maternal education (in years), and annual household income[1] (measured with an ordinal scale ranging from 1 [*less than $10,000*] to 10 [*over $90,000*]) were assessed by interview.

Nulliparity. Nulliparity was scored by giving a participant a score of 1 if she was currently pregnant with her first baby or a score of 0 if she had previously given birth. Nulliparity was included in this model because of evidence that it is associated with less favorable birth outcomes (Kramer, 1987) and with greater stress.

Results

We conducted analyses in three steps. First, we examined ethnic differences in the study variables. Second, we conducted structural equation modeling using EQS for Windows (Bentler & Wu, 1995) to evaluate hypothesized interrelationships between the variables. Following conventional procedures (Bentler, 1992), an initial model was specified, its parameters estimated, and its fit tested; then, the model was trimmed using standard procedures, including examination of the multivariate Lagrange Multiplier (LM) test for reducing restrictions on the model and the Wald test for dropping free parameters.[2] Testing the fit of a structural equation model involves examination of the chi-square and the comparative fit index (CFI). Good fit is indicated by a nonsignificant chi-square and a CFI of .90 or greater. One also can examine the ratio between the chi-square and its degrees of freedom, with ratios closer to 1 and less than 3 indicating good fit (Carmines & McIver, 1981). This latter index is useful because the chi-square test is sensitive to sample size. We also conducted analyses to test the possibility that different models were needed to predict birth outcomes for Hispanics and non-Hispanic Whites. In the third step of the analyses, we conducted multiple and logistic regression analyses to test hypothesized interactions.

Ethnic Differences in Study Variables and Birth Outcomes

We performed a one-way multivariate analysis of variance on the continuous dependent variables to test for ethnic differences in infant birth weight (in grams), gestational age at delivery (in weeks), age, years in school, annual household income, parity, mastery, self-esteem, optimism, state anxiety, and pregnancy-related anxiety. With use of the Wilks's criterion, the multivariate test of the combined dependent variables was significant, $F(11, 218) = 15.47$, $p < .001$. Univariate F tests were computed to determine the variables on which the two ethnic groups differed. Dichotomous variables (marital status, nulliparity, LBW, and PTD) were investigated with a series of chi-square tests. Inflated Type I error due to multiple univariate F tests and chi-square tests (14 in total) was controlled by applying Bonferroni correction, resulting in a critical alpha level of .004 per test. The results of these tests are summarized in Table 1.

White women gave birth to babies who were significantly heavier than babies of Hispanic women, $F(1, 228) = 13.80$, $p < .001$, but there were no ethnic differences in gestational age at birth or in rates of LBW or PTD. There also were no ethnic differences in parity or in the percentage of women in each ethnic group who were giving birth for the first time (i.e., nulliparity). In terms of sociodemographics, the Hispanic sample was significantly younger, $F(1, 228) = 47.34$, $p < .001$, and less likely to be married, $\chi^2(1, N = 230) = 11.43$, $p < .001$, than the White sample. Hispanics also had completed fewer years of school, $F(1, 228) = 132.64$, $p < .001$, and had lower annual household incomes, $F(1, 228) = 60.21$, $p < .001$ (for a distribution of annual household incomes for each group, see Table 1). In addition, Hispanics scored lower on mastery, $F(1, 228) = 39.27$, $p < .001$; optimism, $F(1, 228) = 15.62$, $p < .001$; and self-esteem, $F(1, 228) = 24.30$, $p < .001$, than non-Hispanic Whites, but there were no ethnic differences in pregnancy-related anxiety or state anxiety. Given these group differences, ethnicity was controlled in the analyses, and in addition to the full model, separate models were tested for the White and Hispanic samples.

Structural Equation Model Predicting Birth Outcomes

Correlations between the study variables are shown in Table 2. As shown in the hypothesized model (see Figure 1), we expected that state anxiety and pregnancy anxiety would load on a single common factor labeled *Stress*. Because latent factors with only two indicators are underidentified, we randomly split the 10 items from the pregnancy-related anxiety scale into two parcels of 5 items each. Thus, there were three indicators for the Stress latent factor: state anxiety, Pregnancy-Related Anxiety A, and Pregnancy-Related Anxiety B. We expected the Stress latent factor to be negatively related to length of gestation, such that higher stress would predict shorter gestations. In addition, we expected that mastery, optimism, and self-esteem would load on a single common factor, depicted in Figure 1 as *Resources*. This latent factor was expected to predict higher birth weight. We also expected the Resources latent factor to be associated with reduced stress and, in turn, with longer gestations. Ethnicity (1 = *non-Hispanic White,* 0 = *Hispanic*) was expected to predict birth outcomes through its association with resources, and the socioeconomic variables

[1] The income variable was moderately skewed at 1.06 in the entire sample of 230. No transformation (square root, logarithmic, or inverse) changed the outcome of the reported analyses. Consequently, the income variable was used without transformation. Several analyses were conducted to investigate possible ethnic differences in the relationship between income and length of gestation, the birth outcome with which it was most highly associated. None of these analyses provided evidence for ethnic differences.

[2] Because this data set was not large enough to be split into two subsamples for cross-validation, replication will be required to verify the obtained model.

Table 2
Correlations for Major Study Variables (N = 230)

Variable	1	2	3	4	5	6	7	8	9	10	11	12	13
1. Birth weight (in g)	—												
2. Gestational age (in weeks)	.49***	—											
3. Household income	.06	−.17*	—										
4. Education (in years)	.16*	.01	.56***	—									
5. Marital status[a,b]	.13†	−.09	.44***	.27***	—								
6. Maternal age (in years)	.09	−.11	.54***	.45***	.27***	—							
7. Nulliparity[b,c]	−.09	.00	.02	.06	−.14*	−.17**	—						
8. White ethnicity[b,d]	.24***	−.01	.46***	.61***	.22***	.41***	.01	—					
9. Mastery	.19**	.01	.34***	.40***	.27***	.30***	.13*	.38***	—				
10. Optimism	.09	.01	.34***	.26***	.25***	.22***	.07	.25***	.62***	—			
11. Self-esteem	.10	−.05	.40***	.37***	.22**	.25***	.15*	.31***	.60***	.63***	—		
12. State anxiety	−.08	−.16**	−.07	−.01	−.16*	−.03	−.08	−.05	−.36***	−.46***	−.39***	—	
13. Pregnancy-related anxiety	−.08	−.12	−.03	−.05	−.16*	−.16*	.12	−.09	−.35***	−.28***	−.25***	.42***	—

[a]Marital status is coded as 0 for *single* and 1 for *married*. [b]Correlation coefficients shown are Pearson product–moment correlations. Note that product–moment correlations between continuous variables and dummy-coded variables coded as 0 or 1 are the same as point-biserial correlations (i.e., correlations between a continuous and a dichotomous variable). [c]Nulliparity is coded as 0 for *multiparity* and 1 for *nulliparity*. [d]Ethnicity is coded as 0 for *Hispanic* and 1 for *non-Hispanic White*.
†$p < .10$ (marginally significant). *$p < .05$. **$p < .01$. ***$p < .001$.

(income and education) were expected to predict birth outcomes through their association with both resources and anxiety. Finally, nulliparity (1 = *nulliparous,* 0 = *multiparous*), maternal age (in years), and marital status (1 = *married,* 0 = *single*) were included in the model so that their influence on birth outcomes could be controlled. This allowed us to test the association of the two latent factors with birth outcomes independent of the effects of these factors.[3] It is important to note that with length of gestation at birth controlled in this model, the birth weight variable represents fetal growth.

An important issue in analytic strategy was how to treat ethnicity. One option was to include ethnicity in the model as a variable, and another option was to test separate models for Whites and Hispanics. Our primary analyses used the former strategy for several reasons. First, it allowed us to use the full sample size of 230 to estimate parameters, providing greater power to test the hypotheses. It also enabled us to examine the relationships between ethnicity and other variables in the model. Finally, this strategy was consistent with our past research and theory about the effects of stress on birth outcomes, which have been highly consistent across ethnic groups. However, the question of whether these models differ by ethnic group is an important one. Thus, we tested separate models for White and Hispanic women after fitting the hypothesized overall model.

Once the hypothesized overall model was specified as shown in Figure 1, parameters were estimated, and the fit of the model was tested. The results of these analyses indicated that the model exhibited adequate fit to the data, $\chi^2(60, N = 230) = 140.99$, $p < .001$; CFI = .92; $\chi^2/df = 2.35$. However, the LM and Wald tests indicated that substantial improvements in model fit could be obtained by modifying the model in the following ways: (a) fixing the path from age to resources to zero, (b) fixing the path from education to stress to zero, (c) allowing state anxiety to load on resources (in addition to loading on anxiety), (d) freeing the path from nulliparity to Pregnancy-Related Anxiety B, (e) freeing the path from White ethnicity to Pregnancy-Related Anxiety A, (f) freeing the path from education to optimism, and (g) freeing the path from income to length of gestation. Making all of these changes except for allowing state anxiety to load on resources (which we chose not to do for theoretical reasons) resulted in a model with good fit, $\chi^2(58, N = 230) = 105.88$, $p < .01$; CFI = .95; $\chi^2/df = 1.83$. However, because these tests are analogous to post hoc tests, the modified model is not shown here.

The significant relationships illustrated in Figure 2, which shows the hypothesized model with parameter estimates,

[3] A medical risk variable that included 26 medical and obstetrical risk factors was originally included in the model. Although higher medical risk predicted shortened gestations, its presence in the model did not alter or reduce the relationships among stress, resources, and birth outcomes. Thus, in the interest of producing a more parsimonious model, we dropped medical risk from further analyses. Details of the medical risk variable and its association with other variables in the model can be obtained by contacting Christine Killingsworth Rini.

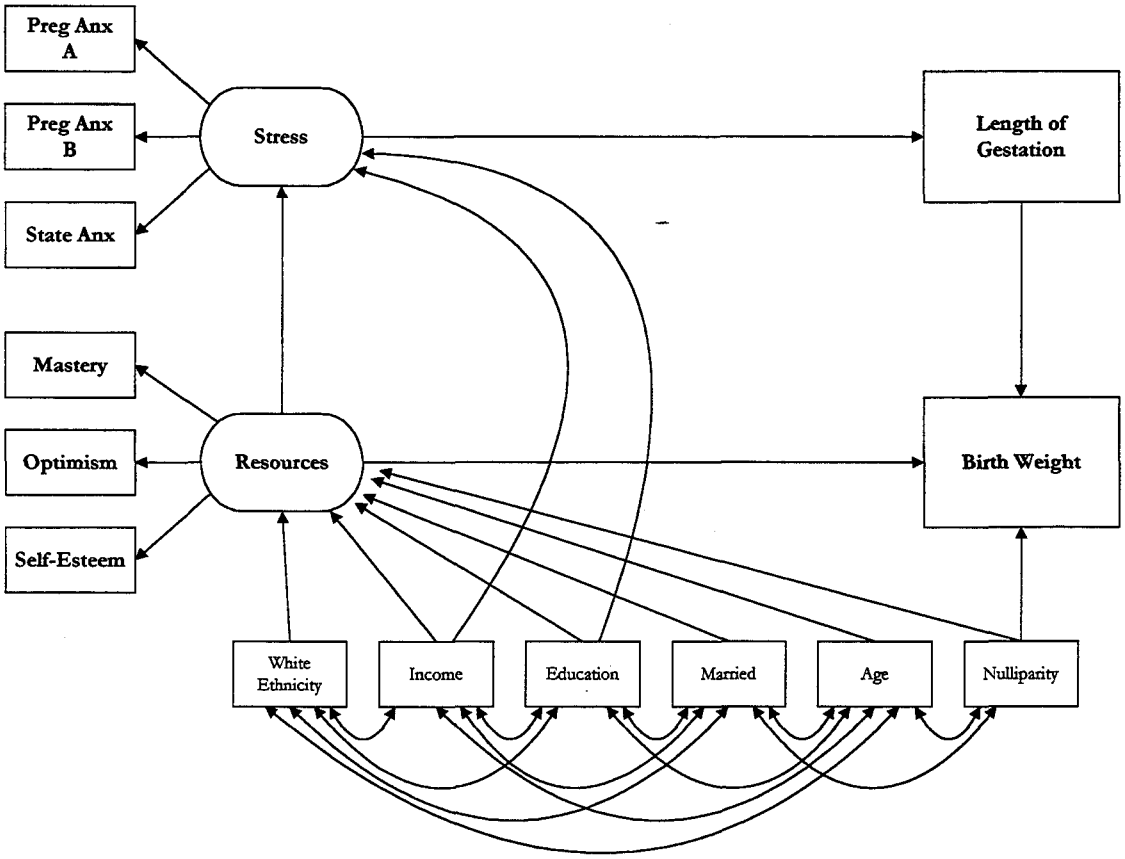

Figure 1. Hypothesized model of the relationship among prenatal psychosocial stress, personal resources, and sociocultural factors that affect adaptation during pregnancy and adverse birth outcomes. Preg = pregnancy-related; Anx = anxiety.

indicate that a longer gestation was strongly predictive of higher birth weight ($\beta = .48$). Furthermore, nulliparity was associated with having lower birth weight ($\beta = -.12$), being unmarried ($r = -.14$), and being of younger age ($r = -.19$). All of these relationships are in the direction expected and are consistent with past research.

Both stress and personal resources predicted birth outcomes independent of the effects of the other variables in the model. Women with higher stress delivered at an earlier gestational age ($\beta = -.20$). Controlling for all other variables in the model, stress was not associated with infant birth weight. Personal resources, in contrast, were directly associated with birth weight and indirectly associated with gestational age through stress reduction. Specifically, women with stronger resources gave birth to heavier babies ($\beta = .21$), controlling for age, marital status, nulliparity, ethnicity, and socioeconomic variables, and having stronger resources was associated with less stress ($\beta = -.67$). The indirect effect of resources on gestational age (mediated by stress reduction) was significant ($\beta = .13$, $z = 2.68$, $p < .05$). Although zero-order correlations suggest that mastery was more strongly associated with birth weight than the other personal resources studied here, examination of the loadings of each of the personal resource variables on the resources latent variable suggests that the relationship between resources and birth weight was almost equally accounted for by all three resources. In addition, although zero-order correlations suggest a stronger association between state anxiety and length of gestation than between pregnancy-related anxiety and length of gestation, the factor loadings in the structural equation model suggest that state anxiety and pregnancy-related anxiety contributed comparably to shortened gestations.

Examination of the sociocultural variables revealed that non-Hispanic Whites had stronger personal resources ($\beta = .19$), with the other variables in the model controlled, but did not differ from Hispanics in stress. Also, non-Hispanic Whites were still more likely to be married ($r = .23$) and older ($r = .41$), with other variables in the model controlled. Ethnicity did not directly predict birth weight after other variables were included in the model. Instead, ethnic differences in infant birth weight appeared to be mediated by other variables. In particular, Hispanics had fewer personal resources than Whites, and women with fewer resources had lower birth weight babies. Ethnic differences in the socioeconomic variables and marital status were also implicated through their association with stronger personal resources. White women had higher household

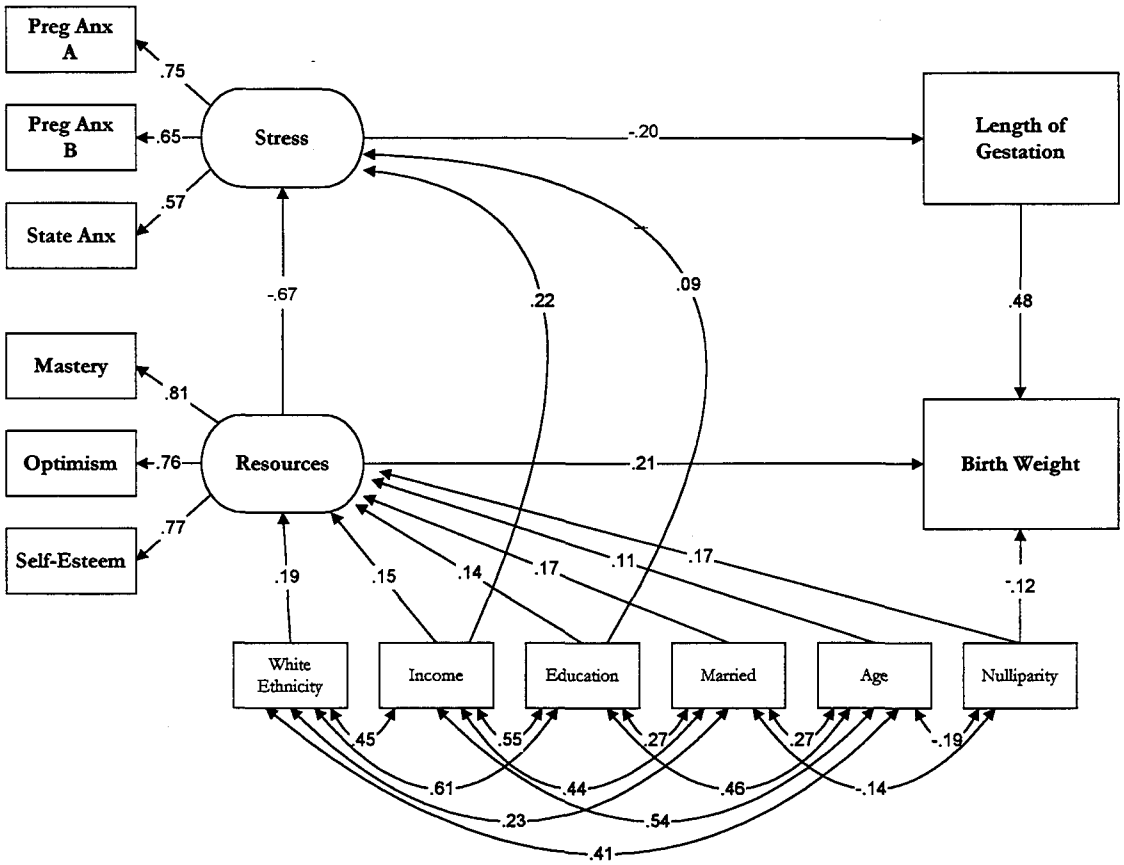

Figure 2. Final overall model. Parameter estimates are standardized. Model fit indices: $\chi^2(60, N = 230) = 140.99, p < .001$; CFI = .92; $\chi^2/df = 2.35$. All paths are significant at $p < .05$ except the paths between (a) age and resources and (b) education and stress. Coding of dichotomous variables: (a) married: 0 = *single,* 1 = *married;* (b) White: 0 = *Hispanic,* 1 = *non-Hispanic White;* (c) nulliparity: 0 = *multiparous,* 1 = *nulliparous.* Preg = pregnancy-related; Anx = anxiety.

incomes and more education and were more likely to be married than were Hispanic women, and these variables were associated with having greater personal resources and, in turn, higher birth weight babies.

Concerning socioeconomic factors, income and education were related to birth outcomes in different ways. Women with more education had stronger personal resources ($\beta = .14$), and personal resources were associated with higher birth weight, as noted above. Income, in contrast, was positively associated with stress ($\beta = .22$) as well as resources ($\beta = .15$). Moreover, there was some indication that income had a direct negative association with length of gestation (i.e., the changes suggested by the LM test). The fact that higher income appeared to contribute to shortened gestations was unexpected and is addressed in the Discussion section.

Not unexpectedly, results also showed that having a higher income was strongly associated with having more education ($r = .55$). Higher income and more education were associated with older age ($rs = .54$ and .46, respectively), being married ($rs = .44$ and .27, respectively), and White ethnicity ($rs = .45$ and .61, respectively).

Ethnicity and Prediction of Adverse Birth Outcomes

To investigate the possibility that the models needed to explain birth outcomes differ for Whites and Hispanics, a separate structural equation model was estimated for each group. The predicted model for Hispanic women was identical to the full model except that ethnicity was not included in the model and income was removed because of the limited variability of income in this sample (i.e., 77% of the Hispanic women in the sample reported an annual household income of less than $20,000). The model exhibited adequate fit for Hispanics, $\chi^2(45, N = 120) = 67.10$, $p < .05$; CFI = .92; $\chi^2/df = 1.49$. The predicted model for White women was identical to the full model except that ethnicity was not included in the model. This model also exhibited adequate fit, $\chi^2(52, N = 110) = 79.23$, $p < .01$; CFI = .93; $\chi^2/df = 1.52$.

Although both ethnic group models fit adequately, the low power of these analyses resulted in a number of parameter estimates failing to reach significance; however, the parameters were of a magnitude comparable to their counterparts in the full model. Thus, these analyses provide little

indication that different models are needed to explain birth outcomes for Whites and Hispanics. One possible exception is the association between resources and birth weight, which was considerably smaller in magnitude in the model estimated for White women ($\beta = .03$) compared with the model estimated for Hispanic women ($\beta = .17$) and the full model ($\beta = .20$). A simultaneous multiple regression analysis was conducted to test the possibility that the effect of resources on birth weight was moderated by ethnicity. However, the interaction between resources and ethnicity did not significantly predict birth weight ($\beta = -.04, p > .10$).

Examination of Interaction Effects

Simultaneous multiple regression analyses were conducted to examine the possibility that personal resources would modify the effect of stress on birth outcomes. An index of personal resources was created by summing standardized scores for mastery, optimism, and self-esteem. Similarly, an index of stress was created by summing standardized scores for state anxiety and pregnancy-related anxiety. Next, an interaction term was created by multiplying the resources index and the stress index. In the first analysis, infant birth weight was the dependent variable, and the predictors were weeks gestation at birth, marital status, maternal age, nulliparity, income, education, ethnicity, personal resources, stress, and the interaction term. As with the structural equation model, with weeks gestation at birth controlled, this model tested predictors of fetal growth. Results of the analysis indicated that the interaction between resources and stress was not significant ($\beta = .04$, *ns*). In the second analysis, length of gestation (in weeks) was the dependent variable, and the predictors were marital status, maternal age, nulliparity, income, education, ethnicity, personal resources, stress, and the interaction term. The interaction between stress and resources did not reach statistical significance in this test either ($\beta = -.03$, *ns*). Thus, there was no evidence that resources buffered stress in this study.

Testing Clinical Outcomes

To assess the ability of the study variables to predict the dichotomous outcomes used in obstetrics (birth weight <2,500 g or birth weight >2,500 g; delivery before 37-weeks gestation or delivery at or after 37-weeks gestation), two logistic regressions were conducted. Besides resources and stress, only predictors that were significant in the multiple regressions for birth weight and length of gestation were included in the corresponding logistic regressions. That is, weeks gestation and ethnicity were included for LBW, and education and income were included for PTD. The only study variable to predict LBW was weeks gestation at birth ($B = -1.38$, odds ratio = 0.25, $p < .001$). Both income ($B = 0.39$, $p < .01$) and stress ($B = 0.46$, $p < .05$) were significant predictors of PTD (odds ratios = 1.48 and 1.59, respectively). In addition, education was a marginally significant predictor of PTD ($B = 0.20$, odds ratio = 0.47, $p = .06$).[4]

Discussion

This study examined several aspects of adaptation during pregnancy and their association with two important birth outcomes. Adaptation was conceptualized as prenatal psychosocial stress, personal resources, and some aspects of the woman's sociocultural context.

One important contribution of this study is to add to the sparse literature on the manner in which personal resources in the form of self-relevant beliefs affect maternal and fetal health. The results provide evidence for a beneficial role of these adaptive resources—self-esteem, optimism, and mastery—in pregnancy and birth. Specifically, these resources were associated with giving birth to larger babies even after controlling for psychosocial stress, length of gestation, marital status, maternal age, income, education, ethnicity, and parity. The mechanisms underlying this direct effect remain unexplained, although behavioral pathways are likely to be a promising avenue for future research. For instance, women with strong personal resources may seek out health-related information more actively or practice preventative health behaviors more often (Aspinwall & Brunhart, 1996; Rodin, 1986; Seeman & Seeman, 1983). Also, they may be more successful at undertaking necessary lifestyle changes such as refraining from smoking, alcohol, and drug use (DiClemente, 1986; Mechanic & Cleary, 1980; Yates & Thain, 1985). Additional research on specific mediators of this relationship is needed.

In addition, personal resources were indirectly associated with length of gestation through stress reduction, with stress operationalized as generalized and pregnancy-related anxiety. This finding is consistent with theory and research linking personal resources in the form of positive beliefs about the self to lower appraised stress (see S. Cohen & Edwards, 1989; Hobfoll, 1989; Jerusalem, 1993; Lazarus & Folkman, 1984; Rodin, 1986) and may tap into processes related to resilience, growth, and thriving (Epel et al., 1998; Park, 1998). Pregnant women with stronger self-esteem, higher mastery, and greater optimism appear to have lower perceived stress, although it is unclear whether this results from lower stress appraisals or better coping and stress management. Both may be operating.

There is no evidence to support a buffering role for personal resources in this study. Thus, having strong resources appears to be health-protective not only for women experiencing high stress but also for those experiencing low stress. Theoretical perspectives on self-esteem, optimism, and mastery suggest that they may be viewed as basic adaptational resources that are useful across a broad range of

[4] Another series of logistic regressions was conducted to investigate the possibility that stress and resources would predict birth outcomes only for women at high medical risk, as suggested in previous research (Dunkel-Schetter, 1998). The medical risk index was dichotomized for these analyses. None of the interaction terms were significant predictors of PTD or LBW.

circumstances (e.g., Hobfoll, 1989; Scheier & Carver, 1992; Skinner, 1995; Taylor & Brown, 1988; Thompson & Spacapan, 1991). The results of this study strongly support this position. However, it is important to note that the lack of findings regarding buffering could be the result of the operationalization of psychosocial stress used here. Buffering may have been more likely if different measures of stress had been used, such as life event stress.

These analyses provide further evidence for an emerging pattern of results linking multidimensional measures of prenatal stress to length of gestation (Lobel, 1994). This replication adds to a growing understanding of the etiology of preterm labor and delivery. Past research has shown an association between stress and activation of the hypothalamic–pituitary–adrenal axis during pregnancy (Wadhwa, Dunkel-Schetter, Chicz-DeMet, Porto, & Sandman, 1996). Furthermore, there is growing evidence that stress hormones such as corticotropin-releasing hormone and cortisol are implicated in the early onset of delivery (Hobel, Dunkel-Schetter, & Roesch, 1998) as well as in suppression of the immune system, which may lead to infections that increase the risk of preterm labor (Paarlberg et al., 1995). This work extends previous research by focusing on a new component of stress (i.e., pregnancy-related anxiety) that appears to play an important role in adverse birth outcomes (Dunkel-Schetter, 1998). Still unknown, however, is what factors other than weak self-relevant beliefs predispose a woman to worry about her pregnancy and her ability to care for her baby, and whether intervening in this process can improve birth outcomes.

In terms of sociocultural factors, the findings of this study suggest that ethnicity is related to several other variables that influence adaptation during pregnancy, thus exerting its influence on birth outcomes indirectly. Specifically, the association of ethnicity to infant birth weight was mediated by Hispanics' lower levels of personal resources. Although future research is needed to investigate why Hispanics in this population reported lower personal resources, difficulties commonly experienced by immigrants may be contributing factors. Language barriers, economic difficulties, separation from friends and family, the need to adjust to new norms, and racial discrimination may lead Mexicans and other Latin American immigrants to experience decrements in beliefs about themselves, their future, and their ability to control important outcomes. As noted earlier, cultural values such as a belief in fatalism may also contribute to lower personal resources.

Ethnicity was also associated with household income and education. These socioeconomic variables, in turn, appeared to influence birth outcomes through their association with age, marital status, resources, and stress. Women with less education and lower incomes had fewer personal resources, which predicted less fetal growth compared with women with more education and higher incomes. Lower income was also associated with less stress. This latter finding is inconsistent with past research (see Williams, 1990). However, univariate analyses of these data showed that income was not correlated with either state anxiety or pregnancy-related anxiety; thus, it seems most likely that this is a suppression effect (J. Cohen & Cohen, 1983).

One strength of this study is its simultaneous consideration of adaptational resources and constraints operating at both the individual and contextual levels. The results provide insight into the interrelationships of these variables, as well as a more comprehensive understanding of their influence on birth outcomes. In particular, little research has been conducted to investigate the role of personal resources in pregnancy. These results, which provide evidence that resources influence birth outcomes both directly and indirectly, merit further attention. Moreover, this study provides some evidence that these processes hold for both Hispanics and non-Hispanic Whites.

In addition, researchers interested in the prediction of FGR, LBW, and PTD have acknowledged the need to recognize the different etiologies of these birth outcomes (e.g., Ernest, Michielutte, Meis, Moore, & Sharp, 1988; Selwyn, 1990). Our results provide evidence that the need to recognize different etiologies holds for psychological as well as biomedical variables. Specifically, the effects of resources and prenatal stress varied with the birth outcome being predicted. Resources were implicated in processes related to fetal growth but not to the timing of delivery, whereas stress was associated with length of gestation but not with fetal growth.

Some limitations of this study must be acknowledged. Although it appears from the evidence that the overall model holds well for both Whites and Hispanics, the ethnic subgroups were too small to be completely confident that the model fits both ethnic groups, and this must be tested in a larger sample. Sample size also limited the ability to detect relationships with dichotomous clinical outcome variables (i.e., LBW, PTD), which would be important to observe for purposes of risk assessment and intervention. These adverse outcomes occur with relatively low frequency in any given data set, and thus a sample of high-risk women is needed to predict them with adequate power. Finally, the study did not assess possible mediators of the relationship between personal resources and fetal growth (e.g., nutrition).

Although much has been made of the importance of studying the biopsychosocial determinants of health, many studies have focused on the biological and psychological aspects of this model, giving relatively little attention to the sociocultural aspects. This study provides evidence that investigating how these factors work together has the potential to help us better understand important health processes and outcomes, including the etiologies of adverse birth outcomes.

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Appendix

Pregnancy-Related Anxiety

1. I am confident of having a normal childbirth.
2. I think my labor and delivery will go normally.
3. I have a lot of fear regarding the health of my baby.
4. I am worried that the baby could be abnormal.
5. I am afraid that I will be harmed during delivery.
6. I am concerned (worried) about how the baby is growing and developing inside me.
7. I am concerned (worried) about losing the baby.
8. I am concerned (worried) about having a hard or difficult labor and delivery.
9. I am concerned (worried) about taking care of a new baby.
10. I am concerned (worried) about developing medical problems during my pregnancy.

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Incidental Findings with Genomic Testing: Implications for Genetic Counseling Practice

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Abstract This paper summarizes the current controversies surrounding the identification and disclosure of "incidental" or "secondary" findings from genomic sequencing and the implications for genetic counseling practice. The rapid expansion of clinical sequencing has influenced the ascertainment and return of incidental findings, while empiric data to inform best practices are still being generated. Using the North Carolina Clinical Genomic Evaluation by Next Generation Exome Sequencing (NCGENES) research project as an example, we discuss the implications of different models of consent and their impact on patient decisions.

Keywords Incidental findings · Secondary findings · Clinical sequencing · Genetic counseling · Medical actionability · Informed consent

Introduction

The question of how to manage the broad range of genomic findings has emerged as one of the more contentious issues in the clinical application of genomic sequencing. In particular, there are concerns surrounding the inevitable generation of what could be considered "incidental findings," frequently defined as a counterpoint to the primarily sought after diagnostic results and collectively described as the "incidentalome" [1••].

Historically used to classify research findings that arise during diagnostic testing [2], and routinely used in medical practice to describe additional findings unrelated to the indication for a particular evaluation, the colloquial meaning of "incidental" can imply something of lesser importance. This value judgment applies to some, but not all, incidental findings and alternative descriptors [3] have been suggested; each has its own promoters and detractors. Following its use by the Presidential Commission on Bioethical Issues, the term "secondary" is now preferred when such findings, unrelated to the diagnostic indication, are deliberately sought [4••].

Classifying secondary results in genetic testing is hardly new, and decisions to disclose their serendipitous discovery using genome-wide testing such as karyotype and microarray have been widely reported [5, 6]. The topic of secondary findings discovered via genomic sequencing has attracted responses from multiple disciplines including social scientists, clinicians, researchers, and bioethicists [7•, 8–12]. Paradoxically, while these variants are ubiquitous in the genome, their presence must be actively sought from among the vast number of other genomic variants in order to be identifiable and reportable.

In 2012, an American College of Medical Genetics and Genomic (ACMG) Working Group on Incidental Findings was assembled due to concerns of the ACMG that rapid expansion of clinical genome-scale sequencing could lead to heterogeneity in practices regarding incidental findings, and the perceived need to establish preliminary guidance for clinical laboratories. In 2013, the Working Group

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published the recommendation that laboratories should routinely analyze and report pathogenic variants from a specific set of genes when clinical diagnostic sequencing was ordered [13•]. They cautioned that patients should be forewarned that sequencing could reveal such findings but, once testing was ordered, the laboratory should analyze a small set of genes and report findings deemed to be "medically actionable" regardless of the proband's phenotype or age.

Extrapolating on attempts to classify genetic tests by parameters such as clinical validity and clinical utility (http://www.cdc.gov/genomics/gtesting/ACCE/index.htm), the Working Group identified a list of 57 genes (later revised to 56) associated with conditions that were considered to reach a high bar of medical actionability. Allowing for differences in the population being sequenced and laboratories' thresholds for asserting pathogenicity, the likelihood that a pathogenic variant will be found resulting in the disclosure of a medically actionable incidental finding has been estimated to be between 1 and 3 % in both research [14, 15•] and clinical studies [16]. These estimates have corresponded to predicted frequencies based on modeling [17]. It was anticipated that the minimal list would provide a focus for further discussions and would be modified in future renditions (Table 1).

Reaction to the ACMG Recommendations on Incidental Findings

The attempt to define the characteristics of conditions that would be sufficient to trigger professional obligations to identify and report secondary findings led to heated discussions of the ethical quandaries that ensue regarding their handling [18, 19]. The attempt to define categories of information within the vast scope of potential genomic findings has allowed genetic professionals to, more or less, coalesce around the general parameters and the idea of listing a minimal core group of genes, albeit without agreement on the particular genes on that list.

Many of the problematic aspects of the recommendations were acknowledged by the ACMG Working Group [13•] but sparked a number of critical commentaries nevertheless. Questions were raised as to whether or not the search for these variants was best viewed as being part of a professional's obligations [20, 21] or if there were legal ramifications [22]. Other concerns included the potential

Table 1 Conditions for which genes and variants are recommended for return of incidental findings in clinical sequencing as proposed by the ACMG

Hereditary breast and ovarian cancer
Li–Fraumeni syndrome
Peutz–Jeghers syndrome
Lynch syndrome
Familial adenomatous polyposis
MYH-associated polyposis
Von Hippel–Lindau syndrome
Multiple endocrine neoplasia type 1
Multiple endocrine neoplasia type 2
Familial medullary thyroid cancer
PTEN hamartoma tumor syndrome
Retinoblastoma
Hereditary paraganglioma–pheochromocytoma syndrome
Tuberous sclerosis complex
WT1-related Wilms tumor
Neurofibromatosis type 2
Ehlers–Danlos syndrome, vascular type
Marfan syndrome, Loeys–Dietz syndromes, and familial thoracic aortic aneurysms and dissections
Hypertrophic cardiomyopathy, dilated cardiomyopathy (including Fabry disease)
Catecholaminergic polymorphic ventricular tachycardia
Arrhythmogenic right-ventricular cardiomyopathy
Romano–Ward long QT syndrome types 1, 2, and 3, Brugada syndrome
Familial hypercholesterolemia
Malignant hyperthermia susceptibility

extra work needed for variant interpretation and confirmation, and whether this effort would be compensated [23], the uncertain accuracy of genotypic predictions in populations in which familial segregation of the phenotype was absent [24], the technological gaps in sequence coverage [25], the potential harms of false positives to unwary patients and their relatives due to errors in the medical literature [26], and the apparent contradiction with the historical recommendations against testing asymptomatic children for adult-onset conditions [27•].

It also became apparent that the initial recommendations did not acknowledge the need for patients to have the opportunity to "opt out" of receiving such information, a position that is largely supported by genetics professionals [28]. A clarification of the 2013 recommendations published by the ACMG Board of Directors [29] addressed this concern by acknowledging the patient's right "not to know" [30] and supported the ability of patients to opt out of categories of results.

Provider Responsibilities, Informed Consent, and Patient Decisions

In addressing the difficult issues of how to account for individual contexts, the ACMG recommendations placed the responsibility as to how, when, and, if results should be communicated on a medical professional's judgment [31]. A group of clinical laboratorians chose to emphasize the patient as the locus of decision making [32]; a stance echoed in research settings where participants are asked to pick and choose among the spectrum of potential genomic results. The choice and, therefore, the responsibility, to decide which results to learn and which results not to learn, are emblematic of a stronger focus on patient-centered decision making that is also affecting other areas of medical care. That being said, enabling a patient to make an informed decision remains the responsibility of the clinical provider, a role that has not changed with the expansion of testing from single gene tests to whole-genome sequencing.

If patients and participants are expected to routinely be asked to decide which secondary findings they want returned and which they do not, what preparation do they need to enable them to make informed decisions? A reflexive response to learn "everything" does not necessarily constitute an informed decision on the matter. The ability to opt out of learning secondary findings entirely is contingent on the recognition that the option exists and in the confidence that declining is a reasonable course of action. The decision to learn one potential finding but not another requires a broader understanding of the scope and magnitude of the universe of potential returnable results. More importantly, the level of understanding needed to decline to learn a potential result is even deeper [33•]. As the menu of gene variants that could potentially be returned grows, the depth of understanding needed for rational decision making also grows due to factors such as pleiotropy; complications often ignored and probably underestimated [34]. How coarsely or finely should the options be divided, and on which attributes can the spectrum of findings be reasonably categorized? How can a rational yet simple enough menu be devised, and communicated, to present understandable categories of clinically valid results, medically actionable or not? And, as variant classification evolves and new treatments become available, genes will inevitably shift from one category to another, further complicating future educational needs.

This patient-centric approach demands a robust informed consent process prior to clinical sequencing. It prompts questions of which information should be included and how it should be tailored to promote patient understanding. In the early days of patient sequencing (circa 2010), authors raised concern over the vast amounts of information they predicted would need to be communicated in order to obtain informed consent [35]. The time estimates to accomplish this supposedly Herculean task topped out at several hours [36], although these estimates were considered unrealistic [37] and, in practice, the approach has become more streamlined [38]. Indeed, in interviews with 29 genetic counselors and research coordinators who obtain consent, they reported spending an average of about 30 min by honing in on information relevant to the return of results [39].

Recommendations of elements to be included on a consent template have been made [40•, 41, 42] but consensus has not yet been achieved (Table 2). The need for a

Table 2 Recommendations for informed consent for genomic sequencing (adapted from Ayuso, et al.; and the ACMG policy statement points to consider for informed consent for genome/exome sequencing)

Testing characteristics
Scope
Description of techniques
Results
Spectrum of returnable vs. non- returnable results
Likelihood of each
How and to whom results will be communicated
Risks, benefits, limitations, and testing alternatives
Special cautions about use in children
Management and choices to opt out of secondary findings
Voluntary participation
Confidentiality and privacy protections
Sample management
De-identification, sharing, and opt-out procedures
Possibility of re-contact with new information

standard consent document template is highlighted by earlier studies of consent forms [43, 44]. To be fair, these forms had been created before enough experience had been gained to be able to achieve a consensus. Still, among sites engaged in translational clinical sequencing exploratory research, striking divergences and omissions were noted in the descriptions of potential diagnostic and secondary findings, the types of results to be returned or not, their inclusion in the medical record, and the role of participant preferences [45].

The broad scope of genomic sequencing creates complex downstream implications depending on the types of results returned. Incomplete appreciation of these implications, and increasingly heightened scrutiny of these complexities, is a source of discrepancies between consent forms. Even standard items on consent forms, such as the expected risks and benefits, the protections for confidentiality, and the future use of the data, can have nuanced meanings. For example, although the voluntary withdrawal from research studies is an obligatory element of consent, it has been argued that this may be a disingenuous promise if results are placed into the medical record where they cannot be removed [46]. Despite the tendency to place different types of information into specific categories, segmentation of genetic information can also be problematic [47].

Which consent elements are considered essential may differ when applied to the potential for learning diagnostic, as compared to learning secondary, results. In particular, the assessment of the risks and benefits of learning diagnostic results by individuals searching for an explanation for their health condition could be expected to differ from the same person's calculation when applied to learning a medically actionable, secondary finding. Alternatively, the decision to learn a medically actionable result may be seen as a form of empowerment by one whose medical condition is stable, as compared to another whose condition is progressive and degenerative; the latter may, instead, find the news distressing and overwhelming. Research participants and clinical patients can also have distinctly different expectations and thus reactions to information [48, 49]. Decisions about learning several kinds of secondary results that span a very broad spectrum of clinical utility could be expected to differ even more widely depending upon many contextual factors.

Making Decisions About Secondary Findings is Complex: Do People Really Want Everything?

Given the complexity of potential secondary findings that could be identified through genome-scale sequencing, it was surprising to learn, as several studies concluded, that although genetic professionals found several extenuating factors that influenced their definition of a returnable result, non-professionals had apparently concluded that the obvious solution would be to just "ask for everything."

Operating on the premise that to find out what people want is simply to ask them, early studies asked many different stakeholders; from clinical [50, 51] and research professionals [52], to those experienced with offering genetic testing [53], to IRB chairs, and to members of the public [54] and, in some cases, combinations of various stakeholders [55]. Since empirical data had yet to be collected about real decisions by people being asked to make them, this information gap was filled by a proliferation of studies that queried populations using hypothetical situations. Although severely limited in their widespread applicability, data from these surveys and focus groups raised conjectures about which kinds of attributes people were seizing on to make decisions. At the same time, beginning with the binning model developed by Berg et al. [7•, 56], clinical research groups expanded and experimented with different ways of categorizing secondary results including what would qualify as returnable and by what mechanisms they could or should be communicated [57•, 58, 59].

Genetic professionals have since come to a general consensus that a limited set of medically actionable results should routinely be returned, with the caveat that an individual's "right not to know" be protected by the informed consent process [60•], perhaps through a formal opportunity to "opt out" of certain kinds of results. Disagreements between groups remained about how best to define and communicate the concept of "medical actionability" and how and if this category should be modified when minors are sequenced [61]. Recommendations about the complicating issues surrounding childhood testing have been made [62••] and qualifiers such as the age of onset of the condition and the child's cognitive status are important [63]. It has been proposed that the identification and return of secondary findings, when identified in a child without a family history, are qualitatively different from the situation in which a child's risk is already known by virtue of the presence of the condition in the family [19]. Christenhusz has advocated that disclosure of medically actionable variants be viewed as the default, allowing for some exceptions, such as the age and status of the patient [11]. Surveys of genetic professionals show increasingly more reservations about return of results when the characteristics of such findings veer further away from the highly penetrant, clearly pathogenic variants strongly associated with medically actionable conditions [28].

Data collected about these issues from parents, individuals with genetic disorders and lay people, on the other hand, tended to show more enthusiasm about the return of a broad spectrum of results [64]. Respondents discriminated

between broad categories across the spectrum of conditions using characteristics such as the seriousness of the disorder, how likely it was to occur, the availability of effective treatments, and the age of onset.

Gathering data from eight focus groups that varied by age, gender, and professional status, Christenhusz found that, although the testing of minors was given special consideration, the mantle of parental responsibility was often the ultimate deciding factor [65••]. Even when they had second thoughts about learning information that could be ambiguous, fail to lead to any treatment, and be potentially harmful, most maintained that it was better to know than not to know. Respondents placed value on knowledge itself regardless of whether or not it led to action. They appeared to see only the forest of potential information and not the individual potentially risky trees and most welcomed "information" regardless of its accuracy, validity, or predicted potential for harm. Still, clinical actionability stood out as the benchmark against which all other characteristics were measured. Other scenarios were more contentious, such as evaluating children for adult-onset medically actionable conditions, identifying carrier status, and learning about a variety of other conditions that participants tended to view through a much wider lens of utility than that used by professionals.

This seemingly unanimous agreement that nearly all information was equally welcomed was congruent with reports from other studies that concluded that there was minimal harm, in some populations, in learning genetic information [66]. Taken together, these data might have meant that the difficult job of categorization would be far easier. Some remained uneasy, however, about the limited generalizability of responses from select populations [67, 68], and the inadequacy of psychological measures to detect subtler harms [69••]. There were also questions about how to qualify the degree of risk incurred in returning variants that did not meet a clinical actionability threshold and how to sort out which risks merited more caution than others. One concern that arises with regard to studies that report patient preferences to obtain even the "uncertain" genomic information is whether study participants truly understand the magnitude of uncertain findings that could be discovered. Furthermore, few studies have explored informational preferences related to the amount and quality of information—for example, when given a choice between receiving a handful of well-understood genomic findings or thousands of genomic findings with unknown clinical significance. An important challenge of eliciting patient preferences regarding whether to learn certain categories of information (and presumably not others) is to provide sufficient information to enable an informed choice. The process of setting patient preferences also requires balancing the efficiency of providing categorical choices versus the individualized customization of specific findings to be returned [70].

Assessing Patient Preferences in Research and Clinical Practice

Studies of individuals experienced with sequencing in themselves or their children seemed to echo the prior reports of enthusiasm for genetic information. Reports from the ClinSeq project confirmed that most of the participant population looked to learn about all possible results [71]. This response might be expected, given the atypical characteristics of their select participants; a limitation the authors acknowledged. Parents in their study cited the obligation to learn everything possible about their children and, given their past experiences with their child's rare and etiological mystifying condition, were confident they could withstand and incorporate any information regardless of its predictive ability [72]. Participants could distinguish how decisions might differ depending upon the category but many remained firm in their own desires to learn as much as possible.

More ambivalence was expressed during interviews conducted with sequenced patients with cancer and parents of children with undiagnosed conditions [73]. These participants expressed a wider variety of preferences but unanimously supported a central role for the patient in the decision-making process. Interviewees expected that additional findings would improve their lives by potentially explaining their diagnosis and they valued information as a way to prevent, or at least prepare, for the future. Even the potential to learn about untreatable conditions had the silver lining of being an opportunity to participate in research. Some participants reported that they would decline to learn information such as carrier status because acting on that information would be contrary to their religious beliefs. They also recognized that learning information is not always an unequivocally positive experience but can be burdensome and cause anxiety.

That so few respondents express anxiety specifically about the potential for genetic discrimination is not unusual; patients often fail to recognize it as a potential risk until after the person obtaining consent specifically raises it [74].

Even as evidence of patient ambivalence about learning secondary findings increased and the chorus advocating a slower pace grew louder [75, 76], the rate of clinical sequencing quickened. Data summarizing the sequencing experience of 200 patients who had been presented with an option of learning secondary findings were published [77•]. In this study, there appeared to be no ambivalence among those studied, as 93.5 % indicated that they desired secondary findings in any of four categories for which pathogenic variants were returned. Children, who made up 81 % of the population, were only eligible for results in the

category of predisposition to early disease, while adults were eligible for three additional categories: carrier status, predisposition to later onset disease, and predisposition to cancer. Somewhat disturbingly, 15 % of those consenting for a child's test requested results in categories for which they were not eligible, irrespective of the required pre-test genetic counseling and information in the consent form. Whether this result reflected a true desire for information on the part of parents, despite being counseled that it was not an option, or whether it indicated failure of clinicians and patients to understand the consent form, is not known. Among adults, 16 % declined at least one category, which was statistically different than the 4 % of declining parents/guardians. As with many candidates for sequencing, the children tested had limited life-spans with little expectation of being able to make autonomous decisions in the future. Such patients have been discussed as perhaps qualifying for a reasonable exception to the usual professional recommendations against testing for adult-onset conditions in children, but consensus has not been achieved on this point. Others have noted that when sequencing is done to explain a chronic health problem, individuals may not be ready or be able to think through the implications of learning secondary findings until after they learn their diagnostic results, even following a discussion about it [78].

The NCGENES Experience with Secondary Findings

One barrier to describing the spectrum of results that could be learned from genomic sequencing is the sheer heterogeneity of potential information. Berg and colleagues developed a categorization scheme used in the North Carolina Clinical Genomic Evaluation by Next Generation Exome Sequencing (NCGENES) project [7•, 56, 58]. NCGENES is designed to investigate the performance of NGS technologies in the diagnosis of patients with suspected genetic disorders to determine their validity and best use in clinical care. The project, part of the NIH-funded Clinical Sequencing Exploratory Research (CSER) consortium, also seeks to evaluate the impact on participants of receiving diagnostic results, medically actionable secondary findings, and non-medically actionable secondary findings.

Defining the criteria by which conditions can be classified as medically actionable or, by contrast, non-medically actionable has been challenging [79] and discrepancies arise between what providers mean and what patients assume by this term. The term "medically actionable" focuses on actions that can be taken by a medical professional rather than the spectrum of actions that may be taken by patients regardless of their efficacy. The term "medically actionable" is narrowly defined in NCGENES as pathogenic or highly likely pathogenic variants that "confer a high likelihood of disease, for which knowledge of their presence allows medical interventions that can significantly reduce morbidity and mortality."

Since most secondary findings have limited medical actionability, thereby leading to lack of consensus regarding their routine disclosure, the NCGENES project is specifically studying the potential benefits and harms of learning such information. Adult participants in NCGENES who are not cognitively impaired are randomized to either a group that learns diagnostic results and any medically actionable findings, or a group that is asked to decide, in addition, whether or not to learn any combination of six additional categories of non-medically actionable secondary findings. Both groups are followed to learn the impact of these results. Adults in the group randomized to make decisions about additional non-medically actionable findings are educated about the characteristics of each type including the implications of learning them and the eligibility of the results to be placed in the medical record (Table 3). Education occurs both by written information sent prior to their return of their diagnostic and medically actionable result visit and by an in-person discussion with a medical geneticist and genetic counselor at that visit. Importantly, this decision making occurs after the return of results visit, and participants are specifically asked not to make a decision at that time and are instead given the ability to initiate analysis at any subsequent time by contacting the study.

In our preliminary experience with NCGENES participants who have been randomized to make a decision about non-medically actionable secondary findings, it appears that only a minority is requesting them. This result is in contrast to the expectation that most participants would request everything. It suggests that even when participants express an intention to learn secondary findings, these initial predictions may not reflect an unequivocal desire for them. It may be that, as in previous studies, participants are optimistic about the value of genetic information for future

Table 3 Categories of secondary findings and return methods in the NCGENES project

Type of secondary finding		Returned by
A	GWAS risk SNPs	Telephone
B	Pharmacogenomics	Telephone
C	Carrier status	1 visit
D	APOE	1 visit
E	Mendelian disorders	1 visit
F	Severe neurodegenerative disorders	2 visits

use (notwithstanding their limited utility at present), but if so, this optimism does not seem to translate into a desire to immediately learn the information. The endowment of information with an intrinsic power regardless of its expected utility may be more likely for people whose past searches for information to explain their condition have been unsuccessful. Another interpretation of these results is that if NCGENES participants have mixed feelings about learning additional findings, they can simply delay taking action rather than completely shutting the door on their options. This approach to requesting secondary findings is very different than the traditional informed consent model in which patients are required to make their decisions at the time of sequencing, as was the case in the results reported by Shahmirzadi and colleagues [77•].

A discrepancy between stated intentions and actual requests may be more likely to materialize when participants are not asked to decide at the time they consent for diagnostic sequencing. When given time to make their decision, participants have the chance to think through implications they may not have considered before, or to talk to family members or others who were not available at the time of consent. When given space to make their decision, removed from the sphere of influence of a health care professional, participants may feel more freedom to, at the very least, delay the decision if they have doubts. And when empowered to take the first step to initiate the analysis, participants may be convinced that declining is a reasonable option. It may also be expected that these decisions assume lesser importance as time goes by and regular life resumes.

In contrast, when the decision to opt in or out of learning secondary findings is made at the time of consent, participants' assumptions about the process may lead them to opt in, just in case. In research studies, participants may think their results have already been generated and are known to the research team, or they may assume that by declining information they will, in some way, hinder the research. In a clinical setting, patients may view opting out as potentially jeopardizing their ability to learn information that one day may be important. In either case, most individuals have limited experience in being invited to decline a medical test or to assess its risks, and there may be social stigma attached to declining information, regardless of its value. The opportunity for cost-free testing can also be a powerful incentive.

Conclusions and Future Goals

Much of the current controversy over the management of secondary findings in genome-scale sequencing, whether in a research or clinical context, revolves around the perceived differences in the roles and responsibilities of professionals and the rights and preferences of participants. Finding the balance between the appropriate degree of professional guidance and individual choice will require more than vigorous commentary and the reporting of subjective data on hypothetical preferences, but will require empiric data on actual decisions and their outcomes. Traditional modes of informed consent and genetic testing will need to evolve in order to accommodate the increasing complexity of genome-scale sequencing. If, as some anticipate, the role of genomic sequencing in clinical care expands, to become an integral part of medical care, then the roles and responsibilities of clinicians and the rights and preferences of patients may assume a longitudinal nature in which decisions to query information will be made over the course of an individual's life and not necessarily at the moment of consent for sequencing.

Several important tasks remain. Attributes that are central to patients' decisions to learn secondary findings need to be identified. For example, Reiger et al. have conducted a discrete choice experiment to quantify participant preferences by asking them to make trade-offs to rank the relative importance of attributes such as lifetime risk, treatability, seriousness, and cost [80•, 81]. Alternative models of consent and disclosure are being piloted, and staged versions of both may help scale up the genetic counseling process [40•, 57•, 82]. The development of more sensitive tools to identify and track long-term effects of learning genetic information could help define subtler effects associated with better or worse long-term adjustment [69••, 83]. Several groups, such as those in the CSER Consortium and the Electronic Medical Records and Genomic (eMERGE) Network, are collecting data to help inform these tasks.

Finally, there is an urgent need to develop educational strategies to enhance the way people make informed decisions that streamline, yet complement, the genetic counseling process [67]. Electronic decision aids [84, 85] and other tools, both electronic and not, can lay a foundation of knowledge, but the importance of interpersonal dialog to help people reach complex decisions that are right for them should not be underestimated nor discarded. The discrepancies between consent form content and patient comprehension illustrate its importance in promoting understanding, patient autonomy, and shared decision making [86].

As clinical sequencing segues into other populations, such as newborn screening, [87, 88] our definitions and understandings of the risks and benefits of learning genomic findings will evolve, forcing the development of new models of education and counseling. In the era of personalized genomic medicine, genetic counseling has the opportunity to become even more effective and valuable if it can adapt without losing the personalized essence of what it can accomplish.

If it is true that "stories trump numbers and relationships trump stories" [89], educational strategies that touch both the cognitive and the emotional chords in the decision-making process by helping patients forecast their short- and long-term emotional responses to their decisions will help keep genetic counseling relevant regardless of what genomic testing looks like in the future.

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Human and Animal Rights and Informed Consent All studies by MI Roche and JS Berg involving animal and/or human subjects were performed after approval by the appropriate institutional review boards. When required, written informed consent was obtained from all participants.

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