The Genetics of Familial Hypercholesterolemia (FH)

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2019-05-30
Potential COI disclosure

- No conflict of interest (COI) to disclose
Learning Objectives

At the conclusion of this activity, participants will be able to:

- Describe the main known genetic causes of familial hypercholesterolemia (FH)
- Articulate the criteria for genetic testing in FH
- Explain recent developments, utility and limitations of monogenic and polygenic genetic testing
**Introduction – FH**

- **Familial hypercholesterolemia (FH):**
  - An inherited lipid disorder
  - Lifelong exposure to highly elevated LDL levels
  - High risk of premature coronary artery disease (ASCVD)
  - Untreated men are at a 50% risk for a coronary event by age 50 years. Untreated women are at a 30% risk by age 60 years.

- **One of the most common monogenic disorder** encountered in clinical practice (~1/250 individuals)

- **Formal diagnostic criteria** (Western countries):
  - UK Simon Broome FH Registry
  - Dutch Lipid Clinic Network
  - US MEDPED Program
Simplified Canadian Definition for Familial Hypercholesterolemia

FH Screening Criteria

*LDL-C ≥ 5.0 mmol/L (≥ 40 yr)
LDL-C ≥ 4.5 mmol/L (18-39 yr); ≥ 4.0 mmol/L (<18 yr)

+ 

Major Criteria

**DNA Mutation
OR
Tendon xanthomas
OR
LDL-C ≥ 8.5 mmol/L

Yes

Definite FH

No

Minor Criteria

1st-degree relative with high LDL-C
OR
Proband or 1st-degree relative with ASCVD (<55 yr men; <65 yr women)

Yes

Probable FH

No

Severe Hypercholesterolemia
Early diagnosis and treatment can **normalize life expectancy**
The genetics of FH

• Inheritance
  - Autosomal **dominant** (Heterozygous FH [HeFH])
  - Autosomal **recessive** (Homozygous FH [HoFH])
  - "**Polygenic**" inheritance
The genetics of FH

- Heterozygous pathogenic variants in one of 3 genes (LDLR, APOB, PCSK9) in 28-80% of cases
- Rare autosomal recessive form (homozygous FH, HoFH)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Proportion of FH Attributed to Pathogenic Variants in This Gene</th>
<th>Proportion of Pathogenic Variants Detectable by This Method</th>
<th>Gene-targeted Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>APOB</td>
<td>1%-5%</td>
<td>&gt;99%</td>
<td>1 individual</td>
</tr>
<tr>
<td>LDLR</td>
<td>60%-80%</td>
<td>&gt;90%</td>
<td>~2.5%-10%</td>
</tr>
<tr>
<td>PCSK9</td>
<td>0%-3%</td>
<td>~100%</td>
<td>None reported</td>
</tr>
<tr>
<td>Unknown</td>
<td>20%-40%</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Source: GeneReviews
Figure 2. The known mechanisms causing familial hypercholesterolemia linked to low-density lipoprotein (LDL) receptor (LDLR) function. Numbers 1 through 6 correspond to the mechanisms of LDLR dysfunction discussed in the text and Table 3. Familial defective apolipoprotein B (apoB) impairs the ability of the apoB to bind with the LDLR. LDLR adaptor protein (LDLRAP) impairs the ability of the LDLR to interact with LDL particles to extract cholesterol. Proprotein convertase subtilisin/kexin type 9 (PCSK9) gain-of-function (GOF) mutations inhibit LDLR function and increase the degradation of LDLRs.
**LDLR gene**

- Main disease-causing gene
- ~80% of disease-causing variants
- **Loss-of-function** variants
- > 2,000 known pathogenic variants

### TABLE 1 | Overview of common monogenic FH mutations.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Role of normal protein</th>
<th>Type of FH-causative mutation</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDLR</td>
<td>Low-density lipoprotein receptor</td>
<td>Uptake of low-density lipoprotein cholesterol (LDL-c), thus decreasing systemic LDL-c levels</td>
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<td>60–80% of FH-causative monogenic variants. Patients with null LDLR mutations may not benefit from PCSK9 inhibitors or respond well to statin therapy.</td>
</tr>
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<td>Apolipoprotein B-100</td>
<td>Binding of LDL-containing lipoproteins to the LDL receptor</td>
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<td>Up to 5% of FH-causative monogenic variants (may be higher in some populations).</td>
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<td>Proprotein convertase subtilisin/kexin 9</td>
<td>Promotes intracellular LDL receptor degradation</td>
<td>Gain-of-function</td>
<td>Up to 3% of FH-causative monogenic variants.</td>
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</tbody>
</table>

*Detailed overview of pathogenic allelic variants for each gene may be found in ClinVar, HGMD (Human Gene Mutation Database), and LOVD (Leiden Open Variation Database) (16).*
Figure 2. (a) Risk of coronary artery disease (CAD) in patients with LDLR loss-of-function variants for familial hypercholesterolemia relative to unaffected individuals. Studies are combined using a random-effects model. Square sizes are proportional to random-effects study weights with horizontal lines denoting 95% confidence intervals (CIs). (b) Risk of CAD in patients with LDLR hypomorphic variants for familial hypercholesterolemia relative to unaffected individuals. Studies are combined using a random-effects model. Square sizes are proportional to random-effects study weights with horizontal lines denoting 95% CIs. (c) Risk of CAD in patients with loss-of-function variants for familial hypercholesterolemia relative to hypomorphic changes. Odds ratios represent derived ratios of relative risk. Studies are combined using a random-effects model. Square sizes are proportional to random-effects study weights with horizontal lines denoting 95% CIs.
IV: inverse variance
**APOB gene**

- Ligand responsible for LDLR binding during LDL-C uptake
- ~5% of disease-causing variants
- **Loss-of-function** variants
- Typically less severe than *LDLR*

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**PCSK9 gene**

- Responsible for LDLR degradation in liver cells
- ~3% of disease-causing variants
- **Gain-of-function** variants (increased PCSK9 activity & LDLR degradation)

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Other genes

- Rare reports of disease-causing variants in other genes (e.g. *APOE*, *LDLRAP1*, *ABCG5*, and *ABCG8*)
- Mostly autosomal recessive inheritance

**Brief Report**

Description of a Large Family with Autosomal Dominant Hypercholesterolemia Associated with the *APOE* p.Leu167del Mutation

**Human Mutation**

Current Atherosclerosis Reports (2018) 20: 31
https://doi.org/10.1007/s11883-018-0751-0

**Genetics and Genomics (A.J. Marian, Section Editor)**

The Present and the Future of Genetic Testing in Familial Hypercholesterolemia: Opportunities and Caveats

Amanda J. Hooper1,2 • John R. Burnett1,2,3 • Damon A. Bell1,2,3 • Gerald F. Watts2,3
Clinical validity of genetic testing

- Genetic testing of \(LDLR\), \(APOB\) and \(PCSK9\)
- Monogenic forms of FH
- **Severe cases**: sensitivity of \(> 80\%\)
- **Milder presentations** (e.g. no xanthomas): sensitivity of \(~50\%\)
- \(LDL-C > 4.9\ mmol/L\): \(~2\%\ of cases\)

Genetic Testing and Risk Scores: Impact on Familial Hypercholesterolemia

Ashish Sarraju\(^1\) and Joshua W. Knowles\(^{1,2,3*}\)

\(^1\) Division of Cardiovascular Medicine and Cardiovascular Institute, Stanford University, Stanford, CA, United States, \(^2\) The FH Foundation, Pasadena, CA, United States, \(^3\) Stanford Diabetes Research Center, Stanford University, Stanford, CA, United States
Who to test?

- Untreated LDL-C ≥ 5.0 mmol/L for age ≥ 40
- Untreated LDL-C ≥ 4.5 mmol/L for age 18-39
- Untreated LDL-C ≥ 4.0 mmol/L for age ≤ 18
- AND at least one of the following:
  - **Major criteria (definite FH):**
    - Tendon xanthomas in proband
    - Known FH-causing mutation in a 1\textsuperscript{st}-degree relative
    - High LDL-cholesterol in proband (≥ 8.5 mmol/L)
  - **Minor criteria (probable FH):**
    - 1\textsuperscript{st}-degree relative with high LDL-C
    - Proband or 1\textsuperscript{st}-degree relative with early onset atherosclerotic cardiovascular disease (men < 55; women < 65 yr)
Who to test?

- Additional details:
  - Elevated LDL-C **not due to secondary causes**:
    - Severe or untreated hypothyroidism
    - Nephrotic syndrome
    - Hepatic disease (primary biliary cirrhosis)
    - Medication (especially antiretroviral agents)
  
- If baseline LDL-C is unknown:
  - Imputed level using the CardioRisk Calculator
  - [http://www.circl.ubc.ca/cardiorisk-calculator.html](http://www.circl.ubc.ca/cardiorisk-calculator.html)
Who to test?

- **No evidence of the cost-effectiveness of broad population-based screening for FH**
  - *Khera et al.*: FH-causing variant in 1.7% (27/1,386) of cases with an LDL-C > 4.9 mmol/L
  - *Abul-Husn et al.*: FH-causing variant in 2.5% (112/4,433) of cases with an LDL-C > 4.9 mmol/L
Who to test?

- **Confirm diagnosis ≠ Exclude diagnosis**
  - Most genetic tests **do not exclude** a clinical diagnosis
  - This test should be used to confirm, not to rule out a diagnosis of FH
  - “A negative result does not rule out the possibility that this individual harbors a pathogenic variant not detected by this assay”
Who to test?

- **Testing of known familial variants:**
  - All 1\textsuperscript{st} degree and relevant 2\textsuperscript{nd} degree relatives (regardless of the LDL-C level)
  - Testing limited to the known familial variant (“cascade testing”)
  - **Exceptions:**
    - Familial variant not found in symptomatic case (phenocopy) => reflex to full panel
    - Suspicion of homozygous FH (HoFH): 2\textsuperscript{nd} hit to be identified
Clinical utility of genetic testing

- Confirm diagnosis
- Cascade testing
- Risk stratification
- Genotype-phenotype correlation
- Overall management
Clinical utility of genetic testing

- FH is **under-diagnosed**
- Early diagnosis & management is important
- **Cascade testing** of known familial variants
- Known to be highly cost-effective
Clinical utility of genetic testing

- Genetic counseling
- **Cascade genetic testing** to assess the penetrance of familial variants
  - Garcia-Garcia *et al.*: 7% of individuals positive for a FH-causing variant are asymptomatic

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Reduced penetrance of autosomal dominant hypercholesterolemia in a high percentage of families: Importance of genetic testing in the entire family

Ana-Barbara Garcia-García*, Carmen Ivorra, Sergio Martinez-Hervas, Sebastian Blesa, M. José Fuentes, Oscar Puig, Jose Javier Martín-de-Llano, Rafael Carmena, Jose T. Real, Felipe Javier Chaves
Background
Approximately 17% of individuals with familial hypercholesterolemia (FH) develop coronary artery disease (CAD) by a single LDL cholesterol measurement. Determining whether CAD risk varies according to mutation status beyond the observed LDL cholesterol level remains uncertain.

Objectives
This study’s objective was to determine whether CAD risk varies according to mutation status beyond the observed LDL cholesterol level.

Methods
Three genes causative for FH (LDLR, APOB, and PCSK9) were sequenced in 26,025 participants from 7 case-control studies (5,540 CAD case subjects, 8,577 CAD-free control subjects) and 5 prospective cohort studies (11,908 participants). FH mutations included loss-of-function variants in LDLR, missense mutations in LDLR predicted to be damaging, and variants linked to FH in ClinVar, a clinical genetics database.

Results
Among 20,485 CAD-free control and prospective cohort participants, 1,386 (6.7%) had LDL cholesterol ≥190 mg/dl; of these, only 24 (1.7%) carried an FH mutation. Within any stratum of observed LDL cholesterol, risk of CAD was higher among FH mutation carriers than noncarriers. Compared with a reference group with LDL cholesterol <130 mg/dl and no mutation, participants with LDL cholesterol ≥190 mg/dl and no FH mutation had a 6-fold higher risk for CAD (odds ratio: 6.0; 95% confidence interval: 5.2 to 6.9), whereas those with both LDL cholesterol ≥190 mg/dl and an FH mutation demonstrated a 22-fold increased risk (odds ratio: 22.3; 95% confidence interval: 10.7 to 53.2). In an analysis of participants with serial lipid measurements over many years, FH mutation carriers had higher cumulative exposure to LDL cholesterol than noncarriers.

Conclusions
Among participants with LDL cholesterol ≥190 mg/dl, gene sequencing identified an FH mutation in <2%. However, for any observed LDL cholesterol, FH mutation carriers had substantially increased risk for CAD. (J Am Coll Cardiol 2016;67:2578-89) © 2016 by the American College of Cardiology Foundation.
Clinical utility of genetic testing

- Evidence of increased **adherence to treatment**

- **Umans-Eckenhausen et al.**: 37.6% of patients treated at genetic screening, 92.5% and 85.9% 1 and 2 years after screening.

| Review of first 5 years of screening for familial hypercholesterolaemia in the Netherlands |

- **Severe disease-causing variants:**
  - Patient may require more aggressive therapy
  - Early consideration of advanced lipid powering therapy (e.g. PCSK9 inhibitor)?
# Clinical utility of genetic testing

## Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics

### Table 1 Continued

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>MIM disorder</th>
<th>PMID Gene Reviews entry</th>
<th>Typical age of onset</th>
<th>Gene</th>
<th>MIM gene</th>
<th>Inheritance</th>
<th>Variants to report</th>
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<td>610476</td>
<td>PKP2</td>
<td>602861</td>
<td>AD</td>
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<td>TMEM43</td>
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<td>And EP</td>
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<td>PCSK9</td>
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<td>Wilson disease</td>
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<td>ATP7B</td>
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<td>KP</td>
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<td>Ornithine transcarbamylase deficiency</td>
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<td>300461</td>
<td>XL</td>
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<td>CACNA1S</td>
<td>114208</td>
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</tbody>
</table>
Polygenic FH

- Accumulation of **common, small effect variants** affecting LDL-C levels
- Identified by genome-wide association studies (**GWAS**)
- Calculation of a **polygenic risk score** (or GRS)
- **12 alleles** from the Global Lipid Genetic Consortium (GLGC)
Polygenic FH

- Individuals with severe hypercholesterolemia:
  - 20% with a high polygenic risk score
  - 2% with a rare disease-causing variant
Polygenic FH

- **High GRS**: Possible cause in FH patients with no disease-causing variant
- Currently used by direct-to-consumer genetic testing companies
- **Limited clinical applicability**
  - Individual risk difficult to assess
  - Ethnically biased (towards European ancestry)
  - Probable gene-environment (GxE) interactions
  - Unknown cost-effectiveness
  - The absence of a monogenic variant cannot be excluded
Genetic testing in Québec

• What is currently offered in Quebec?
  – Genotyping of 11 recurrent disease-causing \( \text{LDLR} \) variants in the French-Canadian (FC) population
  – Collectively account for up to 90% of FH cases in FC
  – **Limitations:** not tailored to the needs of a more diverse population (e.g. Montreal area)
<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
<th>Reference Details</th>
<th>Type</th>
<th>Supra</th>
<th>Price</th>
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<tbody>
<tr>
<td>55008</td>
<td>Hypercholestérolémie familiale (gène récept. ( \text{LDL} )) (del 5-15 Kb)</td>
<td>(TAAN)</td>
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<td>55010</td>
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<td>Hypercholestérolémie familiale (HF), gène ( R-LDL ) Panel 2 (Glu207Lys, Cys152Trp, Arg329Xaa, Cys347Arg, Tyr468Xaa, Tyr354Cys, 681ins7)</td>
<td>(TAAN)</td>
<td>Rapport</td>
<td>Supra</td>
<td>210,0</td>
</tr>
</tbody>
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**LDLR, APOB and PCSK9 testing**

- **Single-nucleotide variants (SNVs) and small indels**
  - Sequencing of *LDLR, APOB* and *PCSK9* coding regions and flanking intronic regions
  - Now performed by targeted NGS
  - Known familial variants: Sanger sequencing
- **Copy number variants (CNVs) for LDLR**
  - Multiplex ligation-dependent probe amplification (MLPA)
  - Quantitative PCR (qPCR)
  - Gap-PCR (for recurrent CNVs with known breakpoints)
Summary – The genetics of FH

FIGURE 1 | Phenotypic, genotypic, and ASCVD risk spectrum of FH. Lp(a), lipoprotein (a). Other abbreviations as in text. Re-printed with permission from Elsevier (15).
Conclusion & Perspectives

- **Standard or care:**
  - Genetic testing for monogenic FH (*LDLR*, *APOB* and *PCSK9*)
  - Cascade screening in families
  - Should be offered in Québec province

- The use of **polygenic risk scores** requires further studies:
  - Management of FH patients NEG for monogenic testing
  - Risk assessment in FH patients POS for monogenic testing
  - Management of severe hypercholesterolemia patients
  - Clinical outcomes, feasibility, cost-effectiveness, reimbursement, ethics
Thank you!
Questions?