“Nuts and Bolts” of Genetic Testing for Familial Hypercholesterolemia

Joshua W. Knowles, MD, PhD, Diplomate NLA for NLA Clinical Lipid Update
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Conflicts

- CMO for The FH Foundation
- www.thefhfoundation.org
Imagine we are back in 1982:
I have a test that...

- Is accurate and reliable.
- Can identify patients before problems happen.
- Has observational trials to support use.
Goal is to convince you that...

- Genetic testing for FH can be extremely useful.
- It is especially important for cascade screening due to the “overlap problem”.
- The ability to order genetic testing is important part of a lipidologist’s Dx armamentarium.
- Genetic testing is a commodity.
- Genetic testing will become more common.
- And finally...
Genetic testing is not:

Or

Or

Or
CASE PRESENTATION

Familial Hypercholesterolemia
Case 1:

- 24 yo M tennis instructor presented several hours after onset of crushing substernal CP while giving tennis lesson
- PMHx: Nephrotic syndrome
  - Medication non-compliant
- In ED initial vitals showed BP 140s, HR 80s
  - Initial ECG: no concerning ST changes
  - Initial troponin negative
  - Chest CT negative for PE or aortic pathology
- Ongoing CP -> repeat ECG -> STEMI -> Trop 42
  - Taken to cath lab
Cath and echo
Case 1:

- **PMHX:** Nephrotic syndrome (FSGS) diagnosed as a teenager
  - *No lipid panel prior to onset of renal disease*
  - Treated with statins but poor compliance
- **FHx:**
  - Mother and Father both on statins
  - Family of South African Jewish ancestry
- **Labs:** TC 776, LDL 447, TGs 479
What is the diagnosis?

- Renal consult: Nephrotic syndrome?
  - Total cholesterol > 400 mg/dl ~ 25% of the time
- Hematology consult: Hypercoaguuable state?
- Lipidologist consult: FH?

- Is there a test that can arbitrate these competing diagnoses?
GENETIC INFORMATION
Associated Genes
FH is typically caused by mutations in *LDLR*, *APOB*, and *PCSK9*.

**LDL receptor (LDLR):** Binds to Apo B on LDL particle, inducing endocytosis of LDL.

**APOB:** binds LDLR particle to receptor.

**PCSK9 enzyme:** degrades LDL receptors.
Important to know what you are looking for: Pre-test probability

<table>
<thead>
<tr>
<th>GENE</th>
<th>Chr</th>
<th># Causal Mutations</th>
<th>% of FH cases*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LDLR</strong> <em>(LDL Receptor)</em></td>
<td>Chr 19</td>
<td>&gt; 1000</td>
<td>60-80%</td>
</tr>
<tr>
<td><strong>APOB</strong> <em>(Apolipoprotein B)</em></td>
<td>Chr 2</td>
<td>Handful (esp. Arg3500Gln or R3500Q)</td>
<td>1-10%</td>
</tr>
<tr>
<td><strong>PCSK9</strong> <em>(proprotein convertase subtilisin/kexin type 9)</em></td>
<td>Chr 1</td>
<td>Handful</td>
<td>0-3%</td>
</tr>
<tr>
<td>Unknown</td>
<td></td>
<td></td>
<td>20-40%</td>
</tr>
</tbody>
</table>

* Yield for genetic testing is higher in “definite” FH (~80%) vs “probable” and “possible” FH (35-60%)
There’s an app for that!
IMPORTANCE OF GENETIC TESTING

Why not just use lipid panels?
The Overlap Problem
Collaboration Humphries, Kastelein

FH vs. Not FH LDL levels, Ages 5-15

Data on 2469 non-carriers and 825 familial mutation carriers

- 2.2mmol/l (85mg/dl)
- 4.6mmol/l (179mg/dl)
- 3.2mmol/l (124mg/dl)

False +ve = 8%
False –ve = 15%

DNA test avoids false –ve diagnosis

Gets worse with age!

Data from Starr et al 2008
DNA testing for identification of relatives

As LDL-C rises with age in non-FH, overlap increases. DNA testing gives an unambiguous result.
What is “cascade” testing?

- Cascade testing is using **family tracing** to identify and test people who are at high risk of FH.
- Cascade testing using genetic testing plus lipid testing is more **cost effective** than with a lipid panel alone.
  - Mutation +ve pts $\rightarrow$ 50% relatives are FH +ve
    - Pts with mutation have a higher rate of CHD
  - Mutation −ve pts $\rightarrow$ 25-30% relatives have ↑ cholesterol
Whether or not genetic testing is used, cascade testing is a must!

- Use a nationwide, family-based, follow-up system to enable comprehensive identification of affected people.
- Use a combination of DNA testing and LDL-C concentration measurement to identify affected relatives of index individuals with a clinical diagnosis of FH (see below).

**UK algorithm**

Diagram:

- Index individual with a clinical diagnosis of FH
  - Has an FH gene mutation been identified?
    - Yes
      - Use the mutation to identify affected relatives and not LDL-C concentrations.
    - No
      - Use the gender- and age-specific criteria for LDL-C concentration measurements provided in tables 2 and 3 to diagnose FH in affected relatives. Do not use Simon Broome LDL-C criteria for index individuals because this will result in under diagnosis.

- Include at least first- and second-degree relatives. Include third-degree relatives if possible.
- Be aware of the latest guidance on data protection.

Dutch national program has been spectacularly successful

- As of 2012: 5,151 index cases of genetically positive FH identified
- Resulted in screening of 60,000 family members
- In total 27,069 FH cases identified
  - 36% of the family members had a positive genetic test.
- Costs for identifying 1 FH patient: 1200 euro
  - Test almost 3 family members to identify 1 positive FH mutation
- Costs effectiveness: costs per life year saved: 8700 Euro

FH is a “Winnable battle”

Cumulative event-free survival (%) in FH

Follow-up (years)

Statin treatment

No statin treatment
Cascade testing for FH has a “Tier 1” indication
CURRENT GUIDELINES

On genetic testing for FH
Guidelines from NICE (UK)

- Patients clinically diagnosed with FH should be offered a DNA test to confirm diagnosis.
- Cascade testing using DNA tests should be used in families with known mutations, to identify affected individuals.
- Cascade testing using lipid panels should be used to clinically diagnose relatives of FH pts.

Photo credit: nice.org.uk
Guidelines from CSANZ
Cardiac Society of Australia and New Zealand

- Genetic testing can provide certainty of diagnosis where confounding factors make diagnosis unclear.
- Patients requiring genetic tests should be offered genetic counseling prior to genetic analysis.
1. Genetic testing usually not needed for diagnosis, but is useful when clinical lab results are uncertain.

2. Identification of a causal mutation may provide motivation for patients to implement treatment.
In some contexts, genetic information improves outcomes: genetic exclusivity

Lipoprotein Levels at Screening and 2 Years Later in Patients With FH With and Without Cholesterol-Lowering Medication at the Time of Diagnosis*

<table>
<thead>
<tr>
<th>Receiving Cholesterol-Lowering Medication at Screening</th>
<th>No. of Patients</th>
<th>Lipoprotein</th>
<th>Mean (SD) Level, mg/dL</th>
<th>At Screening</th>
<th>2 y After Screening</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>183</td>
<td>Total cholesterol</td>
<td>298 (58)</td>
<td>230 (48)</td>
<td>-22.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Triglycerides</td>
<td>165 (88)</td>
<td>150 (79)</td>
<td>-9.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>HDL cholesterol</td>
<td>44 (11)</td>
<td>47 (12)</td>
<td>+6.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>LDL cholesterol</td>
<td>219 (58)</td>
<td>153 (58)</td>
<td>-30.1</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>118</td>
<td>Total cholesterol</td>
<td>274 (64)</td>
<td>249 (63)</td>
<td>-9.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Triglycerides</td>
<td>169 (36)</td>
<td>157 (117)</td>
<td>-7.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>HDL cholesterol</td>
<td>45 (12)</td>
<td>46 (11)</td>
<td>+2.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>LDL cholesterol</td>
<td>195 (60)</td>
<td>175 (56)</td>
<td>-10.3</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: FH, familial hypercholesterolemia; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

SI conversion factors: To convert lipoprotein levels to millimoles per liter, multiply by 0.0259 for cholesterol and 0.0113 for triglycerides.

*P value (between levels at screening and after 2 years) was calculated by means of a paired t test. For all comparisons, P < .001.

were not receiving cholesterol-lowering medication.

**Results:** The overall percentage of treated patients had risen from 37.6% at screening to 92.5% 1 year later and then decreased to 85.9% 2 years after screening. During follow-up, 6.4% of patients discontinued their medically accepted goals of treatment. This underscores the fact that additional education is required to improve the treatment of individuals with familial hypercholesterolemia.

*Arch Intern Med.* 2003;163:65-68
ORDERING THE TESTS

What to expect
For people that order advanced lipid tests, ordering genetic tests is similar...

**Stanford lab:** All documents after this sheet should be sent to the reference lab (i.e. GeneDx, Ambry, etc.) with the sample.

- Provider information
- Clinic notes
- Insurance information
- Payment information
  - not covered by insurance
Genetic counseling

- Pre-test genetic counseling:
  - Benefits of the test
  - Psychological assessment
  - Limitations of the test (yield of testing, chance of finding a “variant of uncertain significance”, etc)
  - Impact on other family members
  - Implications for insurance
  - Implications for family planning

- Post-test genetic counseling:
  - Test Results
  - Psychological assessment
  - Application of the Information
TESTING INFORMATION

Costs of tests, Laboratory locations, Techniques Used
Overview

- **Many** laboratories around the world test for FH.
- Not all companies test *LDLR, APOB, PCSK9*
  - Many simply look at *LDLR*
  - Some laboratories offer different combinations of the tests
- Some patients may require more than one test.
  - Reflex testing is possible (this means that if one test is unrevealing, a second test is automatically performed).
- The actual technology currently used for most FH testing is not new.
  - But technologies are rapidly evolving
- **Turnaround time** is 1-2 months.
### Genetic testing labs and cost for the index case

<table>
<thead>
<tr>
<th>Gene</th>
<th>% of FH</th>
<th># mutations / cost</th>
<th>US Labs</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDLR</td>
<td>60-80%</td>
<td>&gt;1000 mutations → EXPENSIVE</td>
<td>7 in USA: (20 International)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Whole gene analysis</td>
<td>• Ambry Genetics (CA)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Deletion/Duplication test</td>
<td>• Correlagen Diagnostics (MA)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• ~$1300</td>
<td>• Athena Diagnostics (MA)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Mayo Clinic (MN)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Baylor College of Medicine (TX)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Progenika (MA)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Prevention Genetics (WI)</td>
</tr>
<tr>
<td>APOB</td>
<td>1-10%</td>
<td>1 common mutation → CHEAP</td>
<td>2 in USA: (6 International)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Targeted mutation analysis</td>
<td>• Ambry Genetics (CA)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• ~$400</td>
<td>• ARUP Laboratories (UT)</td>
</tr>
<tr>
<td>PCSK9</td>
<td>&lt;5%</td>
<td>1 common mutation → CHEAP</td>
<td>1 in USA: (2 International)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Targeted mutation analysis</td>
<td>• Ambry Genetics (CA)</td>
</tr>
</tbody>
</table>

**NOTE:** Subsequent testing in family members is inexpensive as causal variant already identified (~$250-$400)
There is no *a priori* reason FH genetic testing is so expensive

- Technology is not a major barrier
  - PCR based
- Some of this is a question of volume and competition

Strachan and Read, *Human Molecular Genetics*, 4th edition
Cost of genome sequencing
2013 Ferrari 458 Spider

Request More Info

First Name*
Last Name*
Preferred Contact*
Email*
Home Phone
Comments

Price $0.40!
Genetics 101

- Mutations come in lots of flavors that affect protein function
  - Promoter
  - Splice site
  - Nonsense
  - Missense
  - Duplication/deletion

Strachan and Read, Human Molecular Genetics, 4th edition
Basic Overview of Techniques

- Entire Coding Region Sequence Analysis
- Deletion/Duplication Analysis
- Targeted Mutation Analysis
Entire Coding Region Sequence Analysis

- Determines the order of DNA nucleotides in all the exons and splice sites of the gene (regions of the DNA that get translated into proteins).
- More expensive and slower than sequencing a single mutation site, but more thorough.
- Recommended for genes with many pathogenic mutations (LDLR).
  - Sequence LDLR exons 1-18 plus at least 20 base pairs into the 5’ and 3’ ends of all introns (for splice sites)

Mechanisms of Disease: genetic causes of familial hypercholesterolemia
Anne K Soutar and Rossi P Naoumova
Nature Clinical Practice Cardiovascular Medicine (2007)
Deletion/Duplication Analysis

- Detects regions of the gene that have either been copied more than once into the genome, or have been left out entirely.
- This method should be used for genes that have high nucleotide repetition (LDLR).
- Deletion/duplication is usually due to unequal crossing over during meiosis.

![Diagram of unequal crossing over](image)
Targeted Mutation Analysis

- Determines the **order of DNA nucleotides** in one **specific site** in the gene.
- **Cheaper and quicker** than sequencing the whole gene.
- Recommended for genes in which only a few common mutations are known (like APOB).
  - For instance, standard would be to sequence exon 26 of APOB containing the known FH causing mutations (only ~ 700 bp)
- Recommended when testing family members of a proband with a known mutation.
### Gene Sequence & Deletion/Duplication Analysis of LDLR, Gene Sequence Analysis of PCSK9 and Partial Gene Analysis of APOB

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutation/Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDLR FULL GENE</td>
<td>Mutation: c.654_666delTGG</td>
</tr>
<tr>
<td></td>
<td>Variants of Unknown Significance: None Detected</td>
</tr>
<tr>
<td>LDLR DEL/DUP</td>
<td>Gross Deletion(s)/Duplication(s): None Detected</td>
</tr>
<tr>
<td>APOB PARTIAL GENE</td>
<td>Mutation(s): None Detected</td>
</tr>
<tr>
<td></td>
<td>Variants of Unknown Significance: None Detected</td>
</tr>
<tr>
<td>PCSK9 FULL GENE</td>
<td>Variant of Unknown Significance: c.63_65dupGCT</td>
</tr>
</tbody>
</table>
Interpretation of results

This patient is heterozygous for the c.654_656delTGG mutation in the LDLR gene. This result is consistent with a diagnosis of familial hypercholesterolemia (FH).

This patient is also heterozygous for the c.63_65dupGCT variant in the PCSK9 gene which may or may not be contributing to clinical symptoms.

The c.654_656delTGG mutation, located in exon 4 of the LDLR gene, results from an in-frame deletion of 3 nucleotides between positions 654 and 656, causing a deletion of the glycine residue at codon 197, located in the LDLR ligand binding domain. This mutation is classified as a class II mutation, which results in impaired intracellular transport and processing of the LDL receptor protein between the endoplasmic reticulum and Golgi complex. This mutation appears to have a founder effect, originating within a Lithuanian Ashkenazi Jewish population, which later immigrated to South Africa, Great Britain, Australia, North America and Israel. The prevalence of this mutation is as high as 35.2% (25/71) in a group Ashkenazi-Israeli patients affected with familial hypercholesterolemia (Meiner V et al. Am J Hum Genet. 1991;49(2):443-449).
How do they come up with their interpretation?

- Each company has a proprietary database of variants from the literature and their own historical tests
- Variants are “graded” by ACMG recommendations
  - Disease causing
    - Well known founder mutations seen in multiple families
    - Tend to lead to very abnormal protein
  - Likely disease causing
    - Not quite as much support in segregation analysis
    - Less data but of the type expected to cause disease
  - Uncertain significance
    - VUS (variants of unknown significance)
    - Minor changes in protein, maybe “novel”
  - Likely benign or benign
    - Seen in normal people
Interpretation of results can require some expertise

This patient is heterozygous for the c.654_656delTGG mutation in the LDLR gene. This result is consistent with a diagnosis of familial hypercholesterolemia (FH).

This patient is also heterozygous for the c.63_65dupGCT variant in the PCSK9 gene, which may or may not be contributing to his clinical symptoms.

- This variant has been found in lots of presumably health people so it is not related to disease
- In other words, this patient is HeFH
- The *LDLR* mutation can be used to screen family members
Implications for insurance

- Under the **ACA**, insurance companies will be prohibited from denying coverage to patients or charging higher premiums for pre-existing conditions.
- Under **GINA**, employers are barred from using genetic information for all decisions regarding employment (hiring, firing, promotions, etc).
FH Foundation and Registry

CASCADe FH REGISTRY

CAscade SCreening for Awareness and DEtection of Familial Hypercholesterolemia
Summary

- FH is a serious cause of morbidity and mortality that is vastly under diagnosed and treated.
- Genetic testing and use of cascade testing can help identify more affected individuals.
- Genetic testing is available at labs around the world, and is proven to be cost effective.
- FH is a very treatable disease, but only if diagnosed early.