Benign or Pathogenic? Genetic Variant Interpretation in the Clinical Laboratory

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From Undetected to Understood
High School Athlete Dies Suddenly

The 16-year-old collapsed moments after making a game-winning shot.

03/05/2011
Genomic testing as Standard of Care for Heritable Cardiomyopathies

Genetics and Cardiovascular Disease: A Policy Statement From the American Heart Association
Euan A. Ashley, Ray E. Hershberger, Colleen Caleshu, Patrick T. Ellinor, Joe G.N. Garcia, David M. Herrington, Carolyn Y. Ho, Julie A. Johnson, Steven J. Kittner, Calum A. MacRae, Gia Mudd-Martin, Daniel J. Rader, Dan M. Roden, Derek Scholes, Frank W. Sellke, Jeffrey A. Towbin, Jennifer Van Eyk and Bradford B. Worrall

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Pan Cardiomyopathy Panel

Please click here for prices, turn around times, and CPT codes.

The Pan Cardiomyopathy (PCM) Panel contains 51 cardiomyopathy genes including Titin (TTN), which encodes the largest human protein. This panel covers genes associated with HCM, DCM, RCM, LVNC, ARVC and CPVT and uses a combination of Next Generation Sequencing technology and conventional Sanger sequencing.

For illustrative reference, click to see one of our images or diagrams. Genes on Pan Cardiomyopathy Panels, Disease-Gene Associations, Gene Cellular Location.

Please select on the disease to read more: HCM, DCM, ARVC/CPVT, or LVNC.

Current Tests:
Pan Cardiomyopathy Panel - 51 genes

Please click on any header to read more.

Genes: 51 genes

Methodology: A combination of next generation sequencing technology and Sanger sequencing

Analytical Sensitivity:
Substitutions: 100% (95%CI=98.5-100)  
Small InDels: 95% (95%CI=83-99)

Clinical Sensitivity: See below.

Additional Links:
- Genetic Basis of Cardiomyopathy Booklet
- How to Order
The benefits, limits of DNA sequencing
One family’s quest to understand hearing loss gave them answers, but also questions they still wonder about

By Carolyn Y. Johnson | GLOBE STAFF | AUGUST 26, 2013
OtoGenome™ Test For Hearing Loss and Usher Syndrome

Please click here for prices, turn around times, and CPT codes.

Background

Hearing loss has an incidence of 1 in 250 births and over half of these children have a genetic etiology.

The comprehensive approach of the OtoGenome Test™ now makes it possible to sequence 70 genes known to cause nonsyndromic hearing loss and syndromes that can present as nonsyndromic such as Usher, Pendred, Jervell and Lange-Nielsen (JLNS), and Branchio-Oto-Renal syndrome (BOR).

- **Nonsyndromic hearing loss**: 63 Genes (see Methods)
- **Maternally-inherited/Amino glycoside-induced**: MTTS1 (tRNAseLUC), MTRNR1 (12S rRNA)
- **Auditory neuropathy/dys-synchrony**: OTOF, DFNB59
- **Jervell and Lange-Nielsen syndrome**: KCNJ1, KCNE1
- **Pendred syndrome/Hearing loss with EVA or Mondini dysplasia**: SLC26A4 (PDS)
- **Usher syndrome (Hearing loss and retinitis pigmentosa)**: CDH23, CLRN1, DFNB31, GPR98, MYO7A, PCDH15, USH1C, USH1G, USH2A

Gene

How to Order Testing

Additional Literature:
- English
  - Understanding The Genetics of Deafness
  - Common Causes of Hearing Loss
- Spanish
  - Comprendiendo La Genética De La Sordera
  - Causas Comunes De La Sordera

http://pcpgm.partners.org/lmm/tests/hearing-loss/OtoGenome
Transformative Technology and Biomedical Research
Increased Sequencing Capability of NextGen Technology

Mardis Nature 2011
Decreased Sequencing Cost of NextGen Technology
Increasing Number of Diseases with Identified Genetic Causes

Beaudet-AL  Nature 2010, with permission
Beyond the headlines...
Groups of genetic disorders

• Mendelian Disorders – 1-2 highly penetrant alleles

• Complex genetic disease – multiple risk alleles

• Somatically mutated cancer – Few to hundreds of mutations with driver and passenger functions
Groups of genetic disorders

• Mendelian Disorders – 1-2 highly penetrant alleles

• Complex genetic disease – multiple risk alleles

• Somatically mutated cancer – Few to hundreds of mutations with driver and passenger functions
Outline

1) Variants in many genes can cause similar symptoms.

2) Some but not all variants in a given disease associated gene can cause disease.
1) Variants in Many Genes can Cause Similar Symptoms
Development of Disease Gene Panels by Clinical Laboratories

- **kb sequenced**
  - 2007: 260
  - 2009: 220
  - 2011: 240

- **Test price**
  - 2007: $5,000
  - 2009: $4,000
  - 2011: $1,000

- **# genes**
  - 2007: 10
  - 2009: 46

- **Development of Disease Gene Panels by Clinical Laboratories**

- **Methods**
  - DCM only
  - DCM only
  - DCM, HCM, ARVC
  - SANGER
  - ARRAY
  - NEXT GEN

Birgit Funke / LMM
Many Genes Contribute to Disease

Dilated Cardiomyopathy

- TTN
- LMNA
- MYH7
- DSP
- TNNT2
Other Genes than Reported Contribute to Disease

- GeneReviews (2012): 80-90% of individuals with the clinical diagnosis of Costello Syndrome carry a mutation in HRAS.”

![Bar chart showing the percentage of cases with a clinical diagnosis of Costello syndrome associated with different genes, including HRAS, BRAF, PTPN11, KRAS, SHOC2, and SOS1.]

LMM testing, n=23

Thomas Mullen, Kat Lafferty / LMM
Clinical Overlap Explains Variants in Genes Associated with Related Disease
Variant Misinterpretation Can Lead to Incorrect Gene-Disease Associations

**MYBPC3?**

DCM Cases (% of cases with likely path/patht variant)

Disease Genes

Birgit Funke / LMM
Curation of Gene-Disease Associations
Nationwide Effort to Curate Gene-Disease Associations

**STAGE 1: DEVELOP CONCEPT**
- Compile gene list
- Initiate curation framework in collaboration with NCBI (JIRA)
- Perform pilot to improve JIRA

**STAGE 2: PILOT**
- Select pilot set of genes to test draft curation framework
- Refine JIRA in collaboration with NCBI
- When robust, evaluate resources needed to curate entire (medical) exome iteratively

**STAGE 3: ENGAGE COMMUNITY**
- Curate large sets of genes by disease area

Institutions involved:
- GeneDx
- Mayo Clinic
- UNC Chapel Hill
- EGL
- LMM
- CHOP

Collaboration partners:
- U41 ICCG
- U01 CRVR

Birgit Funke / LMM
## End Goal: Classification of Medically Relevant Genes

<table>
<thead>
<tr>
<th>Level</th>
<th>Association</th>
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<tbody>
<tr>
<td>Level 3</td>
<td>Definitive association</td>
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<tr>
<td>Level 2</td>
<td>Likely association</td>
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<tr>
<td>Level 1</td>
<td>Weak association</td>
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<tr>
<td>Level 0</td>
<td>Undetermined association - no data</td>
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<tr>
<td>Level -1</td>
<td>Unlikely association</td>
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</table>
Long Term Goal: Phenotype Driven Disease Gene Selection and Testing

EXOME or GENOME → PHENOTYPE DRIVEN GENE SELECTION

MEDICAL EXOME GENE CURATION

GENES OF INTEREST → TEST RESULT

Birgit Funke / LMM
2) Many but not all Variants in a Disease Gene can Cause Disease
Human Genetic Variation
Exclusion of Benign Genetic Variation from in Depth Analysis

Initial list of variants

Exclude common benign variation

Small # of variants - need in-depth assessment

0 - 2 causative variants

Modified from Stitziel et al.
1) Does the variant lead to disrupted protein function?

2) Can disrupted function lead to disease?

3) Is this disrupted gene function causative for my patient’s presentation?
Genetic Variant Interpretation: Work Flow in the Clinical Laboratory

Lab Result

Variant Annotation

- Allele frequency in cases/controls
- Segregation with disease
- Functional studies
- Computational predictions

Variant classification

Clinical Data

Custom knowledge

Benign

Likely Benign

VUS

Likely Pathogenic

Pathogenic

NEGATIVE

INCONCL.

POSITIVE

PHYSICIAN / GENET COUNS

Clinical Report
A Variable Fraction of Variants Are Classified as Likely Path/Pathogenic

TTN
- Likely Pathogenic: 52
- Pathogenic: 1
- Benign: 396
- Unknown Significance: 667
- Likely Benign: 1529

PTPN11
- Likely Benign: 24
- Likely Pathogenic: 19
- Unknown Significance: 14
- Pathogenic: 51
- Benign: 6
The Majority of Variants are Unique to Individual Families

68% (1120/1648) are seen only once

96% of variants are seen <10 times

Number of Probands with Various Heritable Disorders

Heidi Rehm/LMM
New Evidence Results in Changes of Variant Classification

- Benign
- Likely Benign
- Unknown Significance
- Likely Pathogenic
- Pathogenic

~300 category changes over 5 years impacting > 1000 patients with HCM

Heidi Rehm/LMM
Large Gene Panels Increase the Fraction of Positive and Inconclusive Results

Birgit Funke/LMM
INDICATION FOR TEST - DCM and family history of DCM and RCM

RESULTS DNA VARIANTS:
Heterozygous c.427C>T (p.Arg143Trp), Exon 5, MYH7, Unknown Significance
Heterozygous c.4588C>T (p.Arg1530X), Exon 33, MYH7, Unknown Significance

INTERPRETATION: Positive. DNA sequencing of exon 5 of the MYH7 gene revealed the heterozygous Arg143Trp variant, previously identified in this individual's sister. DNA sequencing of exon 33 of the MYH7 gene revealed the heterozygous Arg1530X variant, previously identified in this individual's sister.

Summary: Although it is unclear whether each of the variants detected can cause disease in isolation, it is likely that their combination is causative for disease in this individual as well as the affected sister (see below for individual variant interpretations). Each unaffected parent was found to carry one variant (father: Arg1530X, mother: Arg143Trp), which raises the possibility that they are milder in isolation. The Arg1530X variant is expected to lead to at least some decay of the mRNA (loss of function). On the background of reduced MYH7 expression the effect of the Arg143Trp variant may be exacerbated leading to early heart failure/DCM. The (Arg143Trp) has been previously reported in an individual with HCM and its presence in this individual’s mother as well as maternal aunt whose child was affected with RCM is consistent with a pathogenic role (MYH7 variants have also been reported in patients with RCM). However, there appears to be substantial variability (mother and aunt are reportedly unaffected while the niece has early onset RCM). Additional family studies, particularly of affected individuals may help to clarify the significance of these variants.
Approach:
Health Informatics and Large Collaborations
In Depth Variant Assessment is Time Consuming
Knowledge Database and Reporting Software Enables Collaborative Approach
Automated Communication of Updated Variant Classification

This screenshot was taken from a demonstration system – the content of this screen should not be used for any clinical purpose.
Many Clinical Laboratories Share their Data Publicly

<table>
<thead>
<tr>
<th>Group</th>
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Total = 41923

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The Patients