Does Genetic Testing in FH Impact Clinical Care? Pros and Cons

Jacques Genest MD

Cardiovascular Research Laboratories
McGill University Health Center
Disclosure J. Genest MD 2016

Advisory Board, Speaker’s Bureau, Consultant, Grants, Clinical Trials

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▪ Pfizer
▪ Novartis
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▪ Lilly
▪ Valeant
▪ Aegerion
▪ Ascati

Stock ownership: none;
Off label use: none
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Relevant disclosure: IMPROVE-IT, CANTOS, CAPREE steering Committees; REVEAL, ACCELERATE, AMG145, Lilly Clinical Trials.
Familial Hypercholesterolemia
Franz Hals (1683)

Portrait of a sixty year old woman holding a book
Familial Hypercholesterolemia: Definition
FH is a Condition of Highly Elevated LDL-C

- Common hypercholesterolemia
- FH may be suspected if an adult has LDL-C >5 mmol/L
- HeFH
- HoFH
- Typical LDL-C range

- 5 mmol/L
- 10 mmol/L
- 15 mmol/L
- 20 mmol/L

- 200 mg/dL
- 400 mg/dL
- 500 mg/dL

Severe Hypercholesterolemia
Pedigree of a family with familial hypercholesterolaemia.

Nordestgaard B G et al. Eur Heart J 2013;eurheartj.eht273
LDL-Receptor Pathway
SREBP Pathway

Michael BROWN

Joseph GOLDSTEIN

Nobel Prize 1985
A Century of Cholesterol and Coronaries: From Plaques to Genes to Statins

Joseph L. Goldstein\(^1\),* and Michael S. Brown\(^1\),*

\(^1\)Department of Molecular Genetics, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA

Table 1. A Century of Cholesterol and Coronaries

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1910</td>
<td>Human</td>
</tr>
<tr>
<td>1913</td>
<td>High cholesterol</td>
</tr>
<tr>
<td>1919</td>
<td>Heart attack</td>
</tr>
<tr>
<td>1933</td>
<td>Feedback</td>
</tr>
<tr>
<td>1938</td>
<td>Familial</td>
</tr>
<tr>
<td>1950</td>
<td>Cholesterol</td>
</tr>
<tr>
<td>1951</td>
<td>High-fat bartering</td>
</tr>
<tr>
<td>1953</td>
<td>Risk factor</td>
</tr>
<tr>
<td>1955</td>
<td>LDL identification</td>
</tr>
<tr>
<td>1973</td>
<td>LDL receptor</td>
</tr>
<tr>
<td>1976</td>
<td>HMG CoA synthase</td>
</tr>
<tr>
<td>1981</td>
<td>Statins</td>
</tr>
<tr>
<td>1987</td>
<td>First statins</td>
</tr>
<tr>
<td>1994</td>
<td>Statins</td>
</tr>
<tr>
<td>1997</td>
<td>SREBP pathway elucidated</td>
</tr>
<tr>
<td>2006</td>
<td>PCSK9: Destroyer of LDL receptors</td>
</tr>
</tbody>
</table>

Cell 2015:161; 161-172
**A:** LDL-R pathway in absence of PCSK9

**B:** Intracellular PCSK9 route

**C:** Extracellular PCSK9 route

Mature PCSK9

LDL

apoB

Degradation

Lysosome

Endosome

LDL-R
Familial Hypercholesterolemia
Familial Hypercholesterolemia
Familial Hypercholesterolemia

- Heterozygous FH (HeFH): single copy of mutated gene
- Homozygous FH (HoFH): two copies of mutated gene (either same mutation or compound heterozygous)

This was simple until the 21st century...
Early Diagnosis: Key Signs and Symptoms

• High LDL-C (>5.0 mmol/L) (200 mg/dL)

• Patient or Family History
  – Early CV disease
  – Persistently Elevated LDL-C

• Patient or Family Markers
  – Xanthomata
  – Xanthelasma
  – Arcus corneae
Definition of FH

- MedPed (US)
- Simon-Broome (UK)
- Dutch criteria (Netherlands)
- (Japanese Atherosclerosis Society)
- Canadian Definition

- Based on Age and LDL-C levels
- LDL-C and DNA, Family Hx, Xanthomas
- Point system (Definite, possible, probable)
# FH: Simon-Broome Criteria

## TABLE 1

**Simon Broome criteria for diagnostics of familial hypercholesterolemia**

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>a</strong></td>
<td>Total cholesterol concentration above 7.5 mmol/L in adults or a total cholesterol concentration above 6.7 mmol/L in children aged less than 16 years, or low-density lipoprotein cholesterol concentration above 4.9 mmol/L in adults or above 4.0 mmol/L in children</td>
</tr>
<tr>
<td><strong>b</strong></td>
<td>Tendinous xanthomata in the patient or a first-degree relative</td>
</tr>
<tr>
<td><strong>c</strong></td>
<td>DNA-based evidence of mutation in the <em>LDLR</em> or <em>APOB</em> gene</td>
</tr>
<tr>
<td><strong>d</strong></td>
<td>Family history of myocardial infarction before age 50 years in a second-degree relative or before age 60 years in a first degree relative</td>
</tr>
<tr>
<td><strong>e</strong></td>
<td>Family history of raised total cholesterol concentration above 7.5 mmol/L in a first- or second-degree relative</td>
</tr>
</tbody>
</table>

### Diagnosis

A ‘definite’ FH diagnosis requires either criteria a and b or criterion c
A ‘probable’ FH diagnosis requires criteria a and d or criteria a and e

*Widely used definition. Knowledge of family required*
<table>
<thead>
<tr>
<th>Points</th>
<th>Criteria</th>
<th>Definite FH ( (= \text{or} \geq 8 \text{ points}) )</th>
<th>Probable FH ( (6-7 \text{ points}) )</th>
<th>Possible FH ( (3-5 \text{ points}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 point</td>
<td>1\textsuperscript{st} degree relative with premature cardiovascular disease or LDL-C (&gt;95^{\text{th}}) percentile, or Personal history of premature peripheral or cerebrovascular disease, or LDL-C between 155 and 189 mg/dL (4.01 and 4.89 mmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 points</td>
<td>1\textsuperscript{st} degree relative with tendinous xanthoma or corneal arcus, or 1\textsuperscript{st} degree relative child (&lt;18 \text{ yrs}) with LDL-C (&gt;95^{\text{th}}) percentile, or Personal history of coronary artery disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 points</td>
<td>LDL-C between 190 and 249 mg/dL (4.91 and 6.44 mmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 points</td>
<td>Presence of corneal arcus in patient less than 45 yrs old</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 points</td>
<td>LDL-C between 250 and 329 mg/dL (6.46 and 8.51 mmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 points</td>
<td>Presence of a tendon xanthoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 points</td>
<td>LDL-C above 330 mg/dL (8.53 mmol/L), or Functional mutation in the \textit{LDLR} gene</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Point system, cutaneous manifestations important
Comparisons of HeFH Clinical Signs at first visit: 1979 vs 2000 and 2012

Gaudet D, Quebec, Canada data
Figure 1: Newly proposed Canadian definition of Familial Hypercholesterolemia (FH) based on the Simon-Broome criteria (under discussion)
Performance of the CCS definition on a cohort of 6,923 individuals screened for FH

<table>
<thead>
<tr>
<th>DB1/E21 Database Dx</th>
<th>CCS FH definition (with xanthomas as major criterion)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non FH*</td>
<td>Definite**</td>
</tr>
<tr>
<td>Non FH</td>
<td>4,859</td>
<td>3</td>
</tr>
<tr>
<td>FH</td>
<td>88</td>
<td>921</td>
</tr>
<tr>
<td>Total</td>
<td>4,947</td>
<td>924</td>
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</table>

[95% Confidence Interval]

<table>
<thead>
<tr>
<th></th>
<th>Pr(A)</th>
<th>28%</th>
<th>27%</th>
<th>29.3%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>Pr(+</td>
<td>A)</td>
<td>95.5%</td>
<td>94.5%</td>
</tr>
<tr>
<td>Specificity</td>
<td>Pr(-</td>
<td>N)</td>
<td>97.7%</td>
<td>97.3%</td>
</tr>
<tr>
<td>ROC area</td>
<td>(Sens. + Spec.)/2</td>
<td>.966</td>
<td>.961</td>
<td>.971</td>
</tr>
<tr>
<td>Pr(+</td>
<td>A)/Pr(+</td>
<td>N)</td>
<td></td>
<td>42</td>
</tr>
<tr>
<td>Pr(-</td>
<td>A)/Pr(-</td>
<td>N)</td>
<td></td>
<td>.0462</td>
</tr>
<tr>
<td>LR(+) / LR(-)</td>
<td></td>
<td>910</td>
<td>686</td>
<td>1209</td>
</tr>
<tr>
<td>Pr(A</td>
<td>+)</td>
<td></td>
<td>94.3%</td>
<td>93.2%</td>
</tr>
<tr>
<td>Pr(N</td>
<td>-)</td>
<td></td>
<td>98.2%</td>
<td>97.8%</td>
</tr>
</tbody>
</table>

Dr. D. Gaudet, Chicoutimi Lipid Clinic
Familial Hypercholesterolemia

Imputed LDL-C
- Baseline LDL-C often NOT available
- Use of imputed LDL-C – current LDL-C on Rx, correction for statin and ezetimibe.

- e.g. LDL-C 4.0 mmol/L (160 mg/dL) on Atorva 80 mg + ezetimibe 10 mg → Baseline LDL-C 10.0 mmol/L (400 mg/dL)
Familial Hypercholesterolemia: Molecular Genetics
Genes Causing FH

• Low-density Lipoprotein Receptor (LDLR)
• Apolipoprotein B (APOB)
• Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9)
(Other) Genes Causing FH

- Apolipoprotein E (*APOE*) [del166Leu]
- Signal transducing adaptor family member 1 (*STAP1*)
- LDLR adaptor protein (*LDLRAP1*) [ARH]
- Lysosomal acid lipase (*LIPA*)

Rare, prevalence unknown
Use of low-density lipoprotein cholesterol gene score to distinguish patients with polygenic and monogenic familial hypercholesterolaemia: a case-control study

Philippa J Talmud*, Sonia Shah*, Ros Whittall, Marta Futema, Philip Howard, Jackie A Cooper, Seamus C Harrison, KaWah Li, Fotios Drenos, Frederik Karpe, H Andrew W Neil, Olivier S Descamps, Claudia Langenberg, Nicholas Lench, Mika Kivimaki, John Whittaker, Aroon D Hingorani, Meena Kumari, Steve E Humphries

www.thelancet.com Published online February 22, 2013
Monogenic, Polygenic FH

www.thelancet.com Published online February 22, 2013
LipidSeq + MLPA

LipidSeq: a next-generation clinical resequencing panel for monogenic dyslipidemias

Panel of 73 genes and SNPs identified causing lipoprotein disorders in man, incorporating GWAS data, mouse data.

MLPA: Multiplex Ligation-dependent Probe Amplification for CNV of the LDLR gene

FH Mutation Diagnosis Algorithm

Step 1: Request for DNA diagnosis.
- Check:
  - FH criteria met?
  - Consent form signed?
- Candidate mutation screen (>85% French Canadians)
- Mutation ID?
- LipidSeq, MLPA (*LDLR* gene)
- Mutation ID?
- Exome-sequencing

Step 2: FH Registry
- BioBanking DNA

Step 3: Y

Step 4: N

Step 5: N

FH Registry

BioBanking DNA

DNA

**Notes:**
- FH Canada
- Hypercholestérolémie Familiale
- Familial Hypercholesterolemia
Homozygous FH (HoFH)

**True HoFH**
- Same allele
- Co-sanguinous
- Founder Effect

**Compound HoFH**
- Different Alleles
- Random
- Severity depends on cumulative effect
Homozygous FH: Genetic Heterogeneity, Phenotypic Heterogeneity

Diagnostic Criteria:

Two mutant alleles at the LDLR, APOB, PCSK9, or LDLRAP1 gene locus

OR

An untreated LDL-C >13 mmol/L (500 mg/dL) or treated LDL-C ≥8 mmol/L (300 mg/dL)* together with:

○ Cutaneous or tendon xanthoma before age 10 years or
○ Untreated elevated LDL-C consistent with HeFH in both parents
Spectrum of LDL-R activity

Response to statins (+)

Mutation type: $M = \text{missense}$, $N = \text{Null}$

<table>
<thead>
<tr>
<th></th>
<th>Heterozygotes</th>
<th>Compound Heterozygotes</th>
<th>Homozygotes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$M$</td>
<td>$MN$</td>
<td>$NN$</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>28</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>33.2</td>
<td>13.3</td>
<td>8.7</td>
</tr>
<tr>
<td></td>
<td>21.3</td>
<td>9.7</td>
<td>9.6</td>
</tr>
<tr>
<td></td>
<td>19.8</td>
<td>6.9</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>21.0</td>
<td>3.6</td>
<td>2.3</td>
</tr>
</tbody>
</table>
Homozygous FH

HoFH: \( \frac{1}{250} \times \frac{1}{250} \times \frac{1}{4} = \frac{1}{250,000} \)
LDL Receptor Mutations

15 Kbp CNV (Del) mutation

Deletion in the Gene for LDL-R in a Majority of French Canadians with Familial Hypercholesterolemia
Homozygous FH: Cholesterol Levels

Moorjani S et al.
Lancet 1993;341:1303
Severe Hypercholesterolemia
LDL-C, Familial Hypercholesterolemia
Mutation Status, and Risk for CAD

Amit V. Khera, Hong-Hee Won, Gina M. Peloso,
Sekar Kathiresan, on behalf the
Myocardial Infarction Genetics and CHARGE Consortia
**Background:** The Utility of Genetic Testing in Severe Hypercholesterolemia (LDL ≥ 190 mg/dl) is Uncertain

**Study Objectives:**

1. **Diagnostic Yield**
   What proportion of individuals with LDL ≥ 190 have a FH mutation?

2. **Clinical Importance**
   For any given LDL, does coronary risk vary according to FH mutation status?
**Methods:** Gene Sequencing of *LDLR, APOB,* and *PCSK9* to Identify FH Mutations

1. **Loss of function** variants in *LDLR:*
   a) Premature truncation (nonsense)
   b) Scramble the protein translation (frameshift)
   c) Alter the mRNA splicing process (splice-site)

2. **Missense variants in *LDLR* predicted to be damaging** by each of five computer prediction algorithms

1. Variants in *LDLR, APOB,* or *PCSK9,* annotated as “pathogenic” or “likely pathogenic” in ClinVar, a clinical genetics database
Diagnostic Yield: Fewer than 2% of Individuals with LDL ≥ 190 mg/dl have an Identifiable FH Mutation

Severe Hypercholesterolemia
LDL Cholesterol ≥ 190

1,386 of 20,485 (7%)

FH Mutation Positive

24 of 1,386 (1.7%)
**Clinical Importance:** CAD Risk is Substantially Higher in FH Mutation Carriers with LDL ≥ 190

<table>
<thead>
<tr>
<th></th>
<th>OR for CAD (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL ≥ 190 mg/dl</td>
<td></td>
</tr>
<tr>
<td>FH Mutation – (N = 1,264)</td>
<td></td>
</tr>
<tr>
<td>FH Mutation + (N = 73)</td>
<td></td>
</tr>
<tr>
<td>LDL &lt; 130 &amp; FH Mutation –</td>
<td>Reference</td>
</tr>
</tbody>
</table>

Logistic Regression in Myocardial Infarction Genetics Consortium Studies
Covariates: Gender, Study, 5 principal components of ancestry
Clinical Importance: For a Given Observed LDL, FH Mutation Carriers are at Increased Coronary Risk

Mean LDL

- 203 mg/dl
- 205 mg/dl

Odds Ratio for Coronary Artery Disease (95%CI)

- No
- Yes

LDL Cholesterol Category (mg/dl)

- <130
- ≥130–160
- ≥160–190
- ≥190–220
- ≥220

17.0
(5.3–77.9)

5.2
(4.4–6.2)
Summary

1. Diagnostic Yield

Only about 2% of individuals with LDL ≥ 190 have a FH mutation; remainder likely related to polygenic or environmental causes.

2. Clinical Importance

For any given LDL, risk of coronary artery disease is substantially higher among those with a FH mutation, likely due to increased lifelong exposure to circulating LDL.

Additional Details Available in Online Publication
Methods: FH Mutation Prevalence in MIGen

Myocardial Infarction Genetics Consortium

Controls: 48 of 8,577 (0.6%)
Cases: 116* of 5,540 (2.1%)

*One homozygous carrier
**Results**: LDL and CAD Impact by Variant Class

[Graph showing the relationship between the increase in LDL cholesterol and the odds ratio for coronary artery disease, categorized by variant class.]
Familial Hypercholesterolemia: Prevalence
FH is One of the Most Common of Inherited Diseases

- Heterozygous FH
- Dominant osteosclerosis
- Adult polycystic kidney disease
- Huntington’s disease
- Cystic fibrosis
- Marfan’s syndrome
- Duchenne muscular dystrophy
- Sickle cell anemia
- Phenylketonuria
- Haemophilia

Frequency per 1,000 births

- FH Heterozygote
  - 1 in 270 to 500

- FH Homozygote
  - 1 in 1 million
Title: The Prevalence of Familial Hypercholesterolemia in the 1999-2012 United States National Health and Nutrition Examination Survey (NHANES)

Manuscript number: CIRCULATIONAHA/2015/018791R2

Author(s): Sarah de Ferranti, Children's Hospital Boston

The estimated overall US prevalence of probable/definite FH was 0.40% (95% CI 0.32-0.48) or 1 in 250 (95% CI 1 in 311 to 209); suggesting 834,500 US adults have FH. Prevalence varied by age, being least common in 20-29 year-olds (0.06%, 1 in 1557), and most common in 60-69
Mutations causative of familial hypercholesterolaemia: screening of 98 098 individuals from the Copenhagen General Population Study estimated a prevalence of 1 in 217

Marianne Benn¹,²,³*, Gerald F. Watts⁴, Anne Tybjærg-Hansen²,³,⁵, and Børge G. Nordestgaard²,³,⁶
Familial Hypercholesterolemia: Importance of Diagnosis
FH if Untreated: Major Risks

- CV Disease: 20X higher
- CV Deaths (age 20-59): 8X higher
- Death Rates: 3X higher

Genest et al. *Can J Cardiol* 2014;30:1471-81
LDL accumulates to cause CHD early in life in FH

- Threshold for CHD reached by:
  - Age >60 y in healthy individuals

LDL accumulates to cause CHD early in life in FH

- Threshold for CHD reached by:
  - Age 15 y in HoFH patients
  - Age 40 y in HeFH patients
  - Age >60 y in healthy individuals

LDL cholesterol burden in individuals with or without familial hypercholesterolaemia as a function of the age of initiation of statin therapy.

Nordestgaard B G et al. Eur Heart J 2013;eurheartj.eht273

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Kaplan–Meier curve estimates of cumulative CHD-free survival among individuals with familial hypercholesterolaemia according to statin treatment (P < 0.001 for difference).

Nordestgaard B G et al. Eur Heart J 2013;eurheartj.eht273

© The Author 2013. Published by Oxford University Press on behalf of the European Society of Cardiology.
Familial Hypercholesterolemia: Cascade Screening
Management Recommendations from 2014 CCS Position Statement on FH

- Considering all adults with FH as “high risk” due to lifelong exposure to high LDL-C
- Pharmacotherapy† is recommended to lower LDL by > 50%

<table>
<thead>
<tr>
<th>Initiate therapy</th>
<th>Primary target LDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Consider all patients with FH as being high risk</td>
<td>• &gt; 50% reduction from baseline LDL-C</td>
</tr>
<tr>
<td>• Consider pharmacotherapy</td>
<td>• If CVD, LDL-C target should be &lt;2 mmol/L*</td>
</tr>
</tbody>
</table>

25 year Follow-up of HeFH Patient

Cholestérol-LDL (mmol/L)

Courtoisie Dr. C. Gagné Québec
Why is it Not Working?

Heterozygous FH Patients Fail to Reach Goal on Statins

Starting LDL-C
~5.0 mmol/L

-100%
-90%
-80%
-70%
-60%
-50%
-40%
-30%
-20%
-10%
0%

UNMET TARGETS

Goal
<2 mmol/L

Statin 50% reduction
Double-dose statin +6% reduction
Adjunctive agents +15% reduction
Conclusions

- FH is frequently missed, often detected at first CV event.
- The trajectory of FH toward early CV events and CAD-associated mortality can be modified if LDL-C is controlled.
- Cascade screening is key!
- DNA analysis confirms the clinical diagnosis.
- **Carriers of FH mutation at markedly increased risk**
- Role of the polygenic score under debate. Allele transmission random.