2019 Progress Report

IDE G150258

NCNEXUS (North Carolina Newborn Exome Sequencing for Universal Screening) G150258

Cynthia M. Powell, MD
Professor of Pediatrics and Genetics
Director, Medical Genetics Residency Program
Division of Pediatric Genetics and Metabolism
Department of Pediatrics
University of North Carolina Medical Center
Chapel Hill, NC 27599-7487

December 13, 2019

1. Table of Contents

1. Table of Contents 2
2. General Information 3
3. Study Progress 4
3.1. Brief Summary of Study Progress 4
3.2. List of Investigators and Investigational Sites 4
3.3. Number of Subjects Enrolled 5
3.4. Number of Devices Shipped 6
3.5. Brief Summary of Results 6
3.6. Deviations from the Investigational Plan 11
4. Risk Analysis 13
5. Other Changes 13
6. Future Plans 14

2. General Information

Please state your:

1) IDE number: G150258
2) Device name and indication(s) for use: NCNEXUS (North Carolina Newborn Exome Sequencing for Universal Screening)
3) Sponsor's name address, phone numbers, and fax
   Cynthia M. Powell, MD
   Professor of Pediatrics and Genetics
   Director, Medical Genetics Residency Program
   Division of Pediatric Genetics and Metabolism
   Department of Pediatrics
   University of North Carolina Medical Center
   Chapel Hill, NC 27599-7487
   919-966-4202
4) Sponsor's email address: powellcm@med.unc.edu
5) Contact person: Laura V. Milko, PhD
   NC NEXUS Program Director
   Department of Genetics University of North Carolina at Chapel Hill
   Chapel Hill, NC 27599-7264
   919-843-2878
   laura_milko@med.unc.edu

3. Study Progress

3.1. Brief Summary of Study Progress

The NC NEXUS project has completed its funding cycle and enrollment, and we have summarized the majority of the results in this report, including enrollment and sequencing results.

Next-generation sequencing (if applied correctly) stands to greatly increase the number of rare Mendelian conditions that can be screened for and intervened on presymptomatically, thus representing an evolution of childhood screening exceeding the advent of tandem mass spectrometry. However, this tremendous opportunity also presents considerable challenges, not the least of which is determining which of the thousands of conditions should be screened for in healthy newborns. Our age-based semiquantitative metric (ASQM) provides a method for efficiently identifying conditions that are potentially worthy of inclusion on a next-generation sequencing newborn screening panel and certainly worthy of further rigorous study in order to determine the clinical validity and clinical utility of widespread implementation in newborn care.

While whole exome sequencing is unlikely to replace current methods of newborn screening, it can allow detection of similar conditions for which no standard screening methods exist and that influence the healthcare of a child. It can also detect conditions that will not occur until adulthood but which can indicate the possibility of life-threatening disease in a parent or other family members. Parents are able to make informed decisions about whether or not to seek additional genetic information about their children. While no short term, deleterious consequences occurred as a result of obtaining this information, the long-term impact, positive or negative, to children or their parents cannot be measured.

As identified in the NSIGHT pilot projects, several immediate challenges remain, including development of educational materials for parents to enhance and streamline the informed consent process, methods to increase the sensitivity and specificity of sequencing analysis, standardized protocols for follow-up of individuals identified as having an inherited condition through NGS-NBS, and widespread policy development/ implementation/ dissemination/ coverage of a formal public health approach to screening in newborns and children.

3.2. List of Investigators and Investigational Sites

UNC-CH
Cynthia M. Powell, MD (contact PI)
Jonathan S. Berg, MD, PhD (co-PI)
Bradford Powell, MD, PhD

Myra Roche
Karen Weck, MD
Kirk Wilhelmsen, MD, PhD

RTI
Don Bailey, PhD
Megan Lewis, PhD

3.3. Number of Subjects Enrolled

Table 1 summarizes the final status of enrollment and study completion. 61% of families (i.e., mothers participating independently or couples including a mother and a father) approached for enrollment successfully enrolled in Phase 1 of the project (42% in the diagnosed cohort and 71% in the well-child cohort). Enrollment in Phase 1 involved completing a baseline ("Time 1") questionnaire and completing the online decision aid to learn about NGS-NBS. 55% of families who complete the decision aid completed Visit 1 (80% in the diagnosed cohort and 46% in the well-child cohort).

Table 1: *Status of family enrollment*

	Diagnosed	**Well-child**	**Total**
Approached	153	285	438
Enrolled in Phase 1	64	202	266
Completed Time 1 Questionnaire	56 Couples; 5 Singles	173 Couples; 14 Singles	229 Couples; 19 Singles; 248 Total
Completed Online Decision Aid	59	145	204
Completed Visit 1	47	66	113
Sample Obtained	45	61	106
Results Returned	45	55	100

As summarized in *Table 1*, 204 families completed the online decision aid to learn about sequencing for their child; each "family" was either a mother participating on her own or a couple that included a mother and a father. 113 families completed a study visit to speak with a counselor about sequencing and made a final decision about whether or not to have their child sequenced ("**Visit 1**"). Samples were obtained for 45 children in the diagnosed cohort and 61 in the well-child cohort. A total of 100 families completed Visit 2, 45 in the diagnosed cohort and 55 in the well-child, at which results were returned.

Subject Demographics:

Cumulative Enrollment Table:

	Ethnic Categories						
	Not Hispanic or Latino		**Hispanic or Latino**		**Unknown/ Not Reported Ethnicity**		**Total**
Racial Categories	**F**	**M**	**F**	**M**	**F**	**M**	
American Indian/Alaska Native	2	0	0	1	0	0	3
Asian	20	14	0	0	0	0	34
Native Hawaiian or Other Pacific Islander	1	0	0	0	0	0	1
Black or African American	41	42	3	3	0	0	89
White	149	129	18	26	0	0	322
More than One Race	5	11	7	1	0	0	24
Unknown or Not Reported	0	0	0	0	0	0	0
Total	**218**	**196**	**28**	**31**	**0**	**0**	**473**

Please also see the attached Human Subjects Record.

3.4. Number of Devices Shipped

N/A

3.5. Brief Summary of Results

<u>Project 1 Generation and Analysis of Whole Exome Sequence (WES) Datasets</u>

Aim 1: Generate a high quality whole exome sequence dataset for next-generation newborn screening

The BioSpecimen Processing Facility at the University of North Carolina at Chapel Hill isolated genomic DNA from oral saliva and epithelial cell samples using PureGene chemistry. Exome libraries, including molecular barcoding and exome capture, were prepared using Agilent SureSelect XT kits (Human All Exon V6 probes) according to the manufacturer's low input guidelines. The UNC High-Throughput Sequencing Facility performed exome sequencing on an

Illumina HiSeq2500 with minimum depth of 40X coverage. Raw sequence reads were mapped to the human genome reference GRCh93.p7 using bwa-mem (version 0.7.12) (Li et al., arXiv:1303.3997v1), duplicate reads marked using Picard MarkDuplicates (version 1.130) and variants were called using freebayes (version 1.1.0) (Garrison and Marth, arXiv:1207.3907).

Aim 2: Implement an analytic strategy for NGS-NBS and incidental findings

Variants were prioritized into different classes based on previous classification as Pathogenic or Likely Pathogenic in a ClinVar submission, minor allele frequency in a reference database, and predicted effect of the variant on the protein (frameshift, nonsense, canonical splice-site, missense, synonymous, intronic variants). All rare possibly damaging variants in 466 genes in Category 1 were reviewed by a molecular analyst for each of the 106 participants. For the initial NGS-NBS analysis, molecular analysts were blinded to the participant's cohort (metabolic, hearing loss, or well-child). Variants were classified based on ACMG/AMP interpretation guidelines using collected evidence from population databases, ClinVar and the primary literature. Pathogenic (P) and/or likely pathogenic (LP) variants that were considered a "positive" screen (heterozygous P/LP variants in a gene associated with a dominant condition; two or more P/LP variants in a gene associated with a recessive condition) were flagged for further review by a committee of molecular geneticists and physicians, genetic counselors and researchers. Variants of uncertain significance (VUS) were not returned in the NGS-NBS screen. After completion of the first NGS-NBS analysis, the participant's cohort was revealed to the molecular analyst. If the participant was part of the metabolic cohort or hearing loss cohort, ES data were further analyzed by filtering for variants in a subset of genes within indication-based "diagnostic lists," containing genes associated with the relevant phenotypes. For the indication-based diagnostic analyses, P, LP and VUS were reported. All variants were confirmed in a duplicate sample by Sanger sequencing performed in the UNC Hospitals Molecular Genetics CLIA-certified laboratory.

Aim 3: Develop and evaluate novel bioinformatics approaches for utilization in NGS-NBS

Copy number variant analysis was not conducted due to restrictions based on the study's Investigational Device Exemption approved by the FDA.

<u>Project 2 Clinical Utility of Whole Exome Sequencing in Newborn Screening</u>

Aim 1: Evaluate the use and performance of WES as a diagnostic tool in NBS.

> ***Subaim 1A - Determine the sensitivity of WES in detecting three of the most common genetic conditions currently identified in newborns***

Sequencing and molecular analysis were completed for 17 children who had been diagnosed with inborn errors of metabolism through standard newborn screening. NGS-NBS analysis identified pathogenic or likely pathogenic variants in genes associated with metabolic conditions in 15 of 17 participants in this cohort (88%). Seven participants previously diagnosed with phenylketonuria (PKU) by newborn screening had pathogenic variants in *PAH;* six of them were likely compound heterozygous for two different variants (ranging from missense, canonical

splice-site, nonsense, and/or deletion variants). The seventh participant with a pathogenic variant in *PAH* was homozygous for a missense variant (c.194T>C, p.I65T). Seven participants previously diagnosed with medium-chain acyl-coA dehydrogenase (MCAD) deficiency by newborn screening had pathogenic variants in *ACADM*; five of these seven were homozygous for a well-known pathogenic missense variant (c.985A>G, p.K329) reported previously in individuals with MCAD deficiency. Two of these seven were likely compound heterozygous for two different variants in the *ACADM* gene (p.K329E/p.G267R, p.K329E/p.Y67H). One participant who was previously diagnosed with carnitine deficiency by newborn screening was homozygous for a *SLC22A5* missense variant (c.506G>A, p.R169Q) consistent with his clinical diagnosis. NGS-NBS analysis was deemed "negative" in 2 of 17 participants (12%) in the metabolic cohort. One participant previously diagnosed with maple syrup urine disease (MSUD) was heterozygous for a missense variant in the *BCKDHA* gene (C.1312T>A, p.Y438N) associated with classic, autosomal recessive MSUD but a second pathogenic variant in the other allele was not identified. Another participant previously diagnosed with malonyl-CoA decarboxylase deficiency was homozygous for a missense variant (c.1013T>C, p.L338P) in *MLYCD*, the gene that encodes the malonyl-CoA decarboxylase enzyme, but this variant was classified as a VUS due to insufficient evidence. These results suggest that WES is not as sensitive as tandem mass spectroscopy (the current technology used in public health newborn screening).

Subaim 1B – Determine the impact of WES on the specificity of NBS.

Newborn hearing screening is point of care newborn screening to detect infants with congenital hearing loss. There are many different causes of congenital or prelingual hearing loss, with approximately 50-70% having an underlying genetic etiology. Newborn hearing screening and confirmatory audiology testing only determines the presence of hearing loss but cannot determine an etiology. In the NC NEXUS study, WES and analysis identified a genetic cause in 5 of 28 participants (18%) in the hearing loss cohort. These individuals had a combination of pathogenic and/or likely pathogenic variants in genes implicated in hearing loss that were determined to be reportable in a screening setting. Two participants were each presumed to be compound heterozygous for two variants in the *USH2A* gene (one of the genes associated with Usher syndrome) based on ES data (c.1256G>T, p.C419F and c.3686T>G, p.L1229Ter; c.4338_4339 delCT, p.C1447fs and c.2299delG, p.E767fs). Parental testing confirmed that the variants were *in trans* in both cases. Usher syndrome is associated with hearing loss and progressive vision loss in later childhood. One participant was homozygous for a 1-bp frameshift deletion (c.35delG) in *GJB2* (connexin 26), a common pathogenic variant associated with DFNB1 nonsyndromic hearing loss. One participant was compound heterozygous for 2 pathogenic missense variants (c.626G>T, p.G209V and c.1151A>G, p.E384G) in *SLC26A4*, a gene associated with Pendred syndrome, an autosomal recessive form of syndromic hearing loss associated with thyroid disease in some cases. Finally, one participant was heterozygous for a likely pathogenic variant (c.5597C>T, p.T1866M) in *TECTA*, a gene associated with autosomal dominant hearing loss. We conclude that WES can improve the specificity of newborn hearing screening by identifying a genetic etiology in some cases which can alter clinical management and prognosis, inform parents about recurrence risks in future children, and help with life planning.

Aim 2: Determine the diagnostic capacity and utility of WES to extend the range of current NBS techniques.

Subaim 2A – Assess the use of WES in screening for conditions not identifiable by current NBS testing methods.

Utilizing analysis of 466 gene-disease pairs in the NGS-NBS category, 4 of the 106 participants (0.8%) had positive findings that were not anticipated. One participant in the well-child cohort was heterozygous for a missense *LDLR* variant (c.502G>A, p.D168N) previously reported in autosomal dominant familial hypercholesterolemia. One female participant in the metabolic cohort, diagnosed with PKU, was a carrier of a missense variant (c.1061T>G, p.F354C) in the ornithine transcarbamylase (*OTC*) gene. This variant was previously reported in mild OTC deficiency, an X-linked disease involving the urea cycle associated with potentially life-threatening episodes of hyperammonemia, with variable expressivity in carrier females. One hearing loss cohort participant was heterozygous for a canonical splice-site variant in the *DSC2* gene (c.631-2A>G). This variant has been reported previously in individuals with autosomal dominant arrhythmogenic right ventricular cardiomyopathy. Another hearing loss cohort participant had two variants in the *F11* gene (nonsense variant c.1489C>T, p.R497Ter and missense variant c.1608G>C, p.K536N). Both variants have been reported previously in individuals with autosomal recessive factor XI deficiency (a form of hemophilia). This child has a history of frequent episodes of epistaxis (nosebleeds), one requiring cauterization.

Two-thirds of the participants (65) were randomized into a group that could elect to receive additional findings of WES in their child in the categories of adult-onset actionable and childhood-onset non-actionable conditions and 3 (4.6%) had positive findings. Two participants had additional findings in the adult-onset actionable category. A participant in the metabolic cohort was heterozygous for a deletion variant (c.905-2_905-1delAG) in *RAD51C* that has been reported previously in autosomal dominant familial ovarian cancer. A participant in the well-child cohort was heterozygous for a nonsense c.7480C>T, p.R2494Ter variant in *BRCA2* that is a known pathogenic variant associated with autosomal dominant hereditary breast and ovarian cancer. One participant in the hearing loss cohort had an additional finding for the childhood-onset non-actionable conditions category. This participant was hemizygous for a nonsense variant (c.741G>A, p.W247Ter) in the *OCRL* gene. This gene is associated with Lowe syndrome, and further correlation with clinical findings indicated that the participant has multiple features consistent with Lowe syndrome.

Our findings indicate that WES is able to identify medically actionable conditions that would not otherwise be detected through standard NBS and can also detect conditions that, while not actionable in the traditional sense, can benefit patients and their families through avoidance of a diagnostic odyssey.

Subaim 2B – Assess the ability of WES to illuminate genotype-phenotype correlations and identify novel causes of phenotypic variability.

We had too few participants with documented genetic conditions to assess genotype-phenotype correlations, but we are sharing our genotype/phenotype data with the Newborn Screening

Translational Research Network/Longitudinal Pediatric Data Resource to add to their database of patients and contribute to future clarification of genotype-phenotype correlations. We are also awaiting permission from our IRB to share sequencing data from this study with dbGaP.

Project 3 Ethical and Social Implications of Applying Whole Exome Sequencing in Newborn Screening

Aim 1: Refine and describe, in lay terms, meaningful "bins" or categories of genes to enable parents to make informed decisions about which types of incidental findings they wish to learn.

The NC NEXUS binning committee developed and deployed an age-based, semi-quantitative metric (ASQM) to score and categorize the different types of potential genomic findings to guide informed decision-making and the disclosure of results. In addition to considerations of the age of disease onset, the metric uses the concept of "medical actionability," which includes the likelihood and severity of disease outcomes and the efficacy and potential harms of interventions.

The ASQM was used to score 822 gene-disease pairs, enriched for pediatric onset of disease and suspected actionability, to determine their eligibility to be included in a "NGS-NBS panel". This panel includes genes associated with disorders that are currently in the RUSP, as well as other conditions with onset in infancy or childhood that have treatment, monitoring and/or medical management that can be reasonably expected to improve outcomes (i.e. are "medically actionable"). Of the gene-disease pairs scored, 466 were classified as having childhood onset and high actionability, and were included on the "NGS-NBS" panel. Because the conditions identified by the NGS-NBS panel are comparable to those detected by current NBS screening, the results of the analyses of these "NGS-NBS" genes are disclosed to all study participants. The final version of our gene panel has been applied to the sequencing analysis pipeline and a description of our process and list of categorized gene-disease pairs was published in the Journal of Pediatrics (Milko et al. 2019. PMID: 30851990), accompanied by an editorial entitled, "Large Scale Next Generation Sequencing and Newborn Screening: Are We Ready?" (Phornphutkul and Padbury, 2019. PMID: 30819502).

Aim 2: Develop and evaluate the effectiveness of a decision aid to help parents make an informed decision about study participation and their preference for return of results.

We developed an online decision aid to help parents make an informed decision about study participation and their preference for return of results. Development used principles of good communication and user-centered design; input from an expert steering committee; a multimethod approach to ascertain parents' values, beliefs and informational needs in this context; and best practices for developing decision aids. Formative interviews conducted with parent couples found that many of them reported a collaborative process of talking together, weighing pros and cons, and making joint decisions. We applied these findings in the decision aid to support a parental shared decision-making approach. Following user-centered design principles, decision aid development included iterative audience involvement and pretesting as

well as usability testing with people who were a demographic match with the intended end-users. Results from these interviews were instrumental in ensuring that the decision was understandable and engaging. This development process was reported in a peer-reviewed publication (Lewis et al., 2016, Pediatrics, 137(Supplement 1), S16–S23, PMC4922487). Several additional publications related to decision aid development advanced knowledge regarding its design and features. One publication examined the benefits of including a values clarification exercise in the decision aid (Paquin et al., 2018, Soc Sci Med, pii: S0277-9536(18)30651-8, PMC6509013). A second publication investigated how parental preferences for acquiring information from genome-scale testing is influenced by characteristics of non-medically actionable genetic disorders in children, as well as whether parental preferences differed by gender and race (African-American vs. white) (Lewis et al., 2018, Genet Med, 20(2):181-189, PMC5868968). We then developed an API that allowed the decision aid to communicate decisions to the information workflow system at UNC, in preparation for using the decision aid in our study, with the objective of ensuring the study applied principles of informed decision making for parents considering NGS-NBS and whether to learn additional information from their child's sequencing. Specifically, the decision aid was used in a longitudinal, prospective study of factors associated with parents' willingness to enroll in the study and to accept NGS-NBS for their child, the spectrum of additional results they elect to learn (if randomly assigned to the study arm in which they were offered three categories of additional information), issues surrounding returning additional information from the child's sequencing, and consequences of decision making and results disclosure. The study protocol is described in a peer-reviewed publication (Milko et al., 2018, *Trials*, 19(1), 344, PMC6022715). Completion of the study has positioned us to investigate parent decision making and responses to receiving NGS-NBS and additional findings**.** Data collection is now complete and an analytic dataset is being finalized. We are also finalizing an analysis plan; it includes plans for analyses to describe parental decisions about NGS-NBS and additional findings; parents responses to decision making; and planned secondary analyses evaluated additional outcomes and parents' experience of decision making.

Aim 3: Apply the decision aid and recruitment procedures in a prospective study designed to determine parents' willingness to accept WES for their child; the choices they make regarding the spectrum of results they wish to learn; factors associated with their choices; and the consequences of return of results for both children and families.

Analysis of results of the decision aid tool is in progress. Preliminary analysis presented at the final NSIGHT meeting in June, 2019 suggest that the decision aid mitigated negative emotions that might stem from making decisions to receive this type of information about one's child. Final results will be disseminated via peer-reviewed publication.

3.6. Deviations from the Investigational Plan

1. In January of 2017 a mother enrolled as a single parent but the child's father also came to the consent visit. Both parents provided consent, a sample was obtained, and the family was reclassified as a couple. After that visit, the father failed to participate further by answering the T2 questionnaire. We allowed the family to remain classified as a couple even though data will

be missing from the father.

2. Due to procedural changes in University accounts services that occurred suddenly and without notice, a significant lag time occurred between when we requested funds for gift cards and when those funds were approved. Our study protocol was to send gift cards soon after participants completed the questionnaire. However, for a few months, many participants did not receive these until several weeks later. Currently, all participants have received their gift cards.

3. In April of 2017, two letters to two participants were switched and placed into each other's envelopes, resulting in each participant receiving a letter that included the name and address of the other participant. After being alerted by one of the participants, we spoke to both participants and neither expressed concern about this error. The research assistant was informed of the error and now double checks that the names on the letters ad the envelopes match. This was reported to the IRB on 4/1/17 , who did not require any further action.

4. A family who declined sequencing changed their minds and are in the queue for scheduling. The father had already completed the questionnaire that follows this decision. We will request that he complete it again following the consent visit.

2018

5. The samples of 2 participants (NCX_00002 and NCX_00006) who had the same, biochemically-confirmed, clinical diagnosis (PKU) were apparently swapped. Both participants share one pathogenic variant, PAH c.1315+1G>A, in common. The identification and confirmation of this variant was reported to the parents of NCX 00002. No incorrect variants were reported to either participant's parents. We requested and received new saliva samples from each participant.

6. As part of our study protocol, we obtain two samples of saliva from each participant. One sample is sent to the Biospecimen Processing center (BSP) and the DNA is subsequently sent to Jonathan Berg's research laboratory for whole exome sequencing (WES). The other sample is sent to the CLIA-certified, Molecular Genetics Laboratory (MGL) at UNC Hospitals to allow confirmation of relevant variants identified in the research laboratory. WES and analysis of NCX_00002 was completed first. The PAH c.1315+1G>A mutation, that both children have in common, was identified in the research lab and confirmed by Sanger sequencing in the CLIA-certified, MGL. A second variant, c.782G>A, was identified in the research lab but was not confirmed in the MGL. The sample failed the identity check done by the MGL but was overridden in error. The MGL issued a clinical report that described the c.1315+1G>A pathogenic variant and stated that a second mutation/deletion could be present but had not yet been identified. We reported these results to the parents and emphasized that the results did not change the participant's clinical diagnosis nor did it change the current management for the child.

7. WES and analysis of NCX_00006 was then subsequently performed and the PAH c.1315+1G>A pathogenic variant was identified and confirmed in the MGL by Sanger sequencing. A second mutation identified by research WES, c.284_286delTCA, did not confirm by Sanger sequencing in the MGL. This sample also failed the identity check. At this point the

possibility of a sample swap was considered. The identity SNP genotypes of the two samples were analyzed, confirming a swap between these two participants' samples. In addition, Sanger sequencing in the MGL of the second PAH variant expected in each sample confirmed a swap between these two samples. The family of NCX_00006 had not been contacted with the results and no clinical report had been issued. We subsequently discovered that the swap occurred in the BSP. (See supporting documents: Corrective Action Plan and Preventive Action Statement).

We received and analyzed the second sample from both participants and the results were communicated to both sets of parents.

We will continue to perform identity checks on all samples in the MGL that require confirmation of significant findings from the research sequencing and flag any that do not confirm. In addition, we will discuss any future failing or inconclusive identify check results with the molecular genetics sign-out committee and/or NCNEXUS steering committee. We will also re-iterate the training of relevant study personnel on the importance of flagging identify check discrepancies.

2019

Nothing to report

4. Risk Analysis

No changes to risk analysis.

5. Other Changes

1. In January, we received IRB approval to consent one parent by phone at the time of the consent visit with the other parent, and we also received IRB approval for the well-child cohort recruiter to offer paper copies of the first questionnaire instead of a link to the electronic version. We also began mailing a paper copy of the questionnaire with a stamped return envelope to parents who had not completed it within two weeks of their enrollment.

2. In June, the clinic recruiter began to call families who had not responded to reminders about completing the questionnaire.

3. In July of 2017 we began sending exit letters to parents who had not been able to be scheduled for a consent visit requesting that they complete the second questionnaire. An exit letter informs the parents that we have been trying to reach them unsuccessfully and asks them to contact us if they would like to continue in the study.

4. As of 2018, we are now offering those parents who are in the well-child cohort (at low risk for positive results) AND who have been assigned to the control group (so will not be eligible to request additional results) with the option of learning negative NGS-NBS results by phone and being sent a copy of the research report.

5. As of 2018, we will also offer the option of phone disclosure of results to all parents in the decision group whose only positive results are carrier status. Our initial protocol included being able to relay positive carrier status by phone. The clinical report of carrier status results will be sent to the parents via mail.

6. Future Plans

N/A

7. References

Peer-reviewed publications:

1. Berg JS, Agrawal PB, Bailey DB, et al. Newborn sequencing in genomic medicine and public health. *Pediatrics* 2017;139(2). doi:10.1542/peds.2016-2252.

2. Mollison L, Berg JS. Genetic screening: birthright or earned with age? *Expert Rev Mol Diagn* 2017;17(8):735-738. doi:10.1080/14737159.2017.1346473.

3. Lewis MA, Stine A, Paquin RS, et al. Parental preferences toward genomic sequencing for non-medically actionable conditions in children: a discrete-choice experiment. *Genet. Med.* 2017;20(2):181-189. doi:10.1038/gim.2017.93.

4. Milko LV, Rini C, Lewis MA, et al. Evaluating parents' decisions about next-generation sequencing for their child in the NC NEXUS (North Carolina Newborn Exome Sequencing for Universal Screening) study: a randomized controlled trial protocol. *Trials* 2018;19(1):344. doi:10.1186/s13063-018-2686-4.

5. Paquin RS, Peinado S, Lewis MA, et al. A behavior-theoretic evaluation of values clarification on parental beliefs and intentions toward genomic sequencing for newborns. *Soc. Sci. Med.* 2018. doi:10.1016/j.socscimed.2018.11.017.

6. Lewis, M. A., Bonhomme, N., & Bloss, C. S. A new era, new strategies: Education and communication strategies to manage greater access to genomic information. Hastings Center Report. 2018;48(52), S25–S27. https://doi.org/10.1002/hast.880

7. Milko LV, O'Daniel JM, DeCristo DD, et al. An age-based framework for evaluating genome-scale sequencing results in newborn screening. *J Pediatr.* 2019:68-76. doi: 10.1016/j.jpeds.2018.12.027

8. Powell CM. What Genomic Sequencing Can Offer Universal Newborn Screening Programs. *Hastings Cen Rep*. 2018 July;48 Suppl 2:S18-S19. PubMed PMID: 30133725; PubMed Central PMCID: PMC6863503; DOI: 10.1002/hast.878.

9. Peinado, S., Paquin, R. S., Rini, C., Roche, M., Butterfield, R. M., Berg, J. S., Powell, C.M., Bailey, D.B., Lewis, M. A. (2019). Values clarification and parental decision making about newborn genomic sequencing. Accepted. (Health Psych)

Presentations, Oral and Poster:

Rini, C., Lewis, M. A., Butterfield, R., Souris, K., Powell, C. M. (2019, August). *Women's responses to next-generation sequencing for newborn screening: Psychological predictors of increases in pregnancy-specific anxiety*. Podium presentation at the 33rd Annual Conference of the European Health Psychology Society, Dubrovnik, Croatia.

Milko LV, O'Daniel JM, DeCristo DD, Powell, CM, Berg, JS. (2019, June). *An age-based framework for evaluating genome-scale sequencing results in newborn screening.* Talk presented at Newborn Sequencing in Genomic Medicine and Public Health (NSIGHT) meeting, Bethesda, MD

Roman T.S., Crowley S.B., Roche M.I., Foreman A.K.M., O'Daniel J.M., Seifert, B.A., Lee K., Brandt A., Gustafson C., DeCristo D. M., Strande N.T., Ramkissoon L., Arreola, A., Edgerly, C., Milko L.V., Owen P., Li M., Roy S., Xiong M., Paquin R.S., Butterfield R.M., Lewis M.A., Souris K.J., Bailey Jr. D. B., Rini C., Booker J., Powell B.C., Weck K.E., Powell C.M., Berg J.S. (2019, June). *NC NEXUS exome sequencing molecular analysis results*. Talk presented at Newborn Sequencing in Genomic Medicine and Public Health (NSIGHT) meeting, Bethesda, MD

Roman T.S., Crowley S.B., Roche M.I., Foreman A.K.M., O'Daniel J.M., Seifert, B.A., Lee K., Brandt A., Gustafson C., DeCristo D. M., Strande N.T., Ramkissoon L., Arreola, A., Edgerly, C., Milko L.V., Owen P., Li M., Roy S., Xiong M., Paquin R.S., Butterfield R.M., Lewis M.A., Souris K.J., Bailey Jr. D. B., Rini C., Booker J., Powell B.C., Weck K.E., Powell C.M., Berg J.S (2019, November). *North Carolina newborn exome sequencing for universal screening (NC NEXUS) detects molecular etiologies underlying inborn errors of metabolism and hearing loss*. Poster presented at 2019 Association for Molecular Pathology Annual Meeting, Baltimore, MD

Crowley, S. B., DeCristo, D. M., Wallace, K. E., O'Daniel, J. M., Powell, C. M., & Berg, J. S. (2017, March). *Listening to the Data: an Expert-Curated Gene List to Screen for Hearing Loss in Newborns*. Poster presented at 2017 ACMG Annual Clinical Genetics Meeting, Phoenix, AZ

DeCristo D, Crowley S, Mollison L, Wallace K, Metcalf F, O'Daniel D, Powell C, Berg J. (2017, March). *Better Together: Integrating Genetic Analysis with Biochemical*

Newborn Screening. Poster presented at the 2017 ACMG Annual Clinical Genetics Meeting, Phoenix, AR.

Mollison, L., Crowley, S., DeCristo, D., Wallace, K., O'Daniel, J., Powell, C., Berg, J. (March 2017). *Explicitly Defining Age of Onset and Age of Intervention to Develop Age-Based Targeted Gene Panels for Screening Newborns and Children.* Poster presented at 2017 ACMG Conference, Phoenix, AZ.

O'Daniel, JM and Fayer, S. (September 13, 2017*) Newborn Sequencing In Genomic Medicine and Public Health (NSIGHT): Perspectives from BabySeq and NCGENES.* Invited preconference session presented at the National Society of Genetic Counseling Annual Education Conference, Columbus, OH.

Powell, CM, Roche, MI, Rini C, Lewis, MA, Paquin, RS, Bailey, DB, Margolis, M, Butterfield, R, Milko, L, Powell, B, Berg JS: "The NC NEXUS Project of Newborn Exome Sequencing", platform presentation, 35th Annual Meeting of the Southeastern Regional Genetics Group, Asheville, NC July 21, 2017.

Rini, C. (2017, March) Health Decision Making SIG Presents: Ethical Considerations for Using Online Strategies for Recruiting and Informing Participants in Genomic Sequencing Studies .Symposium conducted at the meeting of the Society for Behavioral Medicine, San Diego, CA.

Wallace, K. E., Crowley, S. B., DeCristo, D. M., Foreman, A. K. M. , Milko, L. V., Mollison, L., O'Daniel, J. M., Powell, B. C., Powell, C. M., & Berg, J. S. (2017, June). *Defining the pediatric actionability of genetic conditions for utility in newborn screening*. Poster presented at 2017 Curating the Clinical Genome, Washington, D.C.

Lewis, M., Butterfield, R., Rini, C., Paquin, R., Roche, M., Berg, J. S., ... Bailey, D. (2017). Parental decision making about genomic sequencing for their children: Ethical and practical considerations. Presented at the 2017 Annual Meeting and Scientific Sessions of the Society of Behavioral Medicine, San Diego, CA. (abstract published in *Annals of Behavioral Medicine, 51*, S2322-S2323)

Peinado, S., Paquin, R. S., Lewis, M., Rini, C., Roche, M., Butterfield, R. M., ... Bailey, D. (2018). Values clarification exercises improved parental decision making about newborn genomic sequencing. Paper presented at the 2018 Annual Meeting and Scientific Sessions of the Society of Behavioral Medicine, New Orleans, LA. (abstract published in *Annals of Behavioral Medicine*, *52*, S206)

Rini, C., Lewis, M., Roche, M., Butterfield, R., Paquin, R., Souris, K., ... Powell, C. (2018). Decision making in couples offered genomic sequencing for newborn screening: Outcomes of a surrogate, joint decision. Presented at the 2018 Annual Meeting and Scientific Sessions of the Society of Behavioral Medicine, New Orleans, LA. (abstract published in *Annals of Behavioral Medicine*, *52*, S665)