Mutations Impacting LDL Receptor Function

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Mutations Impacting LDL Receptor Function

1. Why do genetic testing for individuals with possible hereditary diseases causing hypercholesterolemia?

2. What tests should you order for evaluation of possible familial hypercholesterolemia?

3. What other genetic disorders can be confused with familial hypercholesterolemia?

4. Can genetics give us insights in understanding which drugs may be beneficial for treatment when added to statins?
Mutations Impacting LDL Receptor Function

1. Why do genetic testing for individuals with possible hereditary diseases causing hypercholesterolemia?
Why Perform DNA Testing for Severe Lipid Disorders?

1. Molecular diagnosis of hereditary lipid disorders may improve screening, counseling resulting in more individuals treated earlier in life

2. Individualized therapy depending on the specific mutation
LDL-C Values According to FH Mutation Status:
Myocardial Infarction Genetics Consortium

Prevalence of an FH mutation among severely hypercholesterolemic participants (LDL-C ≥190 mg/dL)

Impact of FH mutation status on CAD according to LDL-C level

Genetic Identification of FH Within a Single US Health Care System (Geisinger Health System, n=50,726 individuals with exome sequencing):

Prevalence of an FH variant in the DiscovEHR cohort and according to recruitment site

<table>
<thead>
<tr>
<th>Population characteristics</th>
<th>FH variant positive/total</th>
<th>Estimated prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>All DiscovEHR participants</td>
<td>229/50,726</td>
<td>1:222</td>
</tr>
<tr>
<td>Participants recruited from cardiac catheterization lab</td>
<td>57/6,747</td>
<td>1:118</td>
</tr>
<tr>
<td>Participants recruited from other sites</td>
<td>172/43,979</td>
<td>1:256</td>
</tr>
</tbody>
</table>
Prevalence of an FH Variant Among Individuals with Severe Hypercholesterolemia (LDL-C ≥ 190 mg/dl)

Participants with severe hypercholesterolemia (LDL-C > 190 mg/dl)
N = 4,435 of 42,696 individuals with LDL-C data available (10.4%)

FH variant positive: 112 of 4,435 (1:40)
Statin Treatment Rates and Outcomes in FH Variant Carriers and Noncarriers

- Currently on statin
  - FH variant negative: 38%
  - FH variant positive: 58%

- Statin-treated with LDL-C < 100 mg/dl
  - FH variant negative: 46%
  - FH variant positive: 77%
FH Variants are Associated with Increased Risk of Premature CAD

Presequencing Likelihood of FH Diagnosis with DLCN Criteria

Percentage of participants meeting clinical criteria for FH diagnosis among living noncarriers (variant-negative; n = 46,070) and carriers (variant-positive; n = 215) of an FH variant

Why Perform DNA Testing for Severe Lipid Disorders?

1. Molecular diagnosis of hereditary lipid disorders may improve screening, counseling resulting in more individuals treated earlier in life

2. Individualized therapy depending on the disorder
**Dutch National Program Has Been Spectacularly Successful**

- As of 2012: 5,151 index cases of genetically positive FH identified
- Resulted in screening of 60,000 family members
- In total **27,069** FH cases identified
  - 36% of the family members had a positive genetic test
- Costs for identifying 1 FH patient: 1200 euro
  - Test almost 3 family members to identify 1 positive FH mutation
- Costs effectiveness: costs per life year saved: 8700 Euro *

In the US, FH is Rarely Diagnosed

<table>
<thead>
<tr>
<th>Number of FH (estimated based on 1/500)</th>
<th>Diagnosed FH (estimated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>33 300 Netherlands</td>
<td>71%</td>
</tr>
<tr>
<td>9900 Norway</td>
<td>43%</td>
</tr>
<tr>
<td>600 Iceland</td>
<td>19%</td>
</tr>
<tr>
<td>15 600 Switzerland</td>
<td>13%</td>
</tr>
<tr>
<td>123 600 UK</td>
<td>12%</td>
</tr>
<tr>
<td>92 200 Spain</td>
<td>6%</td>
</tr>
<tr>
<td>22 200 Belgium</td>
<td>4%</td>
</tr>
<tr>
<td>10 900 Slovak Republic</td>
<td>4%</td>
</tr>
<tr>
<td>11 100 Denmark</td>
<td>4%</td>
</tr>
<tr>
<td>100 000 South Africa</td>
<td>3%</td>
</tr>
<tr>
<td>45 000 Australia</td>
<td>1%</td>
</tr>
<tr>
<td>14 100 Hong Kong</td>
<td>1%</td>
</tr>
<tr>
<td>130 900 France</td>
<td>1%</td>
</tr>
<tr>
<td>46 300 Taiwan</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>121 000 Italy</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>5 700 Oman</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>621 200 USA</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>68 600 Canada</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>254 800 Japan</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>34 300 Chile</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>381 500 Brazil</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>214 900 Mexico</td>
<td>&lt;1%</td>
</tr>
</tbody>
</table>
Genetic Testing and Cost

- Increasing number of labs offer testing in US (>10 labs)
- Tests can be ordered for single gene (*LDLR*) or panels (*LDLR, APOB, PCSK9*)
- Price is dropping; several offer price to patient ≤$500, some < 100$
- Cost may not be covered by insurance
Other Disorders with High LDL-C

1. Familial defective apo B-100: most commonly Arg→Gln substitution at 3500, similar presentation to FH

2. PCSK9 gain-of-function (proprotein convertase subtisilin/kexin 9): autosomal dominant, similar phenotype to FH

3. Autosomal recessive hypercholesterolemia (ARH, gene product ARH adaptor protein): similar to HoFH if pediatric presentation, premature atherosclerosis and CVD

Other Disorders Which Can Be Confused With FH But Require Different Rx

1. Phytosterolemia (ABCG5, ABCG8): ATP-binding cassette G5, G8; autosomal recessive; very high levels of plasma sitosterol and campesterol, like FH, and recurrent joint arthritis, tuberous xanthomas; therapy: ezetimibe

2. Lysosomal acid lipase deficiency (LIPA); AR, can present with high LDL-c, often with low HDL-c and elevated triglycerides, Results in lysosomal accumulation of lipids (cholesteryl esters and triglycerides) and multi-organ system damage (liver, GI tract, and blood vessel walls); therapy: infusions of sebelipase alpha

3. Cerebrotendinous xanthomatosis (CYP27A1); AR, Defect in bile acid metabolism with excess cholestanol levels, xanthomas, neurological disorders, +/- ASCVD: therapy: chenodeoxycholic acid
Cost of Genome Sequencing

Moore’s Law
Case

36 year old white male who has a family history of high cholesterol and premature heart disease was referred by his primary care physician after he had genetic screening because his wife was pregnant. The patient asked to see a specialist because of his test report

LDLR, Variant:798T>A , Heterozygous carriers have a 20 fold increased risk of CHD. Without treatment men have a 50% chance of having an MI by age 50.

He had previously taken a statin but stopped because he had an increase in his LFTs.

Lipid profile: TC 350, TGs 232, LDL-C 261, HDL-C 43 mg/dl
Genetic Testing for FH: Concerns

1. Cost to patient
2. Potential discrimination if mutation is present
3. Inappropriate denial of clinically indicated therapies for patients without definitive results

Randomized Trial of genetic testing in FH

Is Family screening Improved by Genetic Testing for Familial Hypercholesterolemia (I FIGHt FH)

Emil deGoma
Marina Cuchel
Kristen Dilzell
Daniel Rader

FH Foundation
Progenika
HDL, Inc
University of Pennsylvania Precision Medicine Program
**I FIGHT FH: Randomized Trial**

Clinical FH Identified via EHR

- Genetic testing
  - **Mutation-positive (70%)**
    - Cascade screening via mutation testing →
  - **Mutation-negative (30%)**
    - Cascade screening via LDL testing →
- No genetic testing
  - Cascade screening via LDL testing →

<table>
<thead>
<tr>
<th>Week</th>
<th>-4</th>
<th>0</th>
<th>12</th>
<th>24</th>
</tr>
</thead>
</table>

**Primary**
- # Relatives who participate in screening (measured by blood kits returned)

**Secondary**
- # Relatives with a new diagnosis of FH
- # Relatives who initiate statin therapy
- Changes in perception of CV, familial risk
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4. Can genetics give us insights in understanding which drugs may be beneficial for treatment when added to statins?
**PCSK9 Mutation Causing ADH was Identified in a French Family**

- **PCSK9** mutations (625T→A resulting in amino-acid substitution S127R) was found in the 12 affected family members and in individual HC92-IV-3
- Prevalence of **PCSK9** mutations is very low compared with the prevalence of mutations in **LDL-R** and **apoB**\(^2\)

*Filled bars indicate the mutated allele. Age (in years) at lipid measurement, total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C; in g per liter; untreated values for affected individuals) are given.*

PCSK9 LOF Mutations
PCSK9 LOF Mutations Are Associated With Decreased Plasma LDL-C Concentrations

81% of PCSK9R46L allele carriers had mean plasma LDL-C below 50th percentile

Moderate mean plasma LDL-C lowering effect in PCSK9R46L allele carriers

PCSK9 LOF Compound Heterozygote With No Detectable Circulating PCSK9

Paternal allele: PCSK9$^{\Delta R97}$ (disrupted processing/secreton) LDL-C: 39 mg/dL

Maternal allele: PCSK9$^{Y142X}$ (disrupted synthesis) LDL-C: 49 mg/dL

Compound heterozygote
Mutation prevented autocatalytic cleavage and secretion of PCSK9
LDL-C: 14 mg/dL

Apparently healthy, fertile, normotensive, college-educated woman with normal liver and renal function who worked as an aerobics instructor

Mendelian Randomization

Naturally Randomized Trial

Eligible Population

SNP associated with LDL-C
(Naturally Random Allocation of Alleles)

Lower LDL-C Allele
(Treatment Arm)

Other Allele
(Usual Care Arm)

Δ LDL-C

Incident Major Cardiovascular Events

Randomized Controlled Trial

Eligible Population

LDL-C Lowering Therapy
(Random Allocation of Treatment)

Treatment Arm

Usual Care Arm

Δ LDL-C

Incident Major Cardiovascular Events
2x2 Factorial Mendelian Randomization: 108,376 subjects from 14 studies (dbGAP)

- **HMGCR LDL-C score**
  - Above Median (Lower LDL-C)
    - **NPC1L1 LDL-C score**
      - Above Median (Lower LDL-C)
        - **Lower LDL-C via NPC1L1 & HMGCR**
      - Below Median (reference)
        - Reference
          - **Placebo**
        - Ezetimibe
          - **Ezetimibe/Statin**
    - Below Median (reference)
      - Reference
        - **Placebo**
      - Ezetimibe
        - **Ezetimibe/Statin**
  - Below Median (reference)
    - **Reference**
  - Above Median (Lower LDL-C)
    - **Lower LDL-C via NPC1L1**
    - **Statin**

Lifetime Risk of CHD events
2x2 Factorial Mendelian Randomization

<table>
<thead>
<tr>
<th>Group</th>
<th>LDL-C Effect Size (mg/dl)</th>
<th>OR_{CHD} (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both NPC1L1 &amp; HMGCR LDL-C Scores above median</td>
<td>-5.8</td>
<td>0.892 (0.839-0.948)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p = 2.4x10^{-4}</td>
</tr>
<tr>
<td>HMGCR LDL-C Score above median</td>
<td>-2.9</td>
<td>0.947 (0.914-0.982)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p = 3.3x10^{-3}</td>
</tr>
<tr>
<td>NPC1L1 LDL-C Score above median</td>
<td>-2.4</td>
<td>0.952 (0.923-0.983)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p = 2.6x10^{-3}</td>
</tr>
</tbody>
</table>

Ference, BA et al. J Am Coll Cardiol 2015;doi:10.1016/j.jacc.2015.02.020.)
Association Between Presence of Inactivating Mutations in *NPC1L1*, Plasma Lipid Levels, and CHD Risk

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Lipid Mean Difference</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>-13</td>
<td>0.03</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>-12</td>
<td>0.04</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>2</td>
<td>0.29</td>
</tr>
<tr>
<td>Triglycerides (% change)</td>
<td>-12</td>
<td>0.11 (In transformed)</td>
</tr>
</tbody>
</table>

Odds ratio for CHD in mutation carriers vs noncarriers: **0.47 (95% CI 0.25–0.87; P = 0.008)**

on the basis of a meta-analysis of independent samples
Log-Linear Effect of Lower LDL-C on CHD

Log-Linear Effect of Lower LDL-C on CHD

Log-Linear Effect of Lower LDL-C on CHD

Log-Linear Effect of Lower LDL-C on CHD

Log-Linear Effect of Lower LDL-C on CHD


Cumulative Effect of Lifelong LDL-C

Genetically Lower LDL-C

Pharmacologically Lower LDL-C

Proportional Risk Reduction (SE log scale)

lower LDL-C (mg/dl)
Study Design: Naturally Randomized Trial

“Naturally Randomized Trial”

Eligible

ACL variants associated with LDL-C (Naturally Random Allocation of Alleles)

Lower LDL-C Allele (Treatment Arm)

Other Allele (Usual Care Arm)

Δ LDL-C

Incident Major Cardiovascular Events

Randomized Controlled Trial

Eligible

LDL-C Lowering Therapy (Random Allocation of Treatment)

Treatment Arm

Usual Care Arm

Δ LDL-C

Incident Major Cardiovascular Events
Baseline Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ACL Genetic Score below median</th>
<th>ACL Genetic Score above median</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>48,593</td>
<td>52,643</td>
<td></td>
</tr>
<tr>
<td><strong>Lipids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>129.1 (±35.4)</td>
<td>127.1 (±36.1)</td>
<td>2.7E-8</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>51.9 (±14.6)</td>
<td>52.3 (±15.1)</td>
<td>0.21</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>118.8 (83-161)</td>
<td>119.4 (79-155)</td>
<td>0.01</td>
</tr>
<tr>
<td>Non-HDL (mg/dl)</td>
<td>159.1 (±37.5)</td>
<td>157.0 (±37.9)</td>
<td>1.8E-7</td>
</tr>
<tr>
<td><strong>Non-Lipid characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>59.5 (±7.5)</td>
<td>59.8 (±7.1)</td>
<td>0.43</td>
</tr>
<tr>
<td>Women (%)</td>
<td>56.1</td>
<td>55.6</td>
<td>0.58</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>126.9 (±18.2)</td>
<td>126.4 (±17.6)</td>
<td>0.39</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>75.9 (±10.1)</td>
<td>75.6 (±9.8)</td>
<td>0.54</td>
</tr>
<tr>
<td>Weight (lbs)</td>
<td>169.9 (±37.9)</td>
<td>170.7 (±38.2)</td>
<td>0.25</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.2 (±5.1)</td>
<td>28.0 (±5.2)</td>
<td>0.76</td>
</tr>
<tr>
<td>Ever Smoker (%)</td>
<td>54.9</td>
<td>55.2</td>
<td>0.47</td>
</tr>
<tr>
<td>Prevalent Diabetes (%)</td>
<td>5.6</td>
<td>5.7</td>
<td>0.59</td>
</tr>
</tbody>
</table>

*Triglycerides given as median (IQR); all other values are mean (±SD)
Primary Results

<table>
<thead>
<tr>
<th>Outcome</th>
<th>No. Events</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronary death or non-fatal MI</td>
<td>10,144</td>
<td>0.969 (0.95-0.99)</td>
</tr>
<tr>
<td><strong>Secondary</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary death, non-fatal MI or stroke</td>
<td>11,865</td>
<td>0.975 (0.96-0.996)</td>
</tr>
<tr>
<td>Major coronary event</td>
<td>12,037</td>
<td>0.955 (0.92-0.99)</td>
</tr>
<tr>
<td>Major vascular event</td>
<td>13,964</td>
<td>0.958 (0.92-0.99)</td>
</tr>
<tr>
<td><strong>Tertiary</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary death</td>
<td>2,523</td>
<td>0.991 (0.96-1.02)</td>
</tr>
<tr>
<td>non-fatal MI</td>
<td>8,512</td>
<td>0.951 (0.92-0.98)</td>
</tr>
<tr>
<td>Coronary revascularization</td>
<td>4,523</td>
<td>0.937 (0.87-0.98)</td>
</tr>
<tr>
<td>Stroke</td>
<td>3,577</td>
<td>0.982 (0.94-1.02)</td>
</tr>
</tbody>
</table>
Comparison with other LDL-C variants
Assessment of Independent Effect

Genetic Score (monotherapy)
- ACL Score
  - OR: 0.832 (0.727-0.951)
- HMGCR Score
  - OR: 0.844 (0.806-0.885)
- NPC1L1 Score
  - OR: 0.833 (0.755-0.920)
- PCSK9 Score
  - OR: 0.843 (0.805-0.882)

Combination Score (with statin)
- ACL + HMGCR
  - OR: 0.846 (0.804-0.891)
- NPC1L1 + HMGCR
  - OR: 0.837 (0.754-0.876)
- PCSK9 + HMGCR
  - OR: 0.824 (0.785-0.865)

Combination Scores (with EZE)
- ACL + NPC1L1
  - OR: 0.822 (0.772-0.870)
- HMGCR + NPC1L1
  - OR: 0.834 (0.784-0.886)
- PCSK9 + NPC1L1
  - OR: 0.839 (0.795-0.885)

Overall (I-squared = 0.0%, p = 0.997)
- OR: 0.837 (0.820-0.855)
Conclusions

• Lower LDL-C mediated by variants in the ACL gene is causally associated with the risk of cardiovascular events

• Lower LDL-C mediated by variants that mimic the effect of ACL inhibitors has the same effect on the risk of CV events per unit lower LDL-C as variants that mimic the effect of statins, ezetimibe or PCSK9 inhibitors

• Therefore ACL inhibitors (bempedoic acid) should reduce the risk of cardiovascular events by the same amount as statins, ezetimibe, or PCSK9 inhibitors per unit lower LDL-C (whether used alone or in combination with these therapies)
Conclusions

• Molecular diagnosis for familial LDL-c disorders (FH) may improve screening, counseling and earlier initiation of therapy. With newer techniques such as exome sequencing, the majority of individuals with very high LDL-C levels will have mutations or multiple genetic variants that can be identified.

• Personalized therapy for specific genetic variants for FH is not proven superior to using "clinical" phenotyping, however expanded panels could help identify rare disorders confused with FH but which require different Rx.

• Genetic variants and Mendelian randomization studies may help predict which therapies are additive.