Lipid Metabolism in Familial Hypercholesterolemia

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Disclosures

• Honoraria for Speakers Bureau (Pharma)
  AstraZeneca, Sanofi, Pfizer

• Advisory Boards: Sanofi, Aegerion, AstraZeneca

• Research Funding: Pfizer
Effect of LDL-C by Magnitude and Duration of Exposure

Meta-analyses of:
- Mendelian randomization studies
- Prospective cohort studies
- Randomized controlled trials

(N=194,427) Median follow-up 52 years
(N=403,501) Median follow-up 12 years
(N=196,552) Median follow-up 5 years

Familial Hypercholesterolemia: Prevalence and Risk

- FH is caused by genetic mutations passed on by:
  - One parent (heterozygous, HeFH)$^1$
  - Both parents (homozygous, HoFH)$^1$

- **HoFH prevalence ranges from 1 in 160,000 to 1 in 250,000$^{2,3}$**
  - Individuals with HoFH have extremely high LDL-C levels (>500 mg/dL) and premature CV risk$^4$
  - Many with HoFH experience their first coronary event in childhood or adolescence$^4$

- **HeFH prevalence ranges from 1 in 200 to 1 in 250$^3$**
  - Individuals with HeFH can present with LDL-C levels 90 to 500 mg/dL and have premature CV risk$^4$
  - On average, individuals with HeFH experience their first coronary event at age 42 (about 20 years younger than the general population)$^4$

- **Early treatment is recommended for all individuals with FH, with a goal of reducing LDL-C levels by 50% from baseline$^3$**

Abbreviations: CV, cerebrovascular; FH, familial hypercholesterolemia; HeFH, heterozygous familial hypercholesterolemia; HoFH, homozygous familial hypercholesterolemia; LDL-C, low-density lipoprotein cholesterol.

Cumulative LDL-C (mmol) vs. Years Age

- **Coronary disease & death before age 20**
- **Untreated coronary disease before age 55/60**

- **Homozygous FH**
  - Start high dose statin
  - Threshold for CHD
  - 12.5yrs

- **Heterozygous FH**
  - Start low dose statin
  - 35yrs
  - 48yrs
  - 53yrs

- **Without FH**

**Factors influencing LDL-C**:
- Female sex
- Smoking
- Hypertension
- Diabetes
- ↑Triglycerides
- ↓HDL-C
- ↑Lipoprotein(a)

Adapted from Steve Humphries 2013
Lipoprotein Metabolism and Atherosclerosis

Lambert et al. J. Lipid Res. 2012. 53: 2515-2524
Lipoprotein Metabolism and Atherosclerosis

Lambert et al. J. Lipid Res. 2012. 53: 2515-2524
Composition of Lipoproteins

Chylomicron
- Triacylglycerols: 84%
- Cholesterol: 2%
- Phospholipids: 7%
- Proteins: 7%
- Size: 100-1000nm

VLDL
- Triacylglycerols: 54%
- Cholesterol: 9%
- Phospholipids: 19%
- Proteins: 18%
- Size: 30-90nm

LDL
- Triacylglycerols: 45%
- Cholesterol: 21%
- Phospholipids: 22%
- Proteins: 11%
- Size: 20-75nm

HDL
- Triacylglycerols: 50%
- Cholesterol: 22%
- Phospholipids: 24%
- Proteins: 4%
- Size: 5-12nm
Receptor-Mediated Endocytosis of Lipoproteins

- LDL receptor are located at coated pits, which also contain clathrin
- Vesicles fuse with lysosome where cholesterol esters are hydrolyzed into cholesterol & re-esterified by ACAT
- This avoids damaging effects of high concentrations of free cholesterol on membrane
LDL Receptor (apoB-E receptor)

Regulates cholesterol synthesis and plasma cholesterol levels
PCSK9

- Proprotein convertase subtilisin/kexin type 9 (PCSK9)
- the 9th member of the **proprotein convertase** family of proteins that activate other proteins
- involved in the degradation of low-density lipoprotein (LDL) receptors in the liver.
The LDLR Pathway
Laboratory Tests in FH

- Lipid profile (TC, LDL-C, Non-HDL-C, TG)
- Secondary hypercholesterolemia causes
- Other genetic disorders causing hypercholesterolemia
- Genetic diagnosis
- LDL Receptor activity
- PCSK9 levels
- Lp(a) levels
<table>
<thead>
<tr>
<th><strong>FH heterozygotes</strong></th>
<th><strong>FH homozygotes</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Occur in ~ 1 in 500 persons worldwide</td>
<td>Occur in ~ 1 in 1,000,000 persons worldwide</td>
</tr>
<tr>
<td>1 mutated allele</td>
<td>2 mutated alleles</td>
</tr>
<tr>
<td>TC: 350 to 500 mg/dL <strong>(9-12.9 mmol/L)</strong></td>
<td>TC: &gt; 500 to &gt; 1,000 mg/dL <strong>(12.9-25.9 mmol/L)</strong></td>
</tr>
<tr>
<td>LDL-C: 200–400 mg/dL <strong>(5.1-10.3 mmol/L)</strong></td>
<td>LDL-C: &gt; 600 mg/dL <strong>(15.5 mmol/L)</strong></td>
</tr>
<tr>
<td>Half the number of LDLR expressed</td>
<td>LDLR activity absent</td>
</tr>
<tr>
<td>Characterized by 2- to 3- fold elevation in the plasma LDL-C levels and often develop myocardial infarctions as early as 30 to 40 years of age</td>
<td>Characterized by more severe hypercholesterolemia than heterozygotes, with LDL-C levels elevated 6- to 10-fold from birth, and heart attacks in childhood</td>
</tr>
</tbody>
</table>

TC = total cholesterol.

LDL-C Overlap


5-15 years

2.2 mMol/l
4.6 mMol/l
False +ve = 8%, False –ve = 15%

45-54 years

3.1 mMol/l
4.6 mMol/l
4.2 mmol/l
False +ve = 16%, False –ve = 46%

As mean LDL-C rises with age in non-FH, overlap increases. DNA testing gives an unambiguous result.
Broad Spectrum of LDL-C levels in FH

<table>
<thead>
<tr>
<th>Cause</th>
<th>Problem</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analytical</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Novel therapies: very low LDLC concentrations</td>
<td>Magnification of measurement and calculation errors (e.g., Friedewald)</td>
<td>CBR2, CBR3, CBR4</td>
</tr>
<tr>
<td>Nonfasting lipid testing</td>
<td>Postprandial variation of TG in LDLC calculation</td>
<td>CBR4, CBR5</td>
</tr>
<tr>
<td>Increasing prevalence of obesity, diabetes, and moderate or major increases in TG</td>
<td>Nonspecificity bias in hypertriglyceridemic (&gt;175 mg/dL; &gt;2 mmol/L) and dyslipidemic samples</td>
<td>CBR2, CBR3, CBR4, CBR9, FR1, FR2</td>
</tr>
<tr>
<td>High Lp(a)</td>
<td>Overestimation of LDLC</td>
<td>CBR10</td>
</tr>
<tr>
<td>Clinical</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increasing prevalence of obesity and diabetes</td>
<td>LDLC is a less predictive marker</td>
<td>CBR1, CBR5, CBR6, CBR7, FR3</td>
</tr>
<tr>
<td>Residual (on-treatment) CVD risk</td>
<td>Residual risk unexplained by LDLC</td>
<td>CBR8, FR3, FR4</td>
</tr>
<tr>
<td>Personalized medicine</td>
<td>LDLC has low or no diagnostic and predictive performance in certain patients</td>
<td>CBR1, CBR8, FR4, FR5</td>
</tr>
<tr>
<td></td>
<td>LDLC</td>
<td>Non-HDLC</td>
</tr>
<tr>
<td>----------------</td>
<td>----------------------------------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td><strong>Strengths</strong></td>
<td>Widely available laboratory assays for dLDLC measurement or cLDLC calculation</td>
<td>Not dependent on TG variability</td>
</tr>
<tr>
<td></td>
<td>Clinical performance: strong evidence-based, causal risk factor</td>
<td>Can always be calculated in the nonfasting state</td>
</tr>
<tr>
<td></td>
<td>Clinical effectiveness: LDLC-targeted treatment reduces risk</td>
<td>Includes remnant cholesterol</td>
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<tr>
<td><strong>Weaknesses</strong></td>
<td>dLDLC measurement errors in dyslipidemic samples and samples from diseased patients</td>
<td>HDLC measurement errors in dyslipidemic samples</td>
</tr>
<tr>
<td></td>
<td>cLDLC influenced by HDLC measurement errors</td>
<td>Different assays for HDLC affect between-laboratory measurement variability</td>
</tr>
<tr>
<td></td>
<td>cLDLC influenced by postprandial TG variability; invalid at TG &gt;400 mg/dL (4.5 mmol/L)</td>
<td>Arbitrary risk cutpoints and treatment targets, not validated for clinical performance</td>
</tr>
<tr>
<td></td>
<td>cLDLC and dLDLC influenced by increased Lp(a)</td>
<td></td>
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<td></td>
<td>Manufacturer-dependent nonspecificity bias compared with reference method</td>
<td></td>
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</tbody>
</table>
Known Genetic Mutations Associated With Familial Hypercholesterolemia*

<table>
<thead>
<tr>
<th>Gene</th>
<th>LDLR</th>
<th>ApoB</th>
<th>PCSK9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosome</td>
<td>19p13</td>
<td>2p23-24</td>
<td>1p32</td>
</tr>
<tr>
<td>Clinical Effect</td>
<td>LDL-C increased</td>
<td>LDL-C increased</td>
<td>LDL-C increased</td>
</tr>
</tbody>
</table>

*Autosomal Dominant Hypercholesterolemia.
Overlap of clinical and mutation diagnosis of heterozygous familial hypercholesterolaemia

Clinical diagnosis without mutation

Patient: treat LDL
Family: monitor LDL and consider treatment

Mutation without clinical diagnosis

Patient: treat LDL
Family: mutation test, monitor LDL, and consider treatment

Patient: monitor LDL and consider treatment
Family: monitor LDL and consider treatment
<table>
<thead>
<tr>
<th>Affected lipids</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑ Total cholesterol and LDL-C</td>
<td>• Hypothyroidism</td>
</tr>
<tr>
<td></td>
<td>• Nephrosis</td>
</tr>
<tr>
<td></td>
<td>• Dysgammaglobulinemia (systemic lupus erythematosus, multiple myeloma)</td>
</tr>
<tr>
<td></td>
<td>• Progestin\textsuperscript{a} or anabolic steroid treatment</td>
</tr>
<tr>
<td></td>
<td>• Cholostatic diseases of the liver due to abnormal lipoproteins, as in</td>
</tr>
<tr>
<td></td>
<td>primary biliary cirrhosis</td>
</tr>
<tr>
<td></td>
<td>• Protease inhibitors for treatment of HIV infection\textsuperscript{b}</td>
</tr>
</tbody>
</table>
Other genetic disorders

- High Lp(a)
- Lysosomal Acid Lipase Deficiency (Wolman Disease)
- Sitosterolemia (Phytosterolemia)
- Lecithin Cholesterol Acyltransferase Deficiency
LAL-D Presentation in Children and Adults

• Common presenting abnormalities\(^1-3\)
  – Unexplained persistent elevated ALT/AST
  – High/very high LDL-c and low HDL-c

• Diagnosis requires high index of clinical suspicion\(^1\)
  – Many patients diagnosed in childhood
  – Others present with symptoms but are not diagnosed until adulthood

• High potential for mis- or delayed diagnosis; many patients remain undiagnosed\(^3\)

ALT: alanine aminotransferase; AST: aspartate aminotransferase; HDL-c: high-density lipoprotein cholesterol
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ALT: alanine aminotransferase; AST: aspartate aminotransferase; HDL-c: high-density lipoprotein cholesterol

Sitosterolemia, a.k.a. Phytosterolemia

- Autosomal recessive, first described in 1974
- Diagnostically elevated plasma phytosterols
- Rare, <1:1,000,000 (~20 cases in US)
- Associated with premature atherosclerosis
- Increased dietary sterol absorption and failure to excrete sterols into bile
- Need GC or HPLC to make diagnosis

Familial Phytosterolemia

Micelles

ABCG5
ABCG8

Cholesterol

Phytosterols
Lipoprotein (a)

- An LDL + apolipoprotein a
- Different lengths of apo a (kringles) caused by a variable number of kringle IV repeats
- More kringles = lower Lp(a) levels
- Hepatic synthesis
- Lp(a) plasma concentrations are highly heritable and mainly controlled by the apolipoprotein(a) gene [LPA] located on chromosome 6q26-27.

LIPOPROTEIN (a): mechanisms of atherogenesis

Homology with plasminogen (= impaired fibrinolysis)

Binds to macrophages → foam cell formation

Binds to platelets (inhibition or stimulation?)

Deposition of cholesterol into plaques?
Lp(a)-corrected LDLC

- Lp(a)-corrected LDLC can be estimated with the Dahlen modification of the Friedewald formula, which assumes that 30% of Lp(a) weight consists of cholesterol:

\[
cLDLC = TC - HDLC - TG/5 \times [Lp(a) \times 0.30]\]

in mg/dL
Typical distributions of lipoprotein(a) levels in the general population.

Men

Women

Nordestgaard B G et al. Eur Heart J 2010;31:2844-2853
**2016 ESC/EAS Guidelines for the Management of Dyslipidaemias**

The Task Force for the Management of Dyslipidaemias of the European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS)

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**Box 7** Individuals who should be considered for lipoprotein(a) screening

<table>
<thead>
<tr>
<th>Individuals with:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Premature CVD</td>
</tr>
<tr>
<td>• Familial hypercholesterolaemia</td>
</tr>
<tr>
<td>• A family history of premature CVD and/or elevated Lp(a)</td>
</tr>
<tr>
<td>• Recurrent CVD despite optimal lipid-lowering treatment</td>
</tr>
<tr>
<td>• $\geq$5% 10-year risk of fatal CVD according to SCORE</td>
</tr>
</tbody>
</table>
LIPOPROTEIN (a) MEASUREMENT

• Quantitative Lp(a) measurements
  – rocket immunoelectrophoresis
  – rate and endpoint nephelometry
  – turbidimetry
  – radio-immuno assays
  – enzyme immuno assays (ELISA)
  – dissociation-enhanced lanthanide fluorescent immunoassay (DELFIA)
Risk of Myocardial Infarction

<table>
<thead>
<tr>
<th>Lipoprotein(a) (mg/dL)</th>
<th>KIV-2 quartile</th>
<th>Hazard ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 40 30 20 10</td>
<td>1st</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>2nd</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>3rd</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>4th</td>
<td></td>
</tr>
</tbody>
</table>

Trend p<0.001

Kamstrup et al. JAMA 2009; 301: 2331-9
PCSK9 Levels Are Elevated in FH

Raal F et al. J Am Heart Assoc 2013;2:e000028
<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>FH Low</th>
<th>FH High</th>
<th>Control vs. FH Low</th>
<th>Control vs. FH High</th>
<th>FH Low vs. FH High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males [number (%)]</td>
<td>n = 112</td>
<td>n = 94</td>
<td>n = 61</td>
<td>0.20</td>
<td>0.36</td>
<td>0.84</td>
</tr>
<tr>
<td>Age (years)</td>
<td>54 (48)</td>
<td>37 (39)</td>
<td>25 (40)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.054</td>
</tr>
<tr>
<td>Hypertension [number (%)]</td>
<td>10 (9)</td>
<td>5 (5)</td>
<td>4 (7)</td>
<td>0.33</td>
<td>0.63</td>
<td>0.72</td>
</tr>
<tr>
<td>Diabetes [number (%)]</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (2)</td>
<td>—</td>
<td>0.17</td>
<td>0.21</td>
</tr>
<tr>
<td>Current smoker [number (%)]</td>
<td>23 (21)</td>
<td>16 (17)</td>
<td>18 (30)</td>
<td>0.52</td>
<td>0.19</td>
<td>0.066</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.6 ± 4.1</td>
<td>25.6 ± 5.3</td>
<td>24.3 ± 4.8</td>
<td>0.95</td>
<td>0.098</td>
<td>0.097</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>128 ± 15</td>
<td>124 ± 13</td>
<td>122 ± 14</td>
<td>0.038</td>
<td>0.014</td>
<td>0.54</td>
</tr>
<tr>
<td>Lipid profile (mmol/l)</td>
<td></td>
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<td></td>
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<tr>
<td>At genetic FH test</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>pLDL (IQR)</td>
<td>40 (19–65)</td>
<td>45 (20–63)</td>
<td>97 (95–98)</td>
<td>0.89</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>At study visit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>5.3 ± 1.1</td>
<td>5.4 ± 1.1</td>
<td>7.1 ± 1.2</td>
<td>0.24</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-C</td>
<td>3.3 ± 0.9</td>
<td>3.5 ± 1.0</td>
<td>5.3 ± 1.1</td>
<td>0.11</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-C</td>
<td>1.5 ± 0.4</td>
<td>1.5 ± 0.4</td>
<td>1.4 ± 0.4</td>
<td>0.65</td>
<td>0.83</td>
<td>0.55</td>
</tr>
<tr>
<td>Triglycerides (IQR)</td>
<td>0.9 (0.6–1.4)</td>
<td>0.7 (0.5–1.1)</td>
<td>0.7 (0.5–1.1)</td>
<td>0.024</td>
<td>0.012</td>
<td>0.69</td>
</tr>
<tr>
<td>Mean cIMTa (SE) (mm)</td>
<td>0.63 ± 0.008</td>
<td>0.62 ± 0.009</td>
<td>0.67 ± 0.013</td>
<td>0.38</td>
<td>0.009</td>
<td>0.001</td>
</tr>
</tbody>
</table>
LDL-C Levels Correlate With Residual LDLR Activity

![Graph showing the correlation between LDL-CH (mmol/l) and Residual LDL-receptor activity (%).](image)

- **r = -0.655**
- **P = 0.0003**

N=32 HoFH

# Penn HoFH Cohort: LDLR Negative Subjects Have a More Severe Phenotype

<table>
<thead>
<tr>
<th></th>
<th>LDLR negative (n=18)</th>
<th>LDLR defective (n=16)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median (range)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr) at visit 1</td>
<td>11.5 (3.3 - 29)</td>
<td>28.1 (3.3 - 44.6)</td>
<td>0.008</td>
</tr>
<tr>
<td>Age at 1st xanthomas</td>
<td>2.0 (0.25 - 4)</td>
<td>7.0 (1 - 15)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (yr) at FH dx</td>
<td>3.0 (0.5 - 7)</td>
<td>8.0 (2 - 17)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TC (mg/dl) at dx</td>
<td>895 (602-1260)</td>
<td>686 (519-900)</td>
<td>0.002</td>
</tr>
<tr>
<td>Age (yr) at start of Rx</td>
<td>5.0 (1.2 - 10)</td>
<td>16.0 (2 - 31)</td>
<td>0.001</td>
</tr>
<tr>
<td>Age (yr) at CAD</td>
<td>12.5 (6 - 16)</td>
<td>22.0 (16 - 37)</td>
<td>0.004</td>
</tr>
<tr>
<td><strong>Lipid profile at visit 1</strong></td>
<td>Mean (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>618 (238)</td>
<td>453 (162)</td>
<td>0.025</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>535 (214)</td>
<td>393 (159)</td>
<td>0.040</td>
</tr>
<tr>
<td>Apo B (mg/dl)</td>
<td>388 (142)</td>
<td>293 (92)</td>
<td>0.032</td>
</tr>
</tbody>
</table>

Kolansky DM, Am J Cardiol 2008; 102:14-38-43
Conclusion

• The early diagnosis of FH patients (using clinical or genetic diagnosis) and subsequent treatment has proven to be effective in reducing their cardiovascular morbidity and mortality

• The measurement of plasma PCSK9 levels may help explaining the phenotypic variability in FH patients

• Patients with FH should be considered for Lp(a) screening