Cardiomyopathy: clinical diagnostic and research sequencing

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Genetics and Genomics of Disease Pathway

September 15, 2014
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
Clinical case

• 19 year old female
  – 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
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

Incomplete penetrance

Age-dependent penetrance

Phenocopies

Variable expressivity
Mendelian CV syndromes: substantial genetic overlap

- Hypertrophic Cardiomyopathy: 20 genes
- Dilated Cardiomyopathy: 32 genes
- LVNC: 13 genes
- ARVC: 8 genes
- Brugada syndrome: 10 genes
- Short QT syndrome: 5 genes
- CPVT: 5 genes
- Long QT syndrome: 20 genes
## Identifying genetic basis in familial cardiomyopathies

<table>
<thead>
<tr>
<th>Type</th>
<th>Causal mutations in known genes</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

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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, 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

**CardioGene Set**

**Washington University Genomics and Pathology Services**

<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, RYR2</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, TNN1C, TNN3, TNN72, TPM1, TTR</td>
</tr>
<tr>
<td>DCM</td>
<td>ABCC9, ACTC1, ACTN2, ANKR1, BAG3, CSRP3, C7F1, DES, EMD, FHL1, FHL2, GATA1D, LAMP2, LDB3, LMNA, MYBPC3, MYH6, MYH7, NEXN, PLN, RBM20, SCN5A, SGCD, TAZ, TCAP, TMPO, TNN1C, TNN3, TNN72, TPM1, TTN, VCL</td>
</tr>
<tr>
<td>LVNC</td>
<td>ACTC1, CASQ2, DTM2, LDB3, LMNA, MYBPC3, MYH7, TAZ, TNN72, VCL</td>
</tr>
<tr>
<td>ARVC</td>
<td>DES, DSC2, DSG2, DSP, JUP, PKP2, RYR2, TMEM43</td>
</tr>
</tbody>
</table>

**Patient Benefits:***

- 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

**Physicians**

Washington University in St. Louis
### Mendelian forms of dyslipidemia

<table>
<thead>
<tr>
<th>Gene</th>
<th>Locus</th>
<th>Disorder and lipid phenotype</th>
</tr>
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<tbody>
<tr>
<td>ABCA1</td>
<td>9q31.1</td>
<td>Tangier disease: low HDL</td>
</tr>
<tr>
<td>ABCG5</td>
<td>2p21</td>
<td>Sitosterolemia: high LDL</td>
</tr>
<tr>
<td>ABCG8</td>
<td>2p21</td>
<td>Sitosterolemia: high LDL</td>
</tr>
<tr>
<td>APOA1</td>
<td>11q23-q24</td>
<td>ApoA-I deficiency: low HDL</td>
</tr>
<tr>
<td>APOA5</td>
<td>11q23</td>
<td>ApoA-V deficiency: high VLDL and chylomicrons</td>
</tr>
<tr>
<td>APOB</td>
<td>2p24</td>
<td>Familial hypobetalipoproteinemia: low LDL</td>
</tr>
<tr>
<td>APOC2</td>
<td>19q13</td>
<td>Familial ApoC-II deficiency: high chylomicrons</td>
</tr>
<tr>
<td>APOE</td>
<td>19q13</td>
<td>Familial dysbetalipoproteinemia: high VLDL remnants and chylomicrons</td>
</tr>
<tr>
<td>CETP</td>
<td>16q13</td>
<td>Cholesteryl ester transfer protein deficiency: high HDL</td>
</tr>
<tr>
<td>LCAT</td>
<td>16q22</td>
<td>Lecithin-cholesterol acyltransferase deficiency (fish-eye disease): low HDL</td>
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<td>LDLR</td>
<td>19p13</td>
<td>Familial hypercholesterolemia: high LDL</td>
</tr>
<tr>
<td>LDLRAP1</td>
<td>1p36-p35</td>
<td>Autosomal recessive hypercholesterolemia: high LDL</td>
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<td>LIPC</td>
<td>15q22</td>
<td>Familial hepatic lipase deficiency: high VLDL remnants</td>
</tr>
<tr>
<td>LPL</td>
<td>8p21</td>
<td>Lipoprotein lipase deficiency: high chylomicrons</td>
</tr>
<tr>
<td>MTTP</td>
<td>4q24</td>
<td>Abetalipoproteinemia: low LDL</td>
</tr>
<tr>
<td>PCSK9</td>
<td>1p32</td>
<td>Autosomal-dominant hypercholesterolemia: high LDL</td>
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<td>apoC2</td>
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There are additional families with apparent Mendelian forms of dyslipidemia not explained by these genes.

Diagnostic genetic testing “negative”

Instead of using sequencing to diagnose, can we use sequencing to map novel genes?
Through collaborations we have identified 41 families with apparent Mendelian inheritance of dyslipidemias

- 20 LDL > 99% percentile
- 13 LDL < 1% percentile
- 4 HDL > 99% percentile
- 4 HDL < 1% percentile
Mapping causal genes

- Genetic Linkage
- Candidate gene sequencing
- Whole exome sequencing
Mapping causal genes

Advantages
- Covers protein coding regions
- Interpretable variation
- Cost (not for long…)

Disadvantages
- Missing 99% genome coverage

Exome: ~33Mb per individual
## Sequencing results

<table>
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<th>Average / Sample</th>
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</thead>
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<tr>
<td><strong>Total bases</strong></td>
<td>3.9B ± 383M</td>
</tr>
<tr>
<td><strong>Mean target coverage</strong></td>
<td>123x ± 12</td>
</tr>
<tr>
<td><strong>% bases &gt; 20x covered</strong></td>
<td>85% ± 4%</td>
</tr>
<tr>
<td><strong>Coding SNPs</strong></td>
<td>18,964 ± 700</td>
</tr>
<tr>
<td><strong>Percent novel</strong></td>
<td>3%</td>
</tr>
<tr>
<td><strong>Missense SNPs</strong></td>
<td>5,194</td>
</tr>
<tr>
<td><strong>Nonsense SNPs</strong></td>
<td>35</td>
</tr>
</tbody>
</table>
How do we identify the causal variant among thousands of possibilities?
Analytical framework to identify causal variant

Identify variation consistent with phenotypic inheritance

Exclude silent variation

Leverage families exhibiting phenotype of opposite extreme

Use population genetics

Population lipid values

Allele frequency
Identifying causal gene

High-quality variants: 54,031

Missense: 1  Splice site: 1
Putative causal genes

<table>
<thead>
<tr>
<th>Chr</th>
<th>Gene</th>
<th>Functional Class</th>
<th>Protein change</th>
</tr>
</thead>
<tbody>
<tr>
<td>10q23.2</td>
<td>LIPA</td>
<td>Synonymous splice donor</td>
<td>Leads to loss of exon 8 ($\Delta254-277$)</td>
</tr>
<tr>
<td>11p11.2</td>
<td>AGBL2</td>
<td>Missense</td>
<td>P384A</td>
</tr>
</tbody>
</table>

High-quality variants: 54,031

Missense: 1  Splice site: 1
**Deficiency of an Acid Lipase in Wolman’s Disease**

Primary familial xanthomatosis with involvement and calcification of the adrenals, affecting three siblings in early infancy, was first described by Abramov, Schorr and Wolman and further reports have appeared. Failure to thrive, severe malabsorption and hepatosplenomegaly are early manifestations of the disease. Histochemical examination of visceral organs reveals extensive deposition of neutral fat and cholesterol, and the widespread occurrence of foamy lipid-laden cells, while analysis of liver and spleen shows the stored lipids to be chiefly triglycerides and cholesteryl esters.

We have studied three cases of Wolman’s disease (see Table 1) in which as well as a large increase in triglycerides and ester cholesterol of both organs we found a less pronounced increase in free cholesterol and fatty acids particularly in the liver, while the phospholipid content was normal. Serum lipids were within the normal range.

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**Enzyme Deficiency in Cholesteryl Ester Storage Disease**

**HOWARD R. SLOAN and DONALD S. FREDRICKSON**

*From the National Heart and Lung Institute, National Institutes of Health, Bethesda, Maryland 20014*

**ABSTRACT** Cholesteryl ester storage disease has been shown to involve severe deficiency of acid cholesteryl ester hydrolase and triglyceride lipase activity in liver, spleen, and lymph node. The cholesteryl ester hydrolase was also deficient in aorta. Tissue storage of both cholesteryl esters and triglycerides is generalized. Both the lipid and enzymatic changes are very similar to those in Wolman’s disease.

**INTRODUCTION**

Profound tissue accumulation of cholesteryl esters and triglycerides occur in two rare human diseases, Wolman’s disease, and cholesteryl ester storage disease. These diseases involve defective acid lipase activity and have been shown to be inherited as autosomal recessive traits. They have been separated from other lipidoses by enzymatic assay of acid lipase activity in liver, spleen, and lymph node. Acid lipase activity was measured in the tissues of normal individuals and patients with the two diseases. Acid lipase activity in cholesteryl ester storage disease showed a complete loss of enzymatic activity and in Wolman’s disease a partial loss of enzymatic activity.

**METHODS**

Tissues obtained postmortem from a 21 yr old patient with CESD (L. Mc.) (2) who died of aortic stenosis and unrelated to CESD, a 4 month old patient with Wolman’s disease, and controls were stored at -20°C. Tritiated olate (oleate-1-14C) and cholesteryl (palmitate-1-14C) were prepared by reaction of cholesterol with the appropriate acid chloride (-1-14C) (5, 6); glyceryl-α-monoleate was prepared by the analogous reaction with glyceryl-trioleate-1-14C was purchased from Amersham Corp., Arlington Heights, III., and all nonradioactive triglycerides were purified by column and gel chromatography (7, 8). Qualitative and quantitative analyses were performed as described by Kwiterovic and Fredrickson (8). Tissue cholesteryl esters were not previously identified to cause isolated hypercholesterolemia.
LIPA splice mutation causes loss of exon 8

Stitziel et al. ATVB (2013)
Is this CESD?

- CESD reported for other homozygous carriers of this mutation, characterized by hepatic disease
- Family assessed with hepatic MRS

Ruberg et al. 2006
LIPA splice mutation causes subclinical hepatic steatosis

Stitziel et al. ATVB (2013)
1 family down...40 to go

- Remaining 238 individuals have undergone exome sequencing, identical data processing
Results: Analysis of 41 families

- Discovery based on initial exome sequencing: 12%
- Discovery based on followup analysis: 7%

Sitziel et al. Circ CV Genet, Revisions submitted
Refining phenotype using genetics

1) Is there a source of variance that can explain the observed phenotype?
2) Common genetic variation explains ~20% of population heritability for lipid levels.
3) Instead of inheriting a single change explaining the phenotype (Mendelian), are some individuals just really unlucky?

$$LDL = \beta_0 + \beta_1 * SNP_1 + \beta_2 * SNP_2 + ... + \beta_n * SNP_n$$

1) Train model on population (n=12,000)
2) Predict LDL value in family, calculate standardized residual
Reframing phenotype using genetics

Pathogenic PCSK9 mutation segregates with phenotype

Observed LDL = 395
Adjusted p-value = 1x10^{-10}

Observed LDL = 285
Adjusted p-value = 3x10^{-4}

Observed LDL = 257
Adjusted p-value = 3x10^{-3}
Refining phenotype using genetics

Observed LDL = 257
Adjusted p-value = $2 \times 10^{-3}$

Observed LDL = 259
Adjusted p-value = $3 \times 10^{-3}$

Observed LDL = 167
Adjusted p-value = 0.93

Observed LDL = 157
Adjusted p-value = 0.49
Refining phenotype using genetics

Pathogenic \textit{LDLR} mutation identified

15\% of all families appear to have polygenic inheritance

Observed LDL = 257
Adjusted p-value = \(2 \times 10^{-3}\)

Observed LDL = 259
Adjusted p-value = \(3 \times 10^{-3}\)
Results: Analysis of 41 families

- Discovery based on initial exome sequencing: 12%
- Discovery based on followup analysis: 7%
- Discovery based on phenotypic refinement: 2%
- Polygenic inheritance: 15%
- Genetic etiology undiscovered: 63%

Stitziel et al. Circ CV Genet, Revisions submitted
Why are we still missing the answer?

‘Whole’, or ‘hole’ exome sequencing

Shared rare variation

- Targeted bases across exome with ≤ 20 sequencing reads
- Targeted bases across exome with ≤ 10 sequencing reads
- Targeted bases in known lipid genes with ≤ 10 sequencing reads

Stitziel et al. Circ CV Genet, Revisions submitted
## Key scientific challenges

<table>
<thead>
<tr>
<th>Discovery</th>
<th>Identification</th>
<th>Biology</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Causal variant may</td>
<td>• Incomplete penetrance</td>
<td>• Familial clustering may be due to complex genetics, not</td>
</tr>
<tr>
<td>not be coding</td>
<td>• Inadequate sources of controls</td>
<td>monogenic disorder</td>
</tr>
<tr>
<td>• Causal variant may</td>
<td>• Inadequate ability to discern functional from benign</td>
<td>• Incomplete understanding of genotype -&gt; phenotype</td>
</tr>
<tr>
<td>be coding, not captured</td>
<td>variation</td>
<td>relationships</td>
</tr>
<tr>
<td>• Causal variant may</td>
<td></td>
<td></td>
</tr>
<tr>
<td>not be single nucleotide change</td>
<td></td>
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</tr>
</tbody>
</table>
Summary

• Next-generation diagnostic and research sequencing a reality

• Can yield diagnostic clarity and provide insights into disease

• Difficult challenges ahead in both arenas