Clinical genetic testing in the setting of Cardiomyopathies and Arrhythmias

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Clinical Genetic Testing

• Regulated by CAP and CLIA
• Often LDT, not FDA approved
• 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
• 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

**Genes**
- MYBPC3
- MYH7
- MYH6
- TNNT2
- TNNI3
- MYOZ2
Next-Generation Sequencing

- **Sanger sequencing** – 2x read (Bidirectional)

- **Next-generation** – 100-1000x reads at single position
<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>
Genetic basis of inherited arrhythmias and cardiomyopathies

- Hypertrophic Cardiomyopathy: 20 genes
- Dilated Cardiomyopathy: 32 genes
- LVNC: 13 genes
- ARVC: 8 genes
- Brugada Syndrome: 10 genes
- Short QT Syndrome: 5 genes
- Long QT Syndrome: 20 genes
- CPVT: 5 genes

Slide courtesy of Dr. Nate Stitzel
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>$I_{Ks}$ potassium channel alpha subunit (Kv7.1)</td>
<td>30%–35%</td>
</tr>
<tr>
<td>KCNH2 (LQT2)</td>
<td>7q35-q36</td>
<td>$I_{Kr}$ 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>$\beta$-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>
<tr>
<td><strong>Section XIII – Sudden Unexplained Death Syndrome (SUDS)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RYR2</td>
<td>1q42.1-q43</td>
<td>Ryanodine Receptor 2</td>
<td>10%–15%</td>
</tr>
<tr>
<td>KCNQ1</td>
<td>11p15.5</td>
<td>$I_{Ks}$ potassium channel alpha subunit (Kv7.1)</td>
<td>5%–10%</td>
</tr>
<tr>
<td>KCNH2</td>
<td>7q35-q36</td>
<td>$I_{Kr}$ potassium channel alpha subunit (Kv11.1 or hERG)</td>
<td>~5%</td>
</tr>
<tr>
<td><strong>Section XIII – Sudden Infant Death Syndrome (SIDS)</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>3%–5%</td>
</tr>
</tbody>
</table>
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.
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).

http://genomics.agilent.com
Agilent SureSelect Target Capture

- Baits are cRNA
- Multiple biotinylation
- High fidelity 120mers

http://www.genomics.agilent.com/files/Media/SS_Halo/Magnet584.jpg
Washington University CardioGene Set

Mutational analysis of all coding regions of all ordered genes

**LQTS**
- AKAP9, ANK2, CACNA1C, CAV3, KCNE1, KCNE2, KCNH2, KCNJ2, KCNJ5, KCNQ1, SCN4B, SCN5A, SNTA1

**Brugada**
- CACNA1C, CACNB2, GPD1L, HCN4, KCND3, KCNE3, KCNJ8, SCN1B, SCN3B, SCN5A

**CPVT**
- ANK2, CALM1, CASQ2, KCNJ2, RYR2

**SQTS**
- CACNA1C, CACNB2, KCNH2, KCNJ2, KCNQ1

**HCM**
- 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, TNIN3, TNNT2, TPM1, TTR

**DCM**
- ABCC9, ACTC1, ACTN2, ANKR1D1, BAG3, CSRP3, CTF1, DES, EMD, FHL1, FHL2, GATA1, LAMP2, LDB3, LMNA, MYBPC3, MYH6, MYH7, NEXN, PLN, RBM20, SCN5A, SGCD, TAZ, TCA, TMPO, TNNC1, TNIN3, TNNT2, TPM1, TTN, VCL

**LVNC**
- ACTC1, CASQ2, DTNA, LDB3, LMNA, MYBPC3, MYH7, TAZ, TNNT2, VCL

**ARVC**
- DES, DSC2, DSG2, DSP, JUP, PKP2, RYR2, TMEM43
Case Example

Key: Cardiac Dx

- Should genetic testing be performed in this family?

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

- d. Following complications from heart transplant; HCM
ACMG recommendations for standards for interpretation and reporting of sequence variations: Revisions 2007

C. Sue Richards, PhD¹, Sherri Bale, PhD², Daniel B. Bellissimo, PhD³, Soma Das, PhD⁴, Wayne W. Grody, MD, PhD⁵, Madhuri R. Hegde, PhD⁶, Elaine Lyon, PhD⁷, Brian E. Ward, PhD⁸, and the Molecular Subcommittee of the ACMG Laboratory Quality Assurance Committee

• **PATHOGENIC**
  – Sequence variation is previously reported and is a recognized cause of the disorder

• **LIKELY PATHOGENIC**
  – Sequence variation is previously unreported and is of the type which is expected to cause the disorder

• **VUS**
  – Sequence variation is previously unreported and is of the type which may or may not be causative of the disorder

• **LIKELY BENIGN**
  – Sequence variation is previously unreported and is probably not causative of disease

• **BENIGN**
  – Sequence variation is previously reported and is a recognized neutral variant
Lines of Evidence to Aid in Variant Interpretation

• Frequency Data
  – dbSNP, 1000 genomes
  – NHLBI ESP

• Effect on Protein
  – Conservation Data
  – Grantham scores
  – In-silico predictions
    • Protein function
    • Splicing
Lines of Evidence to Aid in Variant Interpretation

• Literature

• Clinical Databases
  – HGMD, ClinVar

• Locus Specific Databases
  – Leiden

• Laboratory Specific Databases
  – EmVClass (Emory)
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).

Key: Cardiac Dx  \hspace{2cm}  Who should be tested next?
Case Study

• 19 y.o. female
• Indication of Obstructive HCM
• Per phone conversation with Nate Stitziel and records in patient notes, consideration of Noonan syndrome
• HCM gene set ordered
  – 31 genes
  – Recent addition of Noonan-associated genes
1- Pathogenic variant
Non-synonymous (Variants found : 1)
RAF1 (chr3:g.12645687G>A)
NM_002880:c.782C>T       NP_002871:p.P261L
Case Study

- A pathogenic variant in **RAF1, p.P261L**, was identified.
- This heterozygous non-synonymous variant is located in the CR2-segment of the **RAF1** proto-oncogene, a serine-threonine protein kinase involved in signaling in the MAPK pathway. Missense alterations within **RAF1** codon 261 including this exact variant have been described in association with Noonan syndrome.

http://www.socialstyrelsen.se/rarediseases/noonansyndrome
Promise of Genetic Testing

• 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 management