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

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Cardiovascular Research Laboratories
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Disclosure J. Genest MD 2016

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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

courtesy of www.frans-hals.org
FH is a Condition of Highly Elevated LDL-C

Common hypercholesterolemia

FH may be suspected if an adult has LDL-C >5 mmol/L

Typical LDL-C range

HeFH

HoFH

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

Severe Hypercholesterolemia
Pedigree of a family with familial hypercholesterolaemia.

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Nordestgaard B G et al. Eur Heart J 2013;eurheartj.eht273
A Century of Cholesterol and Coronaries: From Plaques to Genes to Statins

Joseph L. Goldstein* and Michael S. Brown*  
1Department 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 isolated</td>
</tr>
<tr>
<td>1913</td>
<td>High cholesterol was found</td>
</tr>
<tr>
<td>1919</td>
<td>Heart attacks were linked to cholesterol</td>
</tr>
<tr>
<td>1933</td>
<td>Feedback mechanisms were discovered</td>
</tr>
<tr>
<td>1938</td>
<td>Familial hypercholesterolemia was described</td>
</tr>
<tr>
<td>1950</td>
<td>Cholesterol was linked to heart disease</td>
</tr>
<tr>
<td>1951</td>
<td>High-fat diet was linked to heart disease</td>
</tr>
<tr>
<td>1953</td>
<td>Risk factors were identified</td>
</tr>
<tr>
<td>1955</td>
<td>LDL deficiency was discovered</td>
</tr>
<tr>
<td>1973</td>
<td>LDL receptors were identified</td>
</tr>
<tr>
<td>1976</td>
<td>HMG CoA reductase was discovered</td>
</tr>
<tr>
<td>1981</td>
<td>Statins were introduced</td>
</tr>
<tr>
<td>1987</td>
<td>First statin was released</td>
</tr>
<tr>
<td>1994</td>
<td>Statins became widely used</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>

Acetyl CoA
HMG CoA
Reductase
Mevalonate
Cholesterol
A: LDL-R pathway in absence of PCSK9
B: Intracellular PCSK9 route
C: Extracellular PCSK9 route
Mature PCSK9
LDL
apoB
Degradation
LDL-R
Endosome
Lysosome
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)
TABLE 1
Simon Broome criteria for diagnostics of familial hypercholesterolemia

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</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>b</td>
<td>Tendinous xanthomata in the patient or a first-degree relative</td>
</tr>
<tr>
<td>c</td>
<td>DNA-based evidence of mutation in the LDLR or APOB gene</td>
</tr>
<tr>
<td>d</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>e</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>

**Widely used definition. Knowledge of family required**
## FH: Dutch Lipid Clinics Criteria

<table>
<thead>
<tr>
<th>Points</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 point</td>
<td>1st degree relative with premature cardiovascular disease or LDL-C &gt; 95th 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>
</tr>
<tr>
<td>2 points</td>
<td>1st degree relative with tendinous xanthoma or corneal arcus, or 1st degree relative child (&lt;18 yrs) with LDL-C &gt; 95th percentile, or personal history of coronary artery disease</td>
</tr>
<tr>
<td>3 points</td>
<td>LDL-C between 190 and 249 mg/dL (4.91 and 6.44 mmol/L)</td>
</tr>
<tr>
<td>4 points</td>
<td>Presence of corneal arcus in patient less than 45 yrs old</td>
</tr>
<tr>
<td>5 points</td>
<td>LDL-C between 250 and 329 mg/dL (6.46 and 8.51 mmol/L)</td>
</tr>
<tr>
<td>6 points</td>
<td>Presence of a tendon xanthoma</td>
</tr>
<tr>
<td>8 points</td>
<td>LDL-C above 330 mg/dL (8.53 mmol/L), or functional mutation in the LDLR gene</td>
</tr>
</tbody>
</table>

**Definite FH**
(= or > 8 points)

**Probable FH**
(6-7 points)

**Possible FH**
(3-5 points)

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

Gaudet D, Quebec, Canada data
LDL-C ≥ 5.0 mmol/L  
(≥ 4.0 mmol/L in <18 yo; ≥ 4.5 mmol/L in 18-40 years)

+  

Known DNA Mutation  
or  
Xanthomas

Yes  
Definite FH

No  
1st degree relative with ↑ LDL-C  
or  
1st degree relative with early onset ACVD

Yes  
Probable FH

No  
Hypercholesterolemia  
(Consider DNA testing)

* Secondary causes ruled out (nephrotic syndrome, obstructive jaundice and hypothyroidism)

Figure 1: Newly proposed Canadian definition of Familial Hypercholesterolemia (FH) based on the Simon-Broome criteria (under discussion)
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

- Monogenic cause (75%)
- Polygenic cause (13%)
- Unexplained cause (12%)

- APOE
- LDLR
- CELSR2
- APOB
- ABCG5/8
- HFE
- PCSK9
- ST3GAL4
- MYLIN
- NYNRIN
- SLC22A1

www.thelancet.com Published online February 22, 2013
FH Mutation Diagnosis Algorithm

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

BioBanking DNA
LipidSeq + MLPA

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

Christopher T. Johansen, Joseph B. Dubé, Melissa N. Loyzer, Austin MacDonald, David E. Carter, Adam D. McIntyre, Henian Cao, Jian Wang, John F. Robinson, and Robert A. Hegele

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
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 (+)

$M = \text{missense}$

$N = \text{Null}$

Missense Mutation in the LDLR Gene: A Wide Spectrum in the Severity of FH
Mathilde Varret and Jean-Pierre Rabès. ISBN 978-953-51-0790-3,
Homozygous FH

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

Exon 3 mutation

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>LDL ≥ 190 mg/dl</th>
<th>OR for CAD (95%CI)</th>
</tr>
</thead>
<tbody>
<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

Familial Hypercholesterolemia Mutation

<table>
<thead>
<tr>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
</table>

Odds Ratio for Coronary Artery Disease (95% CI)

Mean LDL

- 203 mg/dl
- 205 mg/dl

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
Familial Hypercholesterolemia: Prevalence
FH More Common than Previously Thought

<table>
<thead>
<tr>
<th>FH Heterozygote</th>
<th>FH Homozygote</th>
</tr>
</thead>
<tbody>
<tr>
<td>• 1 in 270 to 500</td>
<td>• 1 in 1 million</td>
</tr>
</tbody>
</table>

FH is One of the Most Common of Inherited Diseases

 Frequencies per 1,000 births:

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

Frequency chart shows the frequency of each condition per 1,000 births.
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
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

© 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 ASCVD, LDL-C target should be &lt;80 mg/dL*</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%

Statin
50% reduction

Double-dose statin
+6% reduction

Adjunctive agents
+15% reduction

UNMET TARGETS

Goal
<2 mmol/L
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.