Genomic Testing: Actionability, Validation, and Standards for Lab Reports

Reaction: Heidi Rehm, PhD FACMG

eMERGE Consortium Meeting

1/22/2014

Actionability

- **Goal:** Define/discover clinically useful content that can be implemented into the practice of medicine to improve patient outcomes and/or save healthcare costs
- **Observation:** Large focus of eMERGE to date has been on genotypes (PGx, GWAS, PheWAS)
- **Suggestion:** Expand focus to **gene** - phenotype pairs instead of **genotype** - phenotype pairs

OPPORTUNITY FOR COLLABORATION

ClinGen: The Clinical Genome Resource Program

Purpose: Create a centralized repository and interconnected resources of clinically annotated genes and variants to improve our understanding of genomic variation and optimize its use in genomic medicine.

Collaboration between:

- NHGRI U41 Genomic Resource Grant
  - PIs: Ledbetter (Geisinger), Martin (Geisinger), Nussbaum (UCSF), Mitchell (Utah), Rehm (Partners/Harvard)
- NHGRI U01 "Clinically Relevant Variant Resource" Grants
  - Grant 1 PIs: Berg (UNC), Evans (UNC), Ledbetter (Geisinger), Watson (ACMG)
  - Grant 2 PIs: Bustamante (Stanford), Plon (Baylor)
- NCBI
  - ClinVar

THE MEDICAL EXOME INITIATIVE

POSTER # 1585 (Thursday)

FOUNDERS

- Harvard/Partners Lab for Molecular Medicine – *Birgit Funke*
- Emory Genetics Laboratory – *Madhuri Hegde*
- Children's Hospital of Philadelphia – *Avni Santani*



HELP STANDARDIZE MEDICAL EXOME SEQUENCING

- **1:** define medically relevant genes + develop framework for iterative curation
- **2**: develop a "medically enhanced exome" capture kit (all clinically significant genes adequately covered)
- **3:** support evidence-based curation by community experts
  - *Ledbetter/Martin/Nussbaum/Rehm* (U41)
  - *Berg/Evans/Ledbetter/Watson* (U01)
  - *Bustamante/Plon* (U01)
  - *ClinVar Database* (NCBI)

ClinGen Resource

4631 genes

Level 3	**Definitive association**
Level 2	**Likely association**
Level 1	**Weak association**
Level 0	**Uncertain association**
Level -1	**No association**

Evidenced-based Review of Gene-Disease Associations

Disease association evidence level	3
	2
	1
	0
	-1
Age of onset	< 5 yrs
	5-18 yrs
	> 18 yrs
Inheritance	AR
	AD
	XLR
	XLD
	Mitochondrial
Carrier phenotype	Likely (phenotype)
	Possible (phenotype)
	Unlikely

Penetrance	Full penetrance
	High penetrance
	Moderate penetrance
	Low penetrance
	Age-dependent penetrance
Phenotype category	Disease
	Susceptibility to disease
	Pharmacogenetic
	Disease risk modifier
Actionability	Severity of disease
	Likelihood of severe outcome
	Effectiveness of interventions
	Acceptibility of interventions
Clinically tested?	Offered as a clinical test (Lab?)

Estimate of certainty (added to each classification score)	A
	B
	C

UNC SCHOOL OF MEDICINE

GEISINGER

ACMG American College of Medical Genetics and Genomics

ClinGen Grant #2

- U01: Berg (UNC), Evans (UNC), Ledbetter (Geisinger), McLeod (UNC), Watson (ACMG) co-PIs
  - Focus on gene-based clinical actionability
  - Emphasis on expert curation
  - Informatics largely to support curation activities
  - ACMG: logistical/meeting coordination
  - Geisinger: EHR integration pilot project

Developing a Semi-Quantitative "Actionability" Scale

ORIGINAL RESEARCH ARTICLE | Genetics inMedicine

An informatics approach to analyzing the incidentalome

Jonathan S. Berg, MD, PhD[1–3], Michael Adams, MS[1], Nassib Nassar, PhD[4], Chris Bizon, PhD[4], Kristy Lee, MS[1], Charles P. Schmitt, PhD[4], Kirk C. Wilhelmsen, MD, PhD[1,3,4] and James P. Evans, MD, PhD[1–3]

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.

Genet Med advance online publication 20 September 2012

Key Words: clinical informatics; incidental findings; secondary findings; whole-exome sequencing; whole-genome sequencing

Semi-Quantitative "Actionability" Scale

- 5 key parameters of "medical actionability" when considering the case of genomic incidental findings
  - Severity of disease
  - Likelihood of a severe outcome (akin to penetrance)
  - Effectiveness of interventions (for prevention or amelioration of disease prior to developing symptoms)
  - Acceptability of interventions (considering hazards of intervention in an asymptomatic individual)
  - Knowledge base
- These parameters are then scored on a 0-3 scale to yield a final "actionability score"
- EGAPP formalized this concept for an evidence-based method to determine actionability
  - Katrina Goddard's group has a subcontract to generate the streamlined evidence review and provide curations for scoring by experts

Modified from Jonathan Berg

Test Validation

ACMG PRACTICE GUIDELINES | Genetics inMedicine

ACMG clinical laboratory standards for next-generation sequencing

Heidi L. Rehm, PhD[1,2], Sherri J. Bale, PhD[3], Pinar Bayrak-Toydemir, MD, PhD[4], Jonathan S. Berg, MD[5], Kerry K. Brown, PhD[6], Joshua L. Deignan, PhD[7], Michael J. Friez, PhD[8], Birgit H. Funke, PhD[1,2], Madhuri R. Hegde, PhD[9] and Elaine Lyon, PhD[4]; for the Working Group of the American College of Medical Genetics and Genomics Laboratory Quality Assurance Committee

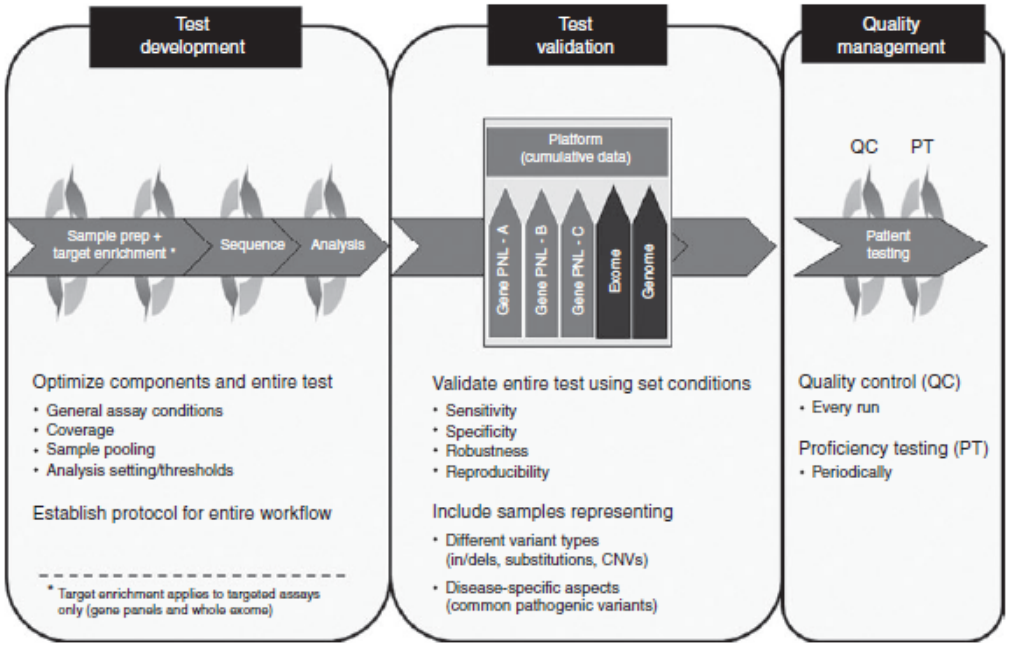


Figure 2 Next-generation sequencing test development and validation process. CNV, copy-number variant; in/dels, insertions and deletions; sample prep, sample preparation.

Validation (cont.)

ACMG PRACTICE GUIDELINES

- Validation must cover all types of rare variants being reported and address homologous regions
- For common variants, validation should be variant-specific
- Orthogonal confirmation may not be necessary if sufficient validation has been performed, quality metrics are high (coverage, mapping quality, etc) and workflow has low-risk for sample swaps

Variant Calling

- Traditional pipelines perform alignment and variant calling to generate a complete vcf file
- Genotyping accuracy can be improved through joint calling (batching many cases) but this is challenging for clinical TATs
- Improved accuracy can also be achieved through targeted “genotype” calling on raw NGS data – more amenable to clinical workflows

Standards for Lab Reports and EHR Deposition

ACMG PRACTICE GUIDELINES

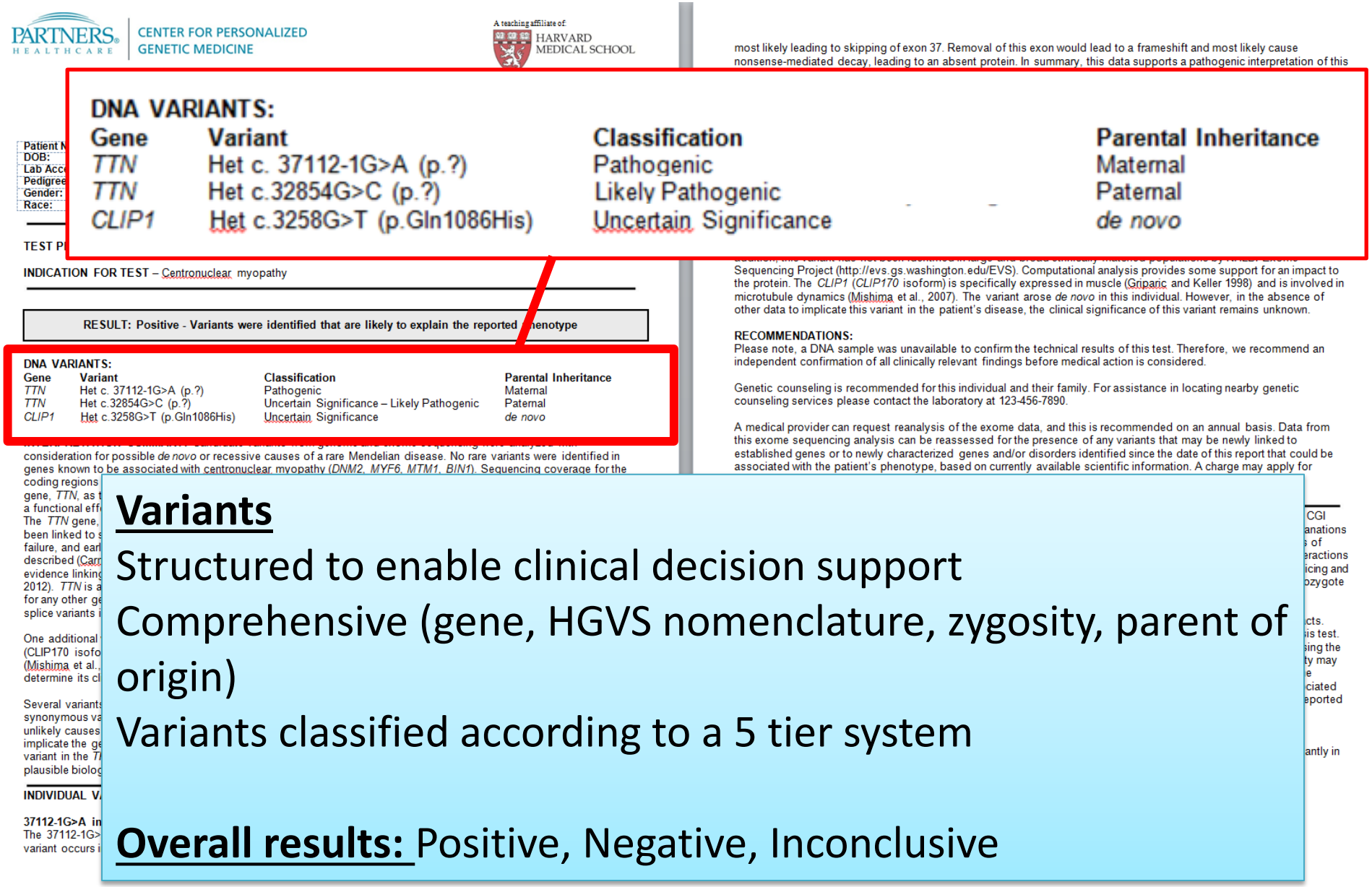
Covers:

- G.1. Turnaround times
- G.2. Data interpretation
- G.3. Reporting of incidental findings
- G.4. Written report
  - Supplement contains samples reports for NGS panels and Exome
- G.5. Data reanalysis

<u>EHR Recommendations:</u>

- Restriction to variants with analytical and clinical validity
- Variants in structured form for CDS (full report can be pdf)

Reports Contain Structured Variants



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most likely leading to skipping of exon 37. Removal of this exon would lead to a frameshift and most likely cause nonsense-mediated decay, leading to an absent protein. In summary, this data supports a pathogenic interpretation of this

DNA VARIANTS:

Gene	Variant	Classification	Parental Inheritance
TTN	Het c. 37112-1G>A (p.?)	Pathogenic	Maternal
TTN	Het c.32854G>C (p.?)	Likely Pathogenic	Paternal
CLIP1	Het c.3258G>T (p.Gln1086His)	Uncertain Significance	*de novo*

Patient N
DOB:
Lab Acc
Pedigree
Gender:
Race:

TEST P

INDICATION FOR TEST – Centronuclear myopathy

RESULT: Positive - Variants were identified that are likely to explain the reported phenotype

DNA VARIANTS:

Gene	Variant	Classification	Parental Inheritance
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TTN	Het c.32854G>C (p.?)	Uncertain Significance – Likely Pathogenic	Paternal
CLIP1	Het c.3258G>T (p.Gln1086His)	Uncertain Significance	*de novo*

consideration for possible *de novo* or recessive causes of a rare Mendelian disease. No rare variants were identified in genes known to be associated with centronuclear myopathy (*DNM2, MYF6, MTM1, BIN1*). Sequencing coverage for the coding regions
gene, *TTN*, as t
a functional effe
The *TTN* gene,
been linked to s
failure, and earl
described (Carr
evidence linking
2012). *TTN* is a
for any other ge
splice variants i

One additional
(CLIP170 isofo
(Mishima et al.,
determine its cl

Several variants
synonymous va
unlikely causes
implicate the ge
variant in the *TI*
plausible biolog

INDIVIDUAL V

37112-1G>A in
The 37112-1G>
variant occurs i

Sequencing Project (http://evs.gs.washington.edu/EVS). Computational analysis provides some support for an impact to the protein. The *CLIP1* (*CLIP170* isoform) is specifically expressed in muscle (Griparic and Keller 1998) and is involved in microtubule dynamics (Mishima et al., 2007). The variant arose *de novo* in this individual. However, in the absence of other data to implicate this variant in the patient's disease, the clinical significance of this variant remains unknown.

RECOMMENDATIONS:
Please note, a DNA sample was unavailable to confirm the technical results of this test. Therefore, we recommend an independent confirmation of all clinically relevant findings before medical action is considered.

Genetic counseling is recommended for this individual and their family. For assistance in locating nearby genetic counseling services please contact the laboratory at 123-456-7890.

A medical provider can request reanalysis of the exome data, and this is recommended on an annual basis. Data from this exome sequencing analysis can be reassessed for the presence of any variants that may be newly linked to established genes or to newly characterized genes and/or disorders identified since the date of this report that could be associated with the patient's phenotype, based on currently available scientific information. A charge may apply for

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Variants

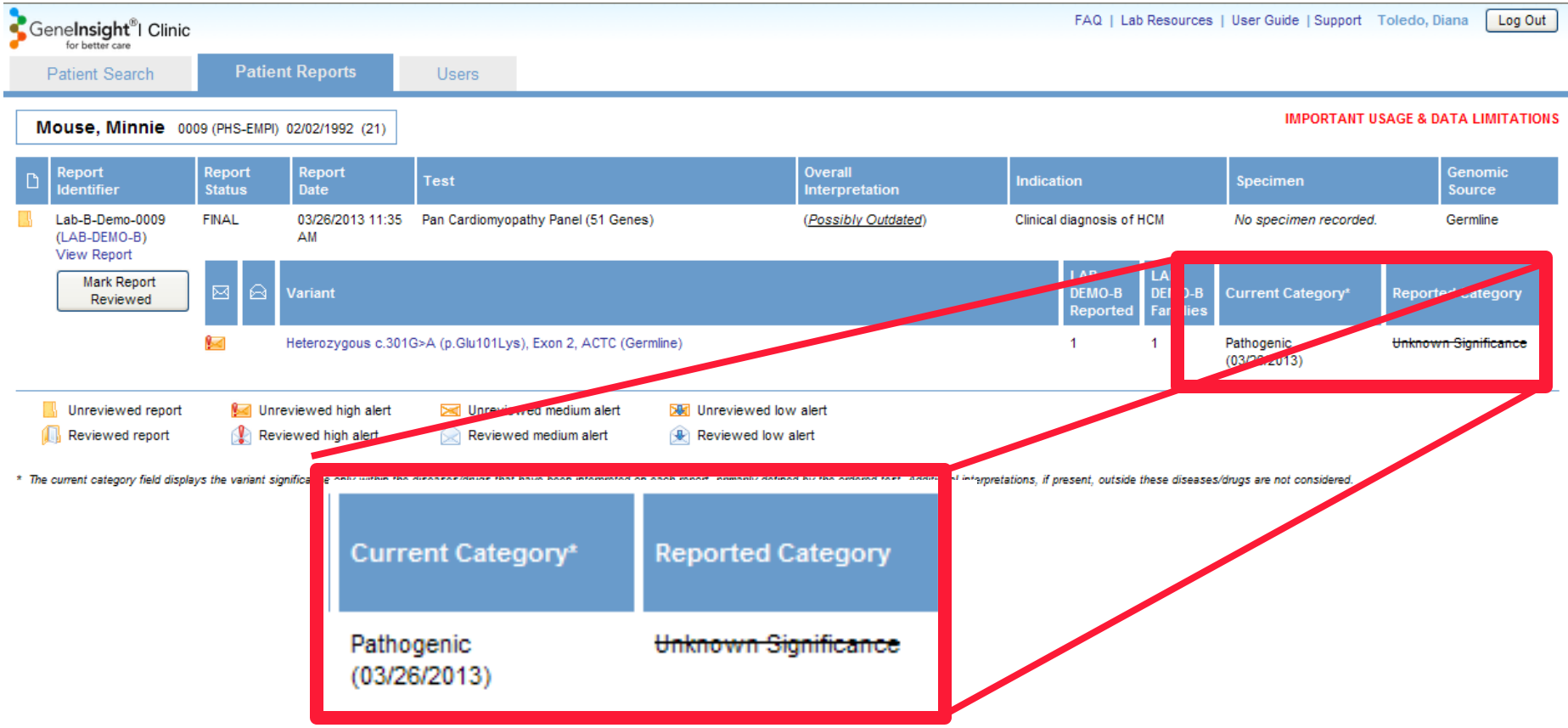
Structured to enable clinical decision support

Comprehensive (gene, HGVS nomenclature, zygosity, parent of origin)

Variants classified according to a 5 tier system

Overall results: Positive, Negative, Inconclusive

GeneInsight Clinic (EHR Integration)



Genome Report

- Generated for all MedSeq subjects in the WGS arm
- One page result summary
  - Monogenic Disease Risk
  - Carrier Risk
  - Pharmacogenomic Associations
  - Blood Groups
- Detailed information for each section provided on later pages:

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Name:
DOB:
Sex: Male
Race:

Accession ID:
MRN:
Specimen:
Received:

Family #:
Referring physician:
Referring facility: MEDSEQ

GENERAL GENOME REPORT

RESULT SUMMARY

Sequencing of this individual's genome was performed and covered 95.8% of all positions at 8X coverage or higher, resulting in over 5.2 million variants compared to reference genome. These data were analyzed to identify previously reported variants of potential clinical relevance as well as novel variants that could reasonably be assumed to cause disease (see methodology below). All results are summarized on page 1 with further details on subsequent pages.

A. MONOGENIC DISEASE RISK: 1 VARIANT IDENTIFIED

This test identified 1 genetic variant that may be responsible for existing disease or the development of disease in this individual's lifetime.

Disease (Inheritance)	Phenotype	Gene (Variant)	Classification
A1. X-linked recessive chondrodysplasia punctata (X-linked)	Abnormal bone and cartilage development	ARSE (c.410G>C p.Gly137Ala)	Uncertain Significance: Favor pathogenic

B. CARRIER RISK: 2 VARIANTS IDENTIFIED

This test identified carrier status for 2 autosomal recessive disorders.

Disease (Inheritance)	Phenotype	Gene (Variant)	Classification	Carrier Phenotype*
B1. Methylmalonic aciduria and homocystinuria,cblC type (Automosal recessive)	Disorder of cobalamin metabolism	MMACHC (c.271_272insA p.Arg91LysfsX14)	Pathogenic	None Reported
B2. Leber congenital amaurosis (Automosal recessive)	Retinal dystrophy and blindness	SPATA7 (c.94+2T>C)	Likely Pathogenic	None Reported

As a carrier for recessive genetic variants, this individual is at higher risk for having a child with one or more of these highly penetrant disorders. To determine the risk for this individual's future children to be affected, the partner of this individual would also need to be tested for these variants. Other biologically related family members may also be carriers of these variants. *Carriers for some recessive disorders may be at risk for certain phenotypes. Please see variant descriptions for more information.

C. PHARMACOGENOMIC ASSOCIATIONS

This test identified the following pharmacogenomic associations. Additional pharmacogenomic results may be requested, but will require additional molecular confirmation prior to disclosure.

Drug	Risk and Dosing Information
C1. Warfarin	Decreased dose requirement
C2. Clopidogrel	Typical response to clopidogrel
C3. Digoxin	Intermediate metabolism and serum concentration of digoxin
C4. Metformin	Intermediate glycemic response to metformin
C5. Simvastatin	Typical risk of simvastatin-related myopathy

D. BLOOD GROUPS

This test identified the ABO Rh blood type as B Positive. Additional blood group information is available at the end of the report.

It should be noted that the disease risk section of this report is limited only to variants with strong evidence for causing highly penetrant disease, or contributing to highly penetrant disease in a recessive manner. Not all variants identified have been analyzed, and not all regions of the genome have been adequately sequenced. These results should be interpreted in the context of the patient's medical evaluation, family history, and racial/ethnic background. Please note that variant classification and/or interpretation may change over time if more information becomes available. For questions about this report, please contact the Genome Resource Center at GRC@partners.org.

Monogenic Disease and Carrier Risk

Detailed Variant Information

Disease (Inheritance)	Gene (Transcript)	Variant (Classification)	Variant Frequency	Disease Prevalence	References
A1. X-linked recessive chondrodysplasia punctata (X-linked)	ARSE (NM_000047.2)	c.410G>C p.Gly137Ala hemizygous (Uncertain Significance)	1/6728 European American	1:500,000	Sheffield 1998, Nino 2008, Franco 1995, Matos-Miranda 2013

VARIANT INTERPRETATION: The Gly137Ala variant in ARSE has been previously identified in 2 males with chondrodysplasia punctata; however, this variant was also identified in one unaffected male family member (Sheffield 1998, Nino 2008). Variants in a paralogous gene (ARSB) at the same position have also been identified in an individual with Maroteux-Lamy syndrome, which also features skeletal abnormalities (Franco 1995). Functional studies indicate that the Gly137Ala variant leads to reduced ARSE activity (Matos-Miranda 2013). In summary, although some data support a disease-causing role, there is currently insufficient evidence for pathogenicity leading to a current classification of uncertain significance.

DISEASE INFORMATION: X-linked chondrodysplasia punctata 1 (CDPX1), a congenital disorder of bone and cartilage development, is caused by a deficiency of the Golgi enzyme arylsulfatase E (ARSE). It is characterized by chondrodysplasia punctata (stippled epiphyses), brachytelephalangy (shortening of the distal phalanges), and nasomaxillary hypoplasia. Although most affected males have minimal morbidity and skeletal findings that improve by adulthood, some have significant medical problems including respiratory compromise, cervical spine stenosis and instability, mixed conductive and sensorineural hearing loss, and abnormal cognitive development. From GeneReviews abstract: http://www.ncbi.nlm.nih.gov/books/NBK1544/

FAMILIAL RISK: X-Linked chondrodysplasia punctata is inherited in an X-linked recessive manner, with primarily males being affected. Each child is at a 50% (or 1 in 2) chance of inheriting the variant from a carrier female, while all daughters will inherit the variant from an affected male.

Pharmacogenomic Associations

C2. Clopidogrel (Anti-coagulation)	**Typical response to clopidogrel**	CYP2C19 rs12248560 rs4244285 rs4986893 Genotype: *1/*1 c.[-806C(;)681G(;)636G]; c.[-806C(;)681G(;)636G]	Patients with the CYP2C19 *1/*1 genotype may have extensive (typical) metabolism of clopidogrel as well as well as typical response to clopidogrel as compared to ultrarapid or poor clopidogrel metabolizers. Additional information and dosing recommendations for this result can be found at: http://www.pharmgkb.org/drug/PA449053.	Tiroch 2010, Sim 2006, Sibbing 2010

CYP2C19 GENOTYPE FREQUENCIES

Metabolism	Genotypes	Frequency
Ultrarapid	*1/*17, *17/*17	5-30%
Extensive	*1/*1	35-50%
Intermediate	*1/*2, *1/*3	18-35%
Poor	*2/*2, *2/*3, *3/*3	2-15%

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Referring physician:
Referring facility: **MEDSEQ**

D. ALLELES CONFERRING SMALL-MODERATE RISK MODIFICATION FOR EIGHT CARDIOVASCULAR PHENOTYPES

Phenotype	Contextual Data: Population Prevalence of Phenotype for Age 56	Contextual Data: Proportion of Variation in Phenotype Liability Explained by Common Genetic Variants	Patient Results: Number of Risk Loci Evaluated	Patient Results: Number of Total Risk Alleles Identified*	Patient Results: Polygenic Relative Risk**	Patient Results: Percentile Rank of Relative Risk**
Abdominal aortic aneurysm	6%	Unknown	3	3/6	1.1	60-70th
Atrial fibrillation	2%	10%	11	7/22	1.2	60-70th
Coronary heart disease	6 %	<10%	60	55/120	1.2	50-60th
Type 2 Diabetes	13%	5-10%	72	71/140	≥3.6	90-100th
Hypertension	52%	<10%	3	3/6	0.9	30-40th
Obesity	37%	1-2%	7	8/14	1.6	80-90th
Platelet aggregation	Unknown	5-10%	4	4/8	≥3.0	90-100th
QT prolongation	Unknown	7%	3	4/6	≤0.8	0-10th

*# of total possible risk alleles = # risk loci x 2 alleles per loci.
** As data utilized in this analysis were derived from non-longitudinal association studies, "Relative Risk from Common Genetic Variation" pertains to near-term risk of developing a phenotype (e.g. approximately 5 year risk), not lifetime risk. "Relative Risk from Common Genetic Variation" and "Percentile Rank of Relative Risk from Common Genetic Variation" values have been estimated using the 1000 Genomes European cohort.

- **Polygenic Predicted Fasting Lipid Profile**
- **Alleles Conferring Small-Moderate Risk for Cardiovascular Traits**

Abdominal aortic aneurysm	6%	Unknown	3	3/6	1.1	60-70th
Atrial fibrillation	2%	10%	11	7/22	1.2	60-70th
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ACMG POLICY STATEMENT | Genetics inMedicine

ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing

Robert C. Green, MD, MPH[1,2], Jonathan S. Berg, MD, PhD[3], Wayne W. Grody, MD, PhD[4–6], Sarah S. Kalia, ScM, CGC[1], Bruce R. Korf, MD, PhD[7], Christa L. Martin, PhD, FACMG[8], Amy L. McGuire, JD, PhD[9], Robert L. Nussbaum, MD[10], Julianne M. O'Daniel, MS, CGC[3], Kelly E. Ormond, MS, CGC[11], Heidi L. Rehm, PhD, FACMG[2,12], Michael S. Watson, PhD, FACMG[13], Marc S. Williams, MD, FACMG[14] and Leslie G. Biesecker, MD[15]

Inherited Cancer Disorders
Hereditary Breast and Ovarian Cancer
Li-Fraumeni Syndrome
Peutz-Jeghers Syndrome
Lynch Syndrome, FAP, MYH-Associated Polyposis
Von Hippel Lindau syndrome
Multiple Endocrine Neoplasia Types 1 & 2
Familial Medullary Thyroid Cancer (FMTC)
PTEN Hamartoma Tumor Syndrome
Retinoblastoma
Hereditary Paraganglioma-Pheochromocytoma Syndrome
WT1-related Wilms tumor
Neurofibromatosis type 2
Tuberous Sclerosis Complex

56 Genes

Cardiac Disorders
Ehlers Danlos Syndrome - vascular type
Marfan Syndrome, Loeys-Dietz Syndromes, and Familial Thoracic Aortic Aneurysms
Hypertrophic, Dilated, and ARV cardiomyopathy
Catecholaminergic polymorphic ventricular tachycardia
Romano-Ward Long QT Syndromes Types 1, 2, and 3 and Brugada Syndrome
Familial hypercholesterolemia

Other: Malignant hyperthermia susceptibility

Incidental Findings Rates:

ClinSeq 2% (ACMG list of 56 genes)

Baylor 4.6% (55/1200) or (2.6% from ACMG list)

U Wash 2.3% (23/1000) from 114 genes

GeneDx 20% (10/50) from ACMG list

mendations, and the background and rationale for these recommen-

on penetrance and clinical utility to fully support these recom-
dations, and we encourage the creation of an ongoing process
pdating these recommendations at least annually as further data
ollected.

t Med 2013:15(7):565–574

Words: genome; genomic medicine; incidental findings; per-
lized medicine; secondary findings; sequencing; whole exome;
whole genome

Variant Analysis for the Genome Report

3-5 million variants

Genes

~20,000 Coding/Splice Variants

Published as Disease-Causing

<1%

Pharmacogenetics

20-40 "Pathogenic" Variants

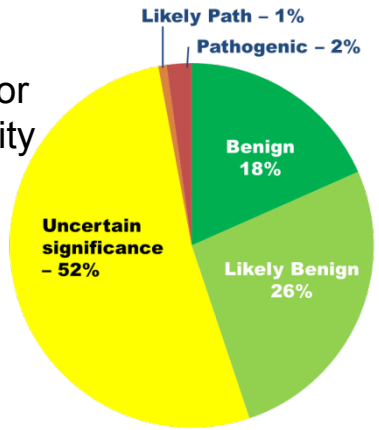
Rare CDS/Splice Variants

5-10 Variants

LOF in Disease Associated Genes

30-50 Variants

Review evidence for variant pathogenicity



97% Excluded

Review evidence for gene-disease association and LOF role

94% Excluded

Acknowledgements

The MedSeq and BabySeq Projects

The ClinGen Resource
- National Human Genome Research Institute
- U41 - BWH/Geisinger/UCSF
- U01 – UNC/ACMG/Geisinger
- U01 – Stanford/Baylor
- NCBI ClinVar

International Collaboration for Clinical Genomics

American College of Medical Genetics

The GeneInsight Team

Laboratory for Molecular Medicine

Genome >>> Report Steps