Cardiomyopathies: clinical diagnostic and research sequencing

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Genetics and Genomics of Disease Pathway
Washington University in Saint Louis

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Promise of genomic medicine
Promise of genomic medicine

Fundamental challenge for human genetics in research and clinical care:

What genetic changes are related to disease?

Clinical case

• 19 year old female
  – Referred to Center for Cardiovascular Genetics for evaluation of hypertrophic cardiomyopathy

  – Past medical history:
    • Cardiac murmur noted at age 2 weeks
    • Diagnosed with hypertrophic cardiomyopathy
    • Surgical septal myectomy at age 10 due to refractory symptoms
Clinical case

• 19 year old female

Premature CAD

Physical exam:
• Short stature (4’10”)
• Hypertelorism
• Slightly triangular mandible
• Harsh systolic murmur
• Asymptomatic bruise upper forearm
Clinical case

• 19 year old female
  – Objective data:
    • Electrocardiogram: Left ventricular hypertrophy
    • Echocardiogram: Left ventricular hypertrophy with significant outflow tract obstruction
    • Review of outside hospital abdominal CT scan (performed for abdominal pain):
      – Incidentally noted duplicated ureter

Clinical case

• 19 year old female with severe left ventricular hypertrophy in the context of short stature, subtle facial abnormalities, and genitourinary malformation
  – Clinical suspicion: Noonan’s Syndrome
    • Had been evaluated at age 12 by medical geneticist and informed she did not have NS
    • Will gene sequencing help inform a diagnosis?
Genetics of cardiovascular disease

Patterns of disease aggregation within families indicate likely genetic influence

- 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  (>30)  1000s of mutations described  MYH7  DCM  HCM  LVNC
Mendelian CV syndromes: substantial genetic overlap

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

Complexity in genetic cardiomyopathies

- Locus heterogeneity
- Allelic heterogeneity
- Genetic overlap

- MYH7
- LMNA
- TTN
- DCM
- (>30)

- 1000s of mutations described

- HCM
- DCM
- LVNC

- Incomplete penetrance
- Age-dependent penetrance
- Phenocopies
- Variable expressivity
Identifying genetic basis in familial cardiomyopathies

**Causal mutations in known genes**

- Hypertrophic ~70%
- Dilated ~35%
- Arrhythmic ~50%
- Non-compacted ~15%

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:
- Detailed patient management through the identification of the genes underlie inheritance of cardiac disorders
- Availability of cardiac-specific gene panels as well as larger comprehensive panels for broader spectrum
- Early genetic testing: investment pre-provocation
- Expert interpretation of genetic results

### Patient Benefits:
- Increased likelihood of finding 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
- Reliable testing, corrected by our research team
- Easier clinical

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**Diseases Subsets**

<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, KCNJ3, KCNJ8, SCNB1, SCNB3, 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>
</tr>
<tr>
<td>HCM</td>
<td>ACTC1, ACTN2, CSRP3, GLA, LAMP2, MYBPC3, MYH6, MYH7, MYL2, MYL3, MYLK2, MYOZ2, NEXN, PLN, PRKAG2, TNNC1, TNX13, TNNT2, TPM1, TTR</td>
</tr>
<tr>
<td>DCM</td>
<td>ABC9, ACTC1, ACTN2, ANKRD1, BAG3, CSRP3, CTF1, DES, EMD, FHL1, FHL2, GATA1D, LAMP2, LDB3, LMNA, MYBPC3, MYH6, MYH7, NEXN, PLN, RBM20, SCN5A, SGCD, TAZ, TCP1, TMPO, TNX1C, TNX3, TNNT2, TPM1, TTN, VCL</td>
</tr>
<tr>
<td>LVNC</td>
<td>ACTC1, CASQ2, DNTA, 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>

[Image of the CardioGene Set and Washington University Genomics and Pathology Services logo]
Clinical translation: challenges

- Payors
  - Probands and relatives

- Genetic complexity
  - Families and populations

- Interpreting incidental findings

NGS-based Evaluation for Cardiomyopathy and Arrhythmia Syndromes: A Clinical Genomics Laboratory Perspective

Jonathan Heusel, M.D., Ph.D.
Chief Medical Officer,
Genomics and Pathology Services
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, aka 23andMe)
- Access to genetic counseling
  - Interpretation
  - Patient management
  - Recurrence risk

Single Locus vs. Multiple Gene Testing

- **Single Locus**
  - Locus specific testing
  - Analyze single gene/locus
  - Determine mutation status of limited region
  - Narrowly targeted
  - Result may trigger additional gene testing
  - Cost effective
  - Efficient/time-saving
  - Yields unexpected findings

- **Multiple Gene Testing**
  - Analyze multiple relevant genes
  - Determine mutation status of all relevant genes simultaneously
Next-Generation Sequencing

- Sanger sequencing – 2x read (Bidirectional)
- Next-generation – 100-1000x reads at single position

Table 1 | Clinically available disease-targeted tests

<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>Aortopathies (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

CPVT
- 5 genes

ARVC
- 8 genes

Brugada syndrome
- 10 genes

Short QT syndrome
- 5 genes

Long QT syndrome
- 20 genes

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>Section I – Long QT Syndrome (LQTS)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KCNQ1 (LQT1)</td>
<td>11p15.5</td>
<td>L_{Q1} potassium channel alpha subunit (Kv7.1)</td>
<td>50%–55%</td>
</tr>
<tr>
<td>KCNH2 (LQT2)</td>
<td>7q35-q36</td>
<td>L_{NH2} 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>Section II – Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT)</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>Section III – Brugada Syndrome (BrS)</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>Section IV – Cardiac Conduction Disease (CCD)</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>Section V – Short QT Syndrome (SQTS)</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>Section VI – Atrial Fibrillation (AF)</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>Section VII – Hypertrophic Cardiomyopathy (HCM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MYH6</td>
<td>11q13.2</td>
<td>Cardiac myosin-binding protein C</td>
<td>20%–40%</td>
</tr>
<tr>
<td>MYH7</td>
<td>14q12.1-2-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%–2%</td>
</tr>
<tr>
<td>TNNI3</td>
<td>19q13.4</td>
<td>Cardiac troponin I type 3</td>
<td>1%–2%</td>
</tr>
<tr>
<td>Section XIII – Sudden Unexplained Death Syndrome (SUDDS)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RYR2</td>
<td>1p42.1-q43</td>
<td>Ryanodine Receptor 2</td>
<td>30%–15%</td>
</tr>
<tr>
<td>KCNJ2</td>
<td>11p15.5</td>
<td>L_{NJ2} potassium channel alpha subunit (Kv7.1)</td>
<td>5%–10%</td>
</tr>
<tr>
<td>KCNH2</td>
<td>7q11-q16</td>
<td>L_{NH2} potassium channel alpha subunit (Kv11.1 or HERG)</td>
<td>~5%</td>
</tr>
<tr>
<td>Section XIII – Sudden Infant Death Syndrome (SIDS)</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>

Heart Rhythm, Vol 8, No 8, August 2011
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

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![CardioGene Set Design Diagram](http://genomics.agilent.com)

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Agilent SureSelect Target Capture

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

![Agilent SureSelect Target Capture Diagram](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, KCNJ5, KCNQ1, SCN4B, SCN5A, SNTA1 |
| Brugada | CACNA1C, CACNB2, GPD1L, HCN4, KCNQ3, 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, MAFK2, MYBPC3, MYH6, MYH7, MYL2, MYLK2, MYOZ2, NEXN, NNRAS, PLN, PRKAG2, PTPN11, RAF1, RIT1, SHOC2, SOS1, TNNT1, TNNT2, TPM1, TRR |
| DCM | ABC9, ACTC1, ACTN2, ANKRD1, BAG3, CSRP3, CTIF1, DES, EMD, FHL1, FHL2, GATA1, LAMP2, LBBD3, LMNA, MYBPC3, MYH6, MYH7, NEXN, PLN, RBM20, SCN5A, SGCD, TAZ, TCAF1, TMPO, TNNT1, TNNT2, TPM1, TTN, VCL |
| LVNC | ACTC1, CASQ2, DNTA, 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?
Case Example

Pathogenic *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?

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Lines of Evidence to Aid in Variant Interpretation

- Literature
- Clinical Databases
  - HGMD, ClinVar
- Locus Specific Databases
  - Leiden
- Laboratory Specific Databases
  - EmVClass (Emory)
Lines of Evidence to Aid in Variant Interpretation

- **Frequency Data**
  - dbSNP, 1000 genomes
  - NHLBI ESP
  - ExAC Browser

- **Effect on Protein**
  - Conservation Data
  - Grantham scores
  - *In-silico* predictions
    - Protein function
    - Splicing

- **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
Return to Case Study

- 19 y.o. female
- Indication of Obstructive HCM
- Per records in patient notes and discussion with the clinician 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
A pathogenic variant in \textit{RAF1}, p.P261L, was identified.

This heterozygous non-synonymous variant is located in the CR2-segment of the \textit{RAF1} proto-oncogene, a serine-threonine protein kinase involved in signaling in the MAPK pathway. Missense alterations within \textit{RAF1} codon 261 including this exact variant have been described in association with Noonan syndrome.


This alteration affects a highly conserved amino acid and is predicted to be deleterious in nature on the basis of \textit{in silico} modeling.

Case Study

- Identifying the pathogenic mutation in this case:
  1. Solidified diagnosis
  2. Revealed additional need for clinical evaluation
  3. Opens potential to disease-specific therapies in future
  4. Allows for molecular evaluation and early diagnosis/management of at-risk relatives
**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?