Human Genetics Impact on Clinical Practice in Lipidology

National Lipid Association
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Human Genetics Impact on Clinical Practice in Lipidology

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DISCLOSURE INFORMATION:
None applicable.

UNLABELED/UNAPPROVED USE:
ISIS-APOCIII

Rx
Educational Objectives

• Review genetic and clinical concepts
• Summarize genomic discoveries in lipids and CHD
• Highlight causal lipid pathways in CHD
• Discuss novel genome-based therapeutics
Genetics - Impact in Clinic

• New discoveries: new targets & biology
  • Causality and directionality
  • Therapeutic translation
  • Precision medicine
Traditional Risk Factors for CHD...

- Age
- Male gender
- Smoking
- Hypertension
- Diabetes mellitus
- High LDL cholesterol / apoB lipoproteins
- Low HDL cholesterol

explain only about 60-70% of early heart attacks
Coronary Disease is Influenced by Genetics

If your mother or father had early-onset cardiovascular disease, your risk of early heart disease is:

Men 3.2 times average
Women 2.9 times average

Lloyd-Jones, JAMA 2004
Genomic Discovery Strategies

- Common variants
- Low-frequency variants
- Rare variants/Mendelian

Effect Size:
- Strong
- Moderate
- Weak

Allele Frequency:
- 50%
- 5%
- 0.5%
- 0.05%
An Exomic View of the Genome
(Exome-seq Identifies Low Frequency and Rare Variants in Protein Coding Genes)

1-2% genome encodes protein coding genes (~20K) but ~80% of genome is functional
DNA Variants

New biology
New therapeutic targets

Lipids and other Cardiovascular risk factors

Coronary disease and atherosclerosis
DNA Variants

New biology
New therapeutic targets

Lipids and other cardiovascular risk factors

Coronary disease and atherosclerosis

??
Mendelian Causes of Extreme High LDL-C Levels Helped Confirm the “LDL Hypothesis”
Familial Hypercholesterolemia

12 Y.O. Female

LDL cholesterol 760 mg/dL (normal < 130 mg/dL)
Inherited Syndromes of Extremes of LDL-C: PCSK9 Mutations

Loss of function mutations in PCSK9

Gain of function mutations in PCSK9
## Mendelian Lipid Disorders

<table>
<thead>
<tr>
<th>Gene</th>
<th>Locus</th>
<th>GWAS SNP</th>
<th>Disorder and lipid phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCA1</td>
<td>9q31.1</td>
<td>rs1883025</td>
<td>Tangier disease: low HDL</td>
</tr>
<tr>
<td>ABCG5</td>
<td>2p21</td>
<td>rs4299376</td>
<td>Sitosterolemia: high LDL</td>
</tr>
<tr>
<td>ABCG8</td>
<td>2p21</td>
<td>rs4299376</td>
<td>Sitosterolemia: high LDL</td>
</tr>
<tr>
<td>APOA1</td>
<td>11q23-q24</td>
<td>rs964184</td>
<td>ApoA-I deficiency: low HDL</td>
</tr>
<tr>
<td>APOA5</td>
<td>11q23</td>
<td>rs964184</td>
<td>ApoA-V deficiency: high VLDL and chylomicrons</td>
</tr>
<tr>
<td>APOB</td>
<td>2p24</td>
<td>rs515135</td>
<td>Familial hypobetalipoproteinemia: low LDL</td>
</tr>
<tr>
<td>APOC2</td>
<td>19q13</td>
<td>rs4420638</td>
<td>Familial ApoC-II deficiency: high chylomicrons</td>
</tr>
<tr>
<td>APOE</td>
<td>19q13</td>
<td>rs4420638</td>
<td>Familial dysbetalipoproteinemia: high VLDL remnants and chylomicrons</td>
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<tr>
<td>CETP</td>
<td>16q13</td>
<td>rs173539</td>
<td>Cholesteryl ester transfer protein deficiency: high HDL</td>
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<tr>
<td>LCAT</td>
<td>16q22</td>
<td>rs2271293</td>
<td>Lecithin-cholesterol acyltransferase deficiency (fish-eye disease): low HDL</td>
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<tr>
<td>LDLR</td>
<td>19p13</td>
<td>rs6511720</td>
<td>Familial hypercholesterolemia: high LDL</td>
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<tr>
<td>LDLRAP1</td>
<td>1p36-p35</td>
<td>rs12027135</td>
<td>Autosomal recessive hypercholesterolemia: high LDL</td>
</tr>
<tr>
<td>LIPC</td>
<td>15q22</td>
<td>rs10468017</td>
<td>Familial hepatic lipase deficiency: high VLDL remnants</td>
</tr>
<tr>
<td>LPL</td>
<td>8p21</td>
<td>rs12678919</td>
<td>Lipoprotein lipase deficiency: high chylomicrons</td>
</tr>
<tr>
<td>MTTP</td>
<td>4q24</td>
<td>N/A</td>
<td>Abetalipoproteinemia: low LDL</td>
</tr>
<tr>
<td>PCSK9</td>
<td>1p32</td>
<td>rs11206510</td>
<td>Autosomal-dominant hypercholesterolemia: high LDL</td>
</tr>
<tr>
<td>SAR1B</td>
<td>5q31.1</td>
<td>N/A</td>
<td>Chylomicron retention disease: low chylomicrons</td>
</tr>
</tbody>
</table>
# GWAS in > 100,000 Individuals: 95 Lipid Loci

<table>
<thead>
<tr>
<th>LDL-C</th>
<th>HDL-C</th>
<th>Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCG5/8</td>
<td>ABCA1</td>
<td>ACSS2</td>
</tr>
<tr>
<td>ABO</td>
<td>ABCA8</td>
<td>AFF1</td>
</tr>
<tr>
<td>ANGPTL3</td>
<td>ADM</td>
<td>ANGPTL3</td>
</tr>
<tr>
<td>APOA</td>
<td>ANGPTL4</td>
<td>ANKRD55</td>
</tr>
<tr>
<td>APOB</td>
<td>APOA</td>
<td>ANKRDS55</td>
</tr>
<tr>
<td>APOE</td>
<td>APOB</td>
<td>ANKRD55</td>
</tr>
<tr>
<td>BRAP</td>
<td>APOE</td>
<td>ANKRD55</td>
</tr>
<tr>
<td>BTNL2</td>
<td>APOE</td>
<td>ANKRD55</td>
</tr>
<tr>
<td>CBLN3</td>
<td>C6orf106</td>
<td>ANKRD55</td>
</tr>
<tr>
<td>CETP</td>
<td>CETP</td>
<td>ANKRD55</td>
</tr>
<tr>
<td>CILP2</td>
<td>CITED2</td>
<td>ANKRD55</td>
</tr>
<tr>
<td>CYP7A1</td>
<td>CMIP</td>
<td>ANKRD55</td>
</tr>
<tr>
<td>DNAH11</td>
<td>COBLL1</td>
<td>ANKRD55</td>
</tr>
<tr>
<td>FADS</td>
<td>DOCK6</td>
<td>ANKRD55</td>
</tr>
<tr>
<td>FRK</td>
<td>FADS</td>
<td>ANKRD55</td>
</tr>
<tr>
<td>GPAM</td>
<td>GALNT2</td>
<td>ANKRD55</td>
</tr>
</tbody>
</table>

**Total Cholesterol**

| ERGIC3 | EVI5 | FUT2 | RAB3GAP1 | RAF1 | SPTY2D1 | ZNF664 |

Teslovich, et al Nature 2010
The Role of PCSK9 in the Regulation of LDL Receptor Expression
DNA Variants

New biology
New therapeutic targets

Lipids and other Cardiovascular risk factors

Coronary disease and atherosclerosis
Replicated GWAS Loci for MI/CHD: Ongoing Functional Genomics

CARDIoGRAM. Nature Genet. 2011
Summary: GWAS, MetaboChip and Exome-seq Loci in CHD

• ~50 CHD loci at genome-wide significance
• 12 loci for lipid traits and 5 for blood pressure
• ADAMTS7, COL4A1, TCF21, PDGF implicate VSMC, matrix
• CXCL12, IL6R, IL5 implicate inflammation and immunity
• FLT1/VEGFR1, EDNRA, GUCY1A implicate endothelium
• 13 CHD loci with consistent mouse knock out model finding
• Exomes: known loci, TGRL loci (ANGPTL4), novel loci (SEVP1)
• Mega GWAS, large exome & whole genome seq on-going

CARDIoGRAMplusC4D Nature Genet 2013 and Nature Genet 2015; MIGen ExSeq Nature 2015
Impact on Clinic

• New discoveries: new targets & biology
• Causality and directionality in CHD
• Therapeutic translation
• Precision medicine
Exome-seq Identifies Mutations in Protein Coding Genes

Exomes (dark blue)

Why Sequence Exons?
Identify Rare Functional Variants & Establish Causal Direction
PCSK9 Mutations: Causality and Directionality

- **Gain of function mutations in PCSK9**
- **Loss of function mutations in PCSK9**

Frequency (¥)

LDL-C
Causality – GWAS of LDL-C (Common Variants)

SR-BI−/− mice have elevated HDL-C levels but impaired RCT and increased atherosclerosis. What about in humans?

The HDL Function Hypothesis

Promoting HDL functions (not raising HDL-C levels) will reduce CV events?
Mendelian Randomization Studies

Randomized Controlled Trial

- Randomization Method
  - Exposed: Intervention
  - Control: No intervention
  - Confounders equal between groups
  - Outcomes compared between groups

Mendelian Randomization

- Random Segregation and Assortment of Alleles
  - Exposed: Variant Allele
  - Control: Reference Allele
  - Confounders equal between groups
  - Outcomes compared between groups
  - e.g., Lp(a) positive
  - CRP and HDL-C negative
Mendelian Randomization Studies

Candidate gene approach
Documented or suspected association or mechanism

Unbiased approach
Common variant (GWAS)
Rare mutation (WES)

Genetic variant(s)
Associated with triglycerides, but not with other risk factors

Triglycerides

Coronary disease

Jansen. Eur Heart J 2014;35:1917
APOC3 LOF Mutations Reduce TG levels and Protect against CAD

Loss-of-Function Mutations in APOC3, Triglycerides, and Coronary Disease

The TG and HDL Working Group of the Exome Sequencing Project, National Heart, Lung, and Blood Institute*

Loss-of-Function Mutations in APOC3 and Risk of Ischemic Vascular Disease

Loss-of-function mutations in \( APOC3 \) reduce plasma TG levels and risk of CHD.

**Sequencing**

Apolipoprotein C-III (\( APOC3 \))

Humans with very low TG

- IVS1-2G\( \rightarrow \)A
- IVS2+1G\( \rightarrow \)A
- R19X
- A43T

**Clinical Effect**

-39% -40%

1 in 150 individuals are heterozygous for loss-of-function mutations.

First mechanism of lowering TG shown to reduce MI risk in humans.
**APOA5: Mendelian Randomization Study**

<table>
<thead>
<tr>
<th>Genotype combination (c.-3A&gt;G/c.56C&gt;G/c.*31C&gt;T)</th>
<th>Mean ± SE</th>
<th>% Increase in triglyceride level</th>
<th>Theoretically predicted risk of MI</th>
<th>Observed risk of MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. AA CC TT</td>
<td>P&lt;0.001</td>
<td>0</td>
<td>0</td>
<td>68%</td>
</tr>
<tr>
<td>2. AA CC CT</td>
<td></td>
<td>2</td>
<td>2</td>
<td>1.5</td>
</tr>
<tr>
<td>3. AA CC CC</td>
<td></td>
<td>3</td>
<td>3</td>
<td>1.9</td>
</tr>
<tr>
<td>4. AA CG CT</td>
<td></td>
<td>18</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>5. AG CC CT</td>
<td></td>
<td>19</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>6. AA CG CC</td>
<td></td>
<td>20</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>7. AG CC CC</td>
<td></td>
<td>23</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>8. AA GG CC</td>
<td></td>
<td>48</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>9. AG CG CC</td>
<td></td>
<td>48</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>10. GG CC CC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**LPL: Mendelian Randomization Study**

<table>
<thead>
<tr>
<th>No. of triglyceride-decreasing alleles</th>
<th>Participants</th>
<th>Change in triglyceride levels (%)</th>
<th>Mean (SE)</th>
<th>Theoretically predicted risk</th>
<th>Observed risk</th>
<th>Number of deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–3</td>
<td>692</td>
<td>0</td>
<td></td>
<td></td>
<td>0.86</td>
<td>291</td>
</tr>
<tr>
<td>4</td>
<td>7672</td>
<td>−12</td>
<td></td>
<td></td>
<td></td>
<td>3033</td>
</tr>
<tr>
<td>5</td>
<td>1732</td>
<td>−21</td>
<td></td>
<td></td>
<td></td>
<td>643</td>
</tr>
<tr>
<td>6</td>
<td>112</td>
<td>−31</td>
<td></td>
<td></td>
<td></td>
<td>38</td>
</tr>
</tbody>
</table>

For a 50% decrease in nonfasting triglycerides:

- Observational: 0.73 (0.70–0.77)
- Genetically decreased levels: 0.43 (0.23–0.80)

Thomsen. Clin Chem 2014;60:737
Impact in Clinic

- New discoveries: new targets & biology
- Causality and directionality in CHD
- Therapeutic translation
- Precision medicine
Human genetics leading to smarter and faster development of new medicines
Unmet Medical Needs in the Treatment of Elevated LDL-C

- Unable To Get LDL < 100
- Statin-Intolerant
- LDL > 130
- Apheresis-eligible (LDL > 200)
- HoFH (High risk and on maximal tolerated statin)
Mendelian Syndromes of Low LDL-C Provide New Targets for Therapy
Truncation Mutations in ApoB Cause Low LDL-C Levels
Antisense Oligonucleotide to apoB: a Strategy for Reducing LDL-C

ASO to apoB ( mipomersen )

LDLR

apoB

TG

VLDL

LDL

B B

B

Loss-of-function Mutations in MTP Cause Failure to Assemble and Secrete VLDL
MTP Inhibition: a Strategy for Reducing Hepatic VLDL Secretion and LDL-C

- LDLR
- apoB
- TG
- MTP
- B
- VLDL
- LDL
- Small molecule inhibitor (lomitapide)
Inhibition of PCSK9 as a Novel Strategy for Reducing LDL-C

Antibody, anti-sense oligonucleotide (ASO), siRNA, small molecule inhibitor
PCSK9: From human genetic discovery to new medicine

May 2003

July 2015

- Alirocumab (Approved July 2015)
- Evolocumab (Approved August 2015)

- Indications:
  - Patients with heterozygous familial hypercholesterolemia on maximally tolerated statin therapy with inadequate plasma LDL levels
  - Patients with a history of CHD with inadequate plasma LDL levels

http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm455883.htm
Antisense Inhibition of Apolipoprotein C-III in Patients with Hypertriglyceridemia

Daniel Gaudet, M.D., Ph.D., Veronica J. Alexander, Ph.D., Brenda F. Baker, Ph.D., Diane Brisson, Ph.D., Karine Tremblay, Ph.D., Walter Singleton, M.D., Richard S. Geary, Ph.D., Steven G. Hughes, M.B., B.S., Nicholas J. Viney, B.Sc., Mark J. Graham, M.S., Rosanne M. Crooke, Ph.D., Joseph L. Witztum, M.D., John D. Brunzell, M.D.,* and John J.P. Kastelein, M.D., Ph.D.
APOC3-Targeted Therapeutic: ISIS-APOCIII_{Rx}

A

\[\text{APOC3 (mg/dl)}\]

Treatment period

Study Day

Patient 1

Patient 2

Patient 3

B

\[\text{Triglycerides (mg/dl)}\]

Treatment period

Study Day

C

\[\text{Chylomicron Triglycerides (mg/dl)}\]

Treatment period

Study Day

D

\[\text{Non-HDL Cholesterol (mg/dl)}\]

Treatment period

Study Day
Impact in Clinic

• New discoveries: new biology & targets
• Causality and directionality
• Therapeutic translation
• Precision medicine
Precision Medicine

• Right diagnosis in patient & family
  • Cascade screen, diagnose and treat
  • Precision public health

• Right drug - patient (genetics): efficacy
  • Genome based drug selection
  • “N=1” trials

• Avoid wrong drug: patient safety
  • Gene-drug interactions
  • “N=1” trials
Summary – Take Home Message

• Treasure trove of discoveries for lipids and CHD
  • Most novel CHD loci not related to traditional RF

• Genetic inference of causality and directionality

• LDL-C (but not HDL-C) loci relate to CHD
  • TG loci causal in CHD

• New therapeutics in trials – lipids (not direct CHD)

• Genetics for precision medicine
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Jennie Lin
Rachel Ballantyne

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NHLBI ESP-MI

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