Cardiomyopathies: clinical diagnostic and research sequencing

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Genetics and Genomics of Disease Pathway
Washington University in Saint Louis

September 14, 2016
Promise of genomic medicine

Hegele, Nat Rev Gen 2009
Promise of genomic medicine

Fundamental challenge for human genetics in research and clinical care:

What genetic changes are related to disease?
Clinical case

- 48 year old female
  - Referred to Center for Cardiovascular Genetics seeking genetic testing for hypertrophic cardiomyopathy

- Past medical history:
  - High blood pressure
  - Bilateral hearing loss
Clinical case

• 71 year old mother
  – Apical HCM diagnosed during pre-operative screening
  – Genetic testing revealed “probably causal” mutation in *MYBPC3*
  – Patient is seeking confirmation testing to see if she is a carrier
Clinical case

• What does this mean for me and my family?

No relevant history
71; HCM diagnosed during pre-op screening

13  11  14

48

11; PFO on Echo

46
Genetics of cardiovascular disease

Patterns of disease aggregation within families indicate likely genetic influence
Genetics of cardiovascular disease

- Complex genetic disorders (multiple genes)
- Mendelian disorders (single gene)

Lipids
Blood Pressure
Coronary Heart Disease

Cardiomyopathies
Arrhythmias
Lipids
Vascular syndromes
Inherited cardiomyopathies: Generalizations

(1) Broadly categorized by ventricular geometry and associated arrhythmias
   a) Hypertrophic
   b) Dilated
   c) Non-compacted
   d) Arrhythmic

(2) Autosomal dominant (typically)

(3) Genetically complex
Complexity in genetic cardiomyopathies

Locus heterogeneity

Allelic heterogeneity

Genetic overlap

MYH7
LMNA
TTN
DCM
(>30)

1000s of mutations described

MYH7

HCM
DCM
LVNC
Mendelian CV syndromes: substantial genetic overlap

- **Hypertrophic Cardiomyopathy**: 20 genes
- **Dilated Cardiomyopathy**: 32 genes
- **ARVC**: 8 genes
- **LVNC**: 13 genes
- **Brugada syndrome**: 10 genes
- **Short QT syndrome**: 5 genes
- **CPVT**: 5 genes
- **Long QT syndrome**: 20 genes
Complexity in genetic cardiomyopathies

Locus heterogeneity

Allelic heterogeneity

Genetic overlap

1000s of mutations described

Incomplete penetrance

Age-dependent penetrance

Phenocopies

Variable expressivity

MYH7, LMNA, TTN

DCM

MYH7

HCM

DCM

LVNC

EtOH

CCD

Incomplete penetrance

Age-dependent penetrance

Phenocopies

Variable expressivity
Identifying genetic basis in familial cardiomyopathies

<table>
<thead>
<tr>
<th>Causal mutations in known genes</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertrophic</td>
<td>~70%</td>
</tr>
<tr>
<td>Dilated</td>
<td>~35%</td>
</tr>
<tr>
<td>Arrhythmic</td>
<td>~50%</td>
</tr>
<tr>
<td>Non-compacted</td>
<td>~15%</td>
</tr>
</tbody>
</table>
Idiopathic DCM is not all idiopathic

<table>
<thead>
<tr>
<th>Family evaluation</th>
<th>Estimated prevalence of familial disease in idiopathic DCM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chart review</td>
<td>~2%</td>
</tr>
<tr>
<td>Detailed pedigree construction</td>
<td>~10-25%</td>
</tr>
<tr>
<td>Detailed pedigree construction with screening echocardiography</td>
<td>~30-40%</td>
</tr>
</tbody>
</table>

Yield of genetic screening

Idiopathic DCM ~ Familial DCM

Burkett et al. JACC 2005
Insights from studying inherited basis of cardiomyopathies

**HCM**: A disease of the sarcomere
- Basic understanding of muscle biology
- Focused hypotheses on G+/P- carriers

**ARVC**: A disease of the desmosome

**DCM**: A disease of many diseases
- Force generation, force transmission, energy production, many others to learn
Why test for cardiovascular disease?

1. Diagnostic clarity
   - Potential to end “diagnostic odyssey”
   - HCM vs “athlete’s heart”

2. Identify at risk individuals

3. Genotype guided therapies
   - LongQT syndrome subtypes
   - Enzyme replacement therapy for Fabry’s
   - Promise of cardiovascular genetics
Clinical translation

• Center for Cardiovascular Genetics is:
  – Multidisciplinary clinic focused on evaluation and management of individuals and families with:
    • Known or suspected inherited heart disease (ARVC, DCM, HCM, LQTS, MI/CAD, Lipids, etc)
    • Unclear diagnosis
    • Unknown syndrome
Clinical translation

• Center for Cardiovascular Genetics offers:
  1. Genetic evaluation
  2. Coordinate genetic testing
  3. Determine personalized diagnostic and treatment plans
  4. Genetic counseling and education
  5. IRB-approved research protocols
Clinical translation

Now available: comprehensive genetic testing for disorders that can cause cardiac sudden death.

In collaboration with the Cardiovascular Division at Washington University, Genomics and Pathology Services (GPS) offers a cost-effective analysis of genes with demonstrated importance in the treatment of arrhythmias and cardiomyopathies.

**Physician Benefits:**
- Directed patient management through the identification of the genetic cause and confirmation of diagnosis of cardiac disorders
- Availability of disorder-specific gene subsets as well as larger comprehensive sets for less specific symptoms
- Easy ordering process: insurance preauthorization performed by GPS
- Expert interpretation of genomic results

**Patient Benefits:**
- Increased likelihood of learning the genetic cause of a cardiac disorder compared to single gene tests
- Improved and personalized clinical care with a genetic diagnosis
- Targeted genetic analysis of family members available
- Affordable testing, covered by most insurance plans
- Timely referrals

CardioGene Set
Washington University
Genomics and Pathology Services
Clinical translation

<table>
<thead>
<tr>
<th>Disease Subsets</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long QT Syndrome</td>
<td>AKAP9, ANK2, CACNA1C, CAV3, KCNE1, KCNE2, KCNH2, KCNJ2, KCNJ5, KCNQ1, SCN4B, SCN5A, SNTA1</td>
</tr>
<tr>
<td>Brugada Syndrome</td>
<td>CACNA1C, CACNB2, GPD1L, HCN4, KCND3, KCNE3, KCNJ8, SCN1B, SCN3B, SCN5A</td>
</tr>
<tr>
<td>CPVT</td>
<td>ANK2, CALM1, CASQ2, KCNJ2, KCNQ1</td>
</tr>
<tr>
<td>Short QT Syndrome</td>
<td>CACNA1C, CACNB2, KCNH2, KCNJ2, KCNQ1</td>
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<tr>
<td>HCM</td>
<td>ACTC1, ACTN2, CSRP3, GLA, LAMP2, MYBPC3, MYH6, MYH7, MYL2, MYL3, MYLK2, MYOZ2, NEXN, PLN, PRKAG2, TNNC1, TNNI3, TNNT2, TPM1, TTR</td>
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<td>DCM</td>
<td>ABCC9, ACTC1, ACTN2, ANKRD1, BAG3, CSRP3, CTF1, DES, EMD, FHL1, FHL2, GATAD1, LAMP2, LDB3, LMNA, MYBPC3, MYH6, MYH7, NEXN, PLN, RBM20, SCN5A, SGCD, TAZ, TCAP, TMPO, TNNC1, TNNI3, TNNT2, TPM1, TTN, VCL</td>
</tr>
<tr>
<td>LVNC</td>
<td>ACTC1, CASQ2, DTNA, LDB3, LMNA, MYBPC3, MYH7, TAZ, TNNT2, VCL</td>
</tr>
<tr>
<td>ARVC</td>
<td>DES, DSC2, DSG2, DSP, JUP, PKP2, RYR2, TMEM43</td>
</tr>
</tbody>
</table>
Clinical translation: challenges

• Payors
  – Probands and relatives

• Genetic complexity
  – Families and populations

• Interpreting findings and determining causality
  – Incidental findings
Clinical Genetic Testing

- Regulated by CAP and CLIA
- Often LDT, not FDA cleared
- Performed to aid in:
  - Diagnosis
  - Prognosis
  - Therapeutic decision making
- Utility of testing must be established
  - Impact on clinical care
  - Payors
- Ordered by a clinician
  - Not DTC (direct-to-consumer, ala 23andMe)
- Access to genetic counseling
  - Interpretation
  - Patient management
  - Recurrence risk
Single Locus vs. Multiple Gene Testing

**Locus specific testing**
- Analyze single gene/locus
- Determine mutation status of limited region

**Multiple gene testing**
- Analyze multiple relevant genes
- Determine mutation status of all relevant genes simultaneously

- Narrowly targeted
- Result may trigger additional gene testing
- Cost effective
- Efficient/time-saving
- Yields unexpected findings
Next-Generation Sequencing

- Sanger sequencing – 2x read (Bidirectional)

- Next-generation – 100-1000x reads at single position
NGS Workflow

Specimen

Sequencing

Biofx analysis

Variant review

Concise clinical report

Patient Information:
- Name: Last, First, MI
- MRN: 0000
- Date of Birth: mm/dd/yyyy
- Specimen Type: FFPE
- Indication: Lung adenocarcinoma
- Date Collected: mm/dd/yyyy
- Date Ordered: mm/dd/yyyy
- Date Accessed: mm/dd/yyyy

Test Performed:
- Solid Tumor Gene Set - Targeted next-generation sequencing was performed on this sample of Lung Cancer. See unter Text for more information.

Clinically Relevant Results Summary:
- Variants detected in patient tumor type:
  - EGFR p.E746_A750del

Clinically Relevant Interpretations:
- Variants detected in other tumor types:
  - EGFR p.E746_A750del

References:
Clinical testing for patient care

Cancer (150 genes)
Overgrowth (12 genes)
Cardiac disease (80 genes)
Renal disease (25 genes)
Severe Congenital Neutropenia

Support for clinical trials and research

Cancer
Alzheimer’s
Clinical Trials
Custom project
Exome Panel Carve-Out

Agilent Clinical Research Exome
Medically Enhanced Gene Coverage

Cardio
Renal
Congenital Neutropenia
<table>
<thead>
<tr>
<th>Disease area</th>
<th>Disease type</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer</td>
<td>Hereditary cancers (for example, breast, colon and ovarian)</td>
<td>10–50</td>
</tr>
<tr>
<td>Cardiac diseases</td>
<td>Cardiomyopathies</td>
<td>50–70</td>
</tr>
<tr>
<td></td>
<td>Arrhythmias (for example, long QT syndrome)</td>
<td>10–30</td>
</tr>
<tr>
<td></td>
<td>Aortopathies (for example, Marfan’s syndrome)</td>
<td>10</td>
</tr>
<tr>
<td>Immune disorders</td>
<td>Severe combined immunodeficiency syndrome</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Periodic fever</td>
<td>7</td>
</tr>
<tr>
<td>Neurological, neuromuscular and metabolic disorders</td>
<td>Ataxia</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Cellular energetics, metabolism</td>
<td>656</td>
</tr>
<tr>
<td></td>
<td>Congenital disorders of glycosylation</td>
<td>23–28</td>
</tr>
<tr>
<td></td>
<td>Dementia (for example, Parkinson’s disease and Alzheimer’s disease)</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Developmental delay, autism, intellectual disability</td>
<td>30–150</td>
</tr>
<tr>
<td></td>
<td>Epilepsy</td>
<td>53–130</td>
</tr>
<tr>
<td></td>
<td>Hereditary neuropathy</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>Microcephaly</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Mitochondrial disorders</td>
<td>37–450</td>
</tr>
<tr>
<td></td>
<td>Muscular dystrophy</td>
<td>12–45</td>
</tr>
<tr>
<td>Sensory disorders</td>
<td>Eye disease (for example, retinitis pigmentosa)</td>
<td>66–140</td>
</tr>
<tr>
<td></td>
<td>Hearing loss and related syndromes</td>
<td>23–72</td>
</tr>
<tr>
<td>Other</td>
<td>Rasopathies (for example, Noonan’s syndrome)</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Pulmonary disorders (for example, cystic fibrosis)</td>
<td>12–40</td>
</tr>
<tr>
<td></td>
<td>Short stature</td>
<td>12</td>
</tr>
</tbody>
</table>
Utility of Disease Targeted Sequencing
- Diagnostic Yield

Enhanced Diagnostic Yield with Comprehensive Sequencing
- e.g. Muscular Dystrophies

Cardiac Disease Targeted Sequencing
- Amenable to multigenic phenotypes

Table 2  Summary of Common Cardiac Channelopathy/Cardiomyopathy-Associated Genes (≥5% of Disease)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Locus</th>
<th>Protein</th>
<th>% of Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Section I – Long QT Syndrome (LQTS)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KCNQ1 (LQT1)</td>
<td>11p15.5</td>
<td>IKs, potassium channel alpha subunit (Kv7.1)</td>
<td>30%-35%</td>
</tr>
<tr>
<td>KCNH2 (LQT2)</td>
<td>7q35-q36</td>
<td>IKr, potassium channel alpha subunit (Kv11.1 or hERG)</td>
<td>25%-40%</td>
</tr>
<tr>
<td>SCN5A (LQT3)</td>
<td>3p21</td>
<td>Cardiac sodium channel alpha subunit (NaV1.5)</td>
<td>5%-10%</td>
</tr>
<tr>
<td><strong>Section II – Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RYR2 (CPVT1)</td>
<td>1q42.1-q43</td>
<td>Ryanodine receptor 2</td>
<td>60%</td>
</tr>
<tr>
<td><strong>Section III – Brugada Syndrome (BrS)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCN5A</td>
<td>3p21</td>
<td>Cardiac sodium channel alpha subunit (NaV1.5)</td>
<td>20%-30%</td>
</tr>
<tr>
<td><strong>Section IV – Cardiac Conduction Disease (CCD)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCN5A</td>
<td>3p21</td>
<td>Cardiac sodium channel alpha subunit (NaV1.5)</td>
<td>5%</td>
</tr>
<tr>
<td><strong>Section V – Short QT Syndrome (SQTS)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None of the three known disease-associated genes has been shown to account for ≥5% of this disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Section VI – Atrial Fibrillation (AF)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None of the known disease-associated genes has been shown to account for ≥5% of this disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Section VII – Hypertrophic Cardiomyopathy (HCM)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MYBPC3</td>
<td>11p11.2</td>
<td>Cardiac myosin-binding protein C</td>
<td>20%-45%</td>
</tr>
<tr>
<td>MYH7</td>
<td>14q11.2-q12</td>
<td>β-Myosin heavy chain</td>
<td>15%-20%</td>
</tr>
<tr>
<td>TNNT2</td>
<td>1q32</td>
<td>Cardiac troponin I type 2</td>
<td>1%-7%</td>
</tr>
<tr>
<td>TNNI3</td>
<td>19q13.4</td>
<td>Cardiac troponin I type 3</td>
<td>1%-7%</td>
</tr>
</tbody>
</table>

Heart Rhythm, Vol 8, No 8, August 2011
Cardio – Diagnostic Yield

Heart Rhythm, Vol 8, No 8, August 2011

Table 3  Yield and Signal-to-Noise Associated with Disease-Specific Genetic Testing

<table>
<thead>
<tr>
<th>Section – Disease</th>
<th>Yield of Genetic Test*</th>
<th>% of Controls with a Rare VUS#</th>
<th>Signal-to-Noise (S:N) Ratio+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Section I – LQTS</td>
<td>75% (80%)</td>
<td>4%</td>
<td>19:1</td>
</tr>
<tr>
<td>Section II – CPVT</td>
<td>60% (70%)</td>
<td>3%</td>
<td>20:1</td>
</tr>
<tr>
<td>Section III – BrS</td>
<td>20% (30%)</td>
<td>2% (just SCN5A)</td>
<td>10:1</td>
</tr>
<tr>
<td>Section IV – CCD</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Section V – SQTS</td>
<td>Unknown</td>
<td>3%</td>
<td>Unknown</td>
</tr>
<tr>
<td>Section VI – AF</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Section VII – HCM</td>
<td>60% (70%)</td>
<td>~5% (unpublished data)</td>
<td>12:1</td>
</tr>
<tr>
<td>Section VIII – ACM/ARVC</td>
<td>60%</td>
<td>16%</td>
<td>4:1</td>
</tr>
<tr>
<td>Section IX – DCM</td>
<td>30%</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Section IX – DCM + CCD</td>
<td>Unknown</td>
<td>4% (for SCN5A and LMNA)</td>
<td>Unknown</td>
</tr>
<tr>
<td>Section X – LVNC</td>
<td>17%–41%</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Section XI – RCM</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

*Yield of Genetic Test is a published/unpublished estimate derived from unrelated cases with unequivocal disease phenotype. First number is the yield associated with the targeted major gene scan. The number in parentheses is the total yield when including all known disease-associated genes that have been included in commercial disease gene panels. When only a single percentage is provided, this represents the estimate from a comprehensive disease gene panel. These yield values represent estimates for whites with the particular disease phenotype. Evidence is lacking to establish point estimates for minority populations.

#% of Controls with a Rare Variant of Uncertain Significance (VUS) represents a frequency of rare amino acid substitutions found in whites in the major disease-associated genes that, had it been found in a case, would have been reported as a “possible disease-associated mutation.” This number does not include the frequency of rare genetic variants present in the minor disease-associated genes. Thus, it represents a lower point estimate for the potential false positive rate. A question mark indicates that an otherwise healthy control population has not been systematically examined for the genes of interest. As with the Yield of Genetic Test, these estimates are derived for whites.

+The signal-to-noise (S:N) ratio is derived by dividing the yield by the background rate of VUS in controls. This provides a sense of the positive predictive value of a “positive” genetic test result.
Considerations for Assay Design

• Custom assay
  – Highly specific
  – Can be more costly $$
  – Will require optimization

• Commercial assay
  – Off the shelf option
  – Can be customizable

• Consider wet-lab/bioinformatic modifications

• Consider target space and coverage
Whole Exome Approach for Disease Targeted Sequencing

• Only limited validation steps necessary upon expansion
  • Other genes of interest
  • Other disease areas

• Supplemental/Backfill methodologies may be necessary
  – Dependent on mutational spectrum/coverage

• Some assays more amenable to custom design
CardioGene Set Test Design

• Goal is to create one comprehensive platform with utility for multiple cardiac phenotypes

• Strategic planning
  – Utilize design with ability to encompass multiple clinical tests
  – Whole exome approach
    • Analytic sensitivity, specificity, reproducibility determined for well–characterized reference samples
    • Only limited validation steps necessary upon expansion to include additional genes or separate panels
  – Cardiac, Renal, LGMD, Noonan
CardioGene Set Design

• Target enrichment
  – In solution hybrid capture (Agilent Clinical Research Exome)

• Capture Design
  – Enhanced coverage across exome in disease associated regions
    • OMIM, HGMD, ClinVar

• Sequencing Platforms
  – HiSeq 2500, paired-end 101bp

Figure 2. The performance-optimized design of the SureSelect Clinical Research Exome enables deeper coverage of disease relevant targets from HGMD, OMIM and ClinVar compared to other disease-focused capture solutions in the market when sequenced with the same average coverage (A) while providing a comprehensive exome design based on the high-performing SureSelect Human All Exon V5 (B).
Agilent SureSelect Target Capture

- Baits are cRNA
- Multiple biotinylation
- High fidelity 120mers
# Washington University CardioGene Set

**Mutational analysis of all coding regions of all ordered genes**

<table>
<thead>
<tr>
<th>CARDIOMYOPATHIES</th>
<th>ARRHYTHMIAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>LQTS</td>
<td>AKAP9, ANK2, CACNA1C, CAV3, KCNE1, KCNE2, KCNH2, KCNJ2, KCNJ5, KCNQ1, SCN4B, SCN5A, SNTA1</td>
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<td>Brugada</td>
<td>CACNA1C, CACNB2, GPD1L, HCN4, KCND3, KCNE3, KCNJ8, SCN1B, SCN3B, SCN5A</td>
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<td>CPVT</td>
<td>ANK2, CALM1, CASQ2, KCNJ2, RYR2</td>
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<td>SQTS</td>
<td>CACNA1C, CACNB2, KCNH2, KCNJ2, KCNQ1</td>
</tr>
<tr>
<td>HCM</td>
<td>ACTC1, ACTN2, BRAF, CSRP3, GLA, HRAS, KRAS, LAMP2, MAP2K1, MAK2K2, MYBPC3, MYH6, MYH7, MYL2, MYL3, MYLK2, MYOZ2, NEXN, NRAS, PLN, PRKAG2, PTPN11, RAF1, RIT1, SHOC2, SOS1, TNNC1, TNNI3, TNNT2, TPM1, TTR</td>
</tr>
<tr>
<td>DCM</td>
<td>ABCC9, ACTC1, ACTN2, ANKRD1, BAG3, CSRP3, CTF1, DES, EMD, FHL1, FHL2, GATAD1, LAMP2, LDB3, LMNA, MYBPC3, MYH6, MYH7, NEXN, PLN, RBM20, SCN5A, SGCD, TAZ, TCAP, TMPD, TNNC1, TNNI3, TNNT2, TPM1, TTN, VCL</td>
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<td>LVNC</td>
<td>ACTC1, CASQ2, DTNA, LDB3, LMNA, MYBPC3, MYH7, TAZ, TNNT2, VCL</td>
</tr>
<tr>
<td>ARVC</td>
<td>DES, DSC2, DSG2, DSP, JUP, PKP2, RYR2, TMEM43</td>
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Sanger Backfill Strategy

<table>
<thead>
<tr>
<th>Chr</th>
<th>Start</th>
<th>Stop</th>
<th>Gene</th>
<th>Transcript</th>
<th>Exon</th>
<th>Arrhythmia Gene Set</th>
<th>Long QT Syndrome Gene Set</th>
<th>Brugada Syndrome Gene Set</th>
<th>CPVT Gene Set</th>
<th>Short QT Syndrome Gene Set</th>
<th>Cardiomyopathy Gene Set</th>
<th>HCM Gene Set</th>
<th>DCM Gene Set</th>
<th>LVNC Gene Set</th>
<th>AVRC Gene Set</th>
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<td>chr7</td>
<td>150655147</td>
<td>150655590</td>
<td>KCNH2</td>
<td>NM_000238</td>
<td>4</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
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<tr>
<td>chr7</td>
<td>150644694</td>
<td>150644966</td>
<td>KCNH2</td>
<td>NM_000238</td>
<td>12</td>
<td>x</td>
<td>x</td>
<td>x</td>
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Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology

Sue Richards, PhD1, Nazneen Aziz, PhD2,16, Sherri Bale, PhD3, David Bick, MD4, Soma Das, PhD5, Julie Gastier-Foster, PhD6,7,8, Wayne W. Grody, MD, PhD9,10,11, Madhuri Hegde, PhD12, Elaine Lyon, PhD13, Elaine Spector, PhD14, Karl Voelkerding, MD13 and Heidi L. Rehm, PhD15; on behalf of the ACMG Laboratory Quality Assurance Committee
Evidence use to aid variant interpretation

- **Frequency Data:** ExAC (1000 genomes, NHLBI-GO ESP; dbSNP)

- **Effect on Protein:** Conservation Data, *In-silico* predictions (Protein function (PolyPhen2, SIFT, CONDEL), Splicing)

- **Literature:** Primary articles from quality journals, Recent reviews, Case Studies

- **Clinical Databases:** HGMD, ClinVar, noting evidence for clinical assertions

- **Locus/Disease Specific Databases:** Leiden, UMD, ARVC, aHUS

- **Laboratory Specific Databases:** ARUP, Emory
ACMG Lines of Evidence

**Very strong evidence of pathogenicity**

PVS1 Truncating variant (nonsense, frameshift, affects canonical +/-1 or 2 splice sites, initiation codon, entire exon or multi-exon deletion) in a gene where loss of function is a known mechanism of disease

Caveats:
- Beware of genes where LOF is not a known disease mechanism (e.g. GFAP, MYH7)
- Use caution interpreting LOF variants at the extreme 3’ end of a gene
- Use caution with splice variants that are predicted to lead to exon skipping but leave the remainder of the protein intact
- Use caution in the presence of multiple transcripts

**Strong evidence of pathogenicity**

PS1 Same amino acid change as a previously established pathogenic variant regardless of nucleotide change

Example: Val->Leu caused by either G>C or G>T in the same codon

Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level

PS2 *De novo* (both maternity and paternity confirmed) in a patient with the disease and no family history

Note: Confirmation of paternity only is insufficient. Egg donation, surrogate motherhood, errors in embryo transfer, etc. can contribute to non-maternity

PS3 Well-established *in vitro* or *in vivo* functional studies supportive of a deleterious effect on the gene or gene product
ACMG Lines of Evidence

Stand-Alone evidence of benign impact

BA1  Allele frequency is above 5% in Exome Sequencing Project or 1000 Genomes

Strong evidence of benign impact

BS1  Allele frequency is greater than expected for disorder (see table 6)

BS2  Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder with full penetrance expected at an early age

BS3  Well-established *in-vitro* or *in vivo* functional studies shows no deleterious effect on protein function or splicing

BS4  Lack of segregation in affected members of a family

   Caveat: The presence of phenocopies for common phenotypes (i.e. cancer, epilepsy) can mimic lack of segregation among affected individuals.

Supporting evidence of benign impact

BP1  Missense variant in a gene for which primarily truncating variants are known to cause disease

BP2  Observed in *trans* with a pathogenic variant for a fully penetrant dominant gene/disorder; or observed in *cis* with a pathogenic variant in any inheritance pattern.

BP3  In-frame deletions/insertions in a repetitive region without a known function
Table 5. The Scoring Rules for Classification

<table>
<thead>
<tr>
<th>Pathogenic</th>
<th>Likely Pathogenic</th>
<th>Benign</th>
<th>Likely Benign</th>
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<tr>
<td>a) Very Strong (PVS1) AND</td>
<td>a) 1 Very Strong (PVS1) or 1 Strong (PS1-PS4) AND</td>
<td>a) Stand-Alone (BA1) OR</td>
<td>a) 1 Strong (BS1-BS4) and 1 Supporting (BP1-BP6) OR</td>
</tr>
<tr>
<td>b) 1 Strong (PS1-PS4) OR</td>
<td>b) 1 Moderate (PM1-PM6) OR</td>
<td>b) ≥2 Strong (BS1-BS4)</td>
<td>b) ≥2 Supporting (BP1-BP6)</td>
</tr>
<tr>
<td>c) ≥2 Moderate (PM1-PM6) OR</td>
<td>c) ≥2 Moderate (PM1-PM6) and 1 Supporting (PP1-PP5) OR</td>
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<tr>
<td>d) 1 Moderate (PM1-PM6) and 1 Supporting (PP1-PP5)</td>
<td>d) ≥1 Moderate (PM1-PM6) and ≥4 Supporting (PP1-PP5)</td>
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<tr>
<td>e) ≥2 Supporting (PP1-PP5)</td>
<td>e) ≥2 Moderate (PM1-PM6) and 2 Supporting (PP1-PP5)</td>
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*Variants should be classified as Uncertain Significance if other criteria are unmet or the evidence for benign and pathogenic are contradictory.*
<table>
<thead>
<tr>
<th>Level</th>
<th>Evidence</th>
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<td>Very strong</td>
<td>PVS1 null variant (nonsense, frameshift, canonical ± 1 or 2 splice sites, initiation codon, etc) in a gene where LOF is a known disease mechanism. Caveats: Beware of genes where LOF is not a known disease mechanism (e.g., GFAP, MYH7). Use caution interpreting LOF variants at the extreme 3' end of a gene. Use caution with splice variants that are predicted to lead to exon skipping but leave the remainder of the protein intact.</td>
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<tr>
<td>Strong</td>
<td>PS1 Same amino acid change as a previously established pathogenic variant regardless of nucleotide change. Example: Val-Leu caused by either G&gt;C or G&gt;T in the same codon. Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level.</td>
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<tr>
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<td>PS2 De novo (both maternity and paternity confirmed) in a patient with the disease and no family history. Note: Confirmation of paternity only is insufficient. Egg donation, embryo transfer errors, etc. can contribute to nonmaternity.</td>
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<tr>
<td></td>
<td>PS3 Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product.</td>
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<tr>
<td></td>
<td>PS4 The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls. Note 1: Relative risk or OR, as obtained from case-control studies, is &gt;5.0, and the confidence interval around the estimate of relative risk or OR does not include 1.0. Note 2: In instances of very rare variants where case-control studies may not reach statistical significance, the prior observation of the variant in multiple unrelated patients with the same phenotype, and its absence in controls, may be used as moderate level of evidence.</td>
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<tr>
<td>Moderate</td>
<td>PM1 Located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) w/o benign variation.</td>
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<td></td>
<td>PM2 Absent from controls (or at extremely low frequency if recessive [i.e., patient is homozygous]) in population databases. Caveat: Population data for insertions/deletions may be poorly called by next-generation sequencing.</td>
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<td>PM3 For recessive disorders, detected in trans with a pathogenic variant. Note: This requires testing of parents (or offspring) to determine phase.</td>
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<td>PM4 Protein length changes as a result of in-frame deletions/insertions in a nonrepeat region or stop-loss variants.</td>
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<td>PM5 Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before. Example: Arg156His is pathogenic; now you observe Arg156Cys. Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level.</td>
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<td>PM6 Assumed de novo, but without confirmation of paternity and maternity.</td>
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<tr>
<td>Supporting</td>
<td>PP1 Consanguination with disease in multiple affected family members in a gene definitively known to cause the disease. Note: May be used as stronger evidence with increasing segregation data.</td>
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<td>PP2 Missense variant in a gene with a low rate of benign missense variation and in which missense variants are a common disease mechanism.</td>
</tr>
<tr>
<td></td>
<td>PP3 Multiple computational tools predict deleterious effect: gene/gene product (conservation, evolutionary, splicing impact, etc.). Caveat: Because many in silico algorithms use the same or very similar input for their predictions, each algorithm should not be counted as an independent criterion. PP3 can be used only once in any evaluation of a variant.</td>
</tr>
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<td>PP4 Patient's phenotype or family history is highly specific for a disease with a single genetic etiology.</td>
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<tr>
<td></td>
<td>PP5 Reputable source reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation.</td>
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LOF, loss of function; OR, odds ratio.
Genetic Variant Interpretation Tool

To aid our variant interpretation process, we created an openly-available online tool to efficiently classify variants based on the evidence categories outlined in the article: Richards, et al. Standards and guidelines for the interpretation of sequence variants, 2015. This site displays the evidence categories and descriptions from Table 3 and Table 4 with simple checkboxes for selecting appropriate criteria. The site then incorporates the algorithm in Table 5 to automatically assign the pathogenicity or benign impact based on the selected evidence categories. Since our process often requires analyzing multiple variants per patient, we have also allowed the option of aggregating each variant into an exportable table at the foot of the website for easy documentation of the variant review process for our records. Although this tool is based on the ACMG/AMP Standards and Guidelines, it is not affiliated with ACMG, AMP, or any of the authors of the publication.

Click here to group evidence by category

Patient ID:

Variant ID:

- PV51 null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multistop deletion) in a gene where LOF is a known mechanism of disease

- PS1 Same amino acid change as a previously established pathogenic variant regardless of nucleotide change

- PS2 De novo (both maternally and paternally confirmed) in a patient with the disease and no family history

- PS3 Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product

- PS4 The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls

- PP1 (Strong evidence) Co segregation with disease in multiple affected family members in a gene definitively known to cause the disease

- PM1 Located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation

- PM2 Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium

- PM3 For recessive disorders, detected in trans with a pathogenic variant

- PM4 Protein length changes as a result of in-frame deletions/insertions in a nonrepeat region or stop-loss variants

- PM5 Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before

- PM6 Assumed de novo, but without confirmation of paternity and maternity

- PP1 (Moderate evidence) Co segregation with disease in multiple affected family members in a gene definitively known to cause the disease

- PP2 Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease

- PP3 Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.)

- PP4 Patient’s phenotype or family history is highly specific for a disease with a single genetic etiology

- PP5 Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation

- BP1 Missense variant in a gene for which primarily truncating variants are known to cause disease

- BP2 Observed in trans with a pathogenic variant for a fully penetrant dominant gene/disorder or observed in cis with a pathogenic variant in any inheritance pattern

- BP3 In-frame deletions/insertions in a repetitive region without a known function

- BP4 Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc.)

- BP5 Variant found in a case with an alternate molecular basis for disease

- BP6 Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation

- BP7 A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved
HOW SHOULD HEALTH-CARE PROVIDERS USE THESE GUIDELINES AND RECOMMENDATIONS?

However, it is recommended that all possible follow-up testing, as described above, be pursued to generate additional evidence related to a likely pathogenic variant because this may permit the variant to be reclassified as pathogenic. A variant of uncertain significance should not be used in clinical decision making. Efforts to resolve the classification of the variant as pathogenic or benign should be undertaken. While this effort to reclassify the variant is underway, additional monitoring of the patient for the disorder in question may be prudent. A variant considered likely benign has sufficient evidence that a health-care provider can conclude that it is not the cause of the patient’s disorder when combined with other information, for example, if the variant does not segregate in an affected family member and complex inheritance patterns are unlikely.
Case Example

- d.37y suddenly
- d.26y suddenly
- Presumed cardiomyopathy at autopsy
- HCM, cardiac transplant in 2006
- Arrhythmia

Key: Cardiac Dx

Should genetic testing be performed in this family?
d. Following complications from heart transplant; HCM

d.26y suddenly
Presumed cardiomyopathy at autopsy

Arrhythmia
HCM, cardiac transplant in 2006

Pathogenic \textit{LMNA p.R190W} variant observed.

Carriers of the p.R190W mutation have been described with conduction abnormalities and/or arrhythmias, sudden cardiac death, and heart failure necessitating transplant (Perrot A, et al.; Basic Res Cardiol 104; 90-9; 2009 Jan).

Who should be tested next?
• Mother’s testing reviewed
• \textit{MYBPC3} E619K
• Previously reported in the literature as associated with disease
• Should we confirm in remainder of family?
• **MYBPC3 E619K**
Return to Case Study

- **MYBPC3 E619K**
  - MAF $1.3 \times 10^{-3}$ (NFE)

- Is this population frequency compatible with what we know about the genetic architecture of HCM?

  Disease prevalence $\approx 1:500$
  Maximum genetic contribution of any single allele $\approx 2$
  Penetrance $\approx 50$

Under these assumptions, no single HCM allele should have MAF $> 4 \times 10^{-5}$ (with a corresponding confidence interval)
Return to Case Study

• **MYBPC3 E619K**
  
  • Only reports in literature demonstrate incomplete penetrance or observe as appearing with other pathogenic mutations
### ClinVar Assertions

**Protein change:** E619K

**HGVS:**
- NG_007687.1:g.18523G>A
- NM_000256.3:c.1856G>A
- NC_000011.10:g.47341180C>T (GRCh38)

**Links:**
- dbSNP: [200352229](https://www.ncbi.nlm.nih.gov/snp/200352229)
- NCBi 1000 Genomes Browser: [200352229](https://www.ncbi.nlm.nih.gov/variation/vsdb/200352229)

**Molecular consequence:** NM_000256.3:c.1856G>A: missense variant [Sequence Ontology SO:0001688]

### Assertion and evidence details

**Germline**

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<th>Condition(s) (Mode of inheritance)</th>
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</table>
Return to Case Study

• **MYBPC3 E619K**
  – This allele is likely benign
  – Counseled patient that confirmation testing for this allele would not be informative
  – Currently obtaining clinical testing of mother to review diagnostic evidence for HCM (as opposed to more likely diagnosis of secondary LVH)
Summary: NGS testing in genetic evaluation of inherited diseases

• Genetic testing has utility in
  – Diagnosis
  – Prognosis
  – Therapeutic decision making

• Allows for appropriate patient surveillance and recurrence risk counseling

• Increasingly will be a critical component in many aspects of healthcare
**Summary:** NGS testing in genetic evaluation of inherited diseases

- Clinical genetic testing and reporting using the Washington University CardioGene Set:
  - 80 genes organized into broad or highly focused subpanels
  - Phenotype-based selection
  - Exome-based hybrid capture with enhanced coverage for medically relevant genes
- Dynamic process of continuous re-evaluation, new genes, new disease groupings
- Clinical Utility is improving as knowledge base improves
- Future:
  - Curated variant databases to ensure low VUS rate
  - Full integration with human CNV map (CMA analysis)
Thank you.

Questions?