Clinical Considerations Related to LDL Receptor Dysfunction

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• Editorial—Merck
Is this a rare bird?
Is this a rare bird?

Not if you live in Florida.
Outline

• Diagnosis of familial hypercholesterolemia
• Factors modifying cardiovascular risk in FH
• Utility of genetic testing in FH
Why Focus on Familial Hypercholesterolemia

• Familial hypercholesterolemia (FH) is an inherited genetic disorder causing high cholesterol concentrations and increased risk of premature cardiovascular disease.
• Lifetime exposure to high LDL levels, essentially from birth
• Untreated, FH leads to substantial CVD risk in men and women, with early onset of cardiovascular disease
• Not rare but underdiagnosed
• Treatable but undertreated
• Early diagnosis and treatment eliminate the excess CVD risk
Case 1

- 63 year woman found to have elevated cholesterol about 30 years ago with baseline cholesterol about 385
- Took medications for a long time but later developed muscle problems
- No symptoms or history of cardiovascular disease
- History of obesity, hypertension and some anxiety
- Taking Bystolic, Zoloft, Nexium, nortriptylline, amlodipine
- Retired first grade teacher
- Smoked ½ pack per day age 21 to 31
- One serving of beer or wine daily
Family History

- Mother had heart attacks in her 50’s and died at 60 of MI
- Father had heart valve problems and died at 70
- There is longevity in the father’s family
- Maternal grandmother died at 70 of MI
- Maternal grandfather died at 60—sudden death
- Mother’s brother’s died in 40’s of heart problems
- Sister died at 82 of Alzheimer’s
- Brother died at 61 of colon cancer; stent at 55, hyperlipidemia, diabetes
- Sister’s children: 2 out of 4 have high cholesterol
- Daughter 33: hyperlipidemia, on medication
- Granddaughter 12 and grandson 8—lipids not yet tested
Physical Examination

- BMI 32.3
- Blood pressure 161/80
- Obese woman
- Bilateral arcus corneae upper ring
- Bilateral Achilles tendon xanthomas
Lipids

• Off medication
  • Cholesterol 489
  • Triglycerides 265
  • HDL cholesterol 54
  • LDL cholesterol 382

• Rosuvastatin 10 mg and ezetimibe 10 mg daily
  • Cholesterol 223
  • Triglycerides 160
  • HDL cholesterol 65
  • LDL cholesterol 126
Case 1: Genetic Analysis

LDLR Gene Mutation: p.D221G

“The p.D221G pathogenic mutation (also known as c.662A>G), located in coding exon 4 of the LDLR gene, results from an A to G substitution at nucleotide position 662. The aspartic acid at codon 221 is replaced by glycine, an amino acid with some similar properties. This mutation was originally described in an Italian American with homozygous familial hypercholesterolemia along with another pathogenic allele. This patient's LDLR receptor activity was less than 2% of wild type activity (Hobbs HH, Hum. Mutat. 1992; 1(6):445-66). In addition, this variant was further observed in 79 families and a total of 165 individuals with clinically diagnosed heterozygous familial hypercholesterolemia, which accounted for approximately 7.5% of unrelated individuals in the study (Bertolini S, Atherosclerosis 2013 Apr; 227(2):342-8). In vitro analysis demonstrate that the amount of mature protein is significantly reduced from that of wildtype, remaining in the endoplasmic reticulum (Li et al. Biochemistry 2002 Apr;41(15):4921-8). Two additional probably damaging alterations have also been described in the same codon, p.D221N and p.D221Y. Based on the supporting evidence, p.D221G is interpreted as a disease-causing mutation.”
Definition of Familial Hypercholesterolemia

• Autosomal co-dominant high LDL
  • Most families have only heterozygotes
• Gene dosage effect
  • Homozygotes (or compound heterozygotes) have much higher LDL-C and much earlier CAD onset (childhood and adolescence) than heterozygotes
• Autosomal recessive hypercholesterolemia – very rare, homozygotes for LDL receptor adaptor protein
• FH is not usually associated with extreme hypertriglyceridemia
Prevalence of FH

- Common “single gene” disease
- Heterozygous FH (TC ≈ 300-550 mg/dL)
  - 1 in 200 to 500 people
  - 1 in 100 in French Canadian, S. African, others
- Homozygous FH (TC ≈ 600-1000+ mg/dL)
  - 1 in 250,000 to 1 million people (more common in some groups)
- Over 12 million FH patients worldwide
- In the United States, estimated 620,000 people with FH

Populations with High Prevalence of FH due to Founder Effect

- French Canadian (1 in 100-270)
- Christian Lebanese (1 in 100)
- Several South African populations:
  - Dutch Afrikaner (1 in 100)
  - Ashkenazi Jewish (1 in 100)
  - South Asian Indian
FH Prevalence Based on Recent Genetic Studies

- Historically HoFH 1 in 1 million and HeFH 1 in 500
- Prevalence using genetic testing in a central laboratory in the Netherlands
  - HoFH estimated prevalence 1 in 300,000
- In Denmark, genetic testing of Dutch Lipid Clinic defined cases, HeFH 1 in 200

FH Increases Risk of Premature CVD

• Mean age of onset of cardiovascular events in men with FH is early 40s; women early 50s

• Although <5% of acute MIs occur in persons ≤40 yrs of age, the presence of the familial hypercholesterolemia phenotype is associated with a 20-fold increase in risk of MI by age 40
Non-Fatal CAD in FH (Utah) vs. General U.S. Population

Hopkins PN, et al. Am J Cardiol 2001; 87:547 and unpublished observations
Mutations

- **LDLR**
  - Missense, nonsense, insertions, deletions spread throughout *LDLR* affecting number and function

- **ApoB**
  - Most common is the apoB3500, which affects binding of LDL to the LDL receptor

- **PCSK9**
  - Gain of function leads to increased PCSK9 causing increased degradation of LDL receptors

- **LDLRAP1**
  - facilitates the interaction between the LDL receptor and the cell machinery regulating the endocytic process, LDLR-LDL complex internalization impaired
Diagnosis of FH

- Suspect FH at these LDL cholesterol levels:
  - LDL-C \( \geq 250 \) mg/dL in a patient aged 30 or more
  - LDL-C \( \geq 220 \) mg/dL for patients aged 20 to 29
  - LDL-C \( \geq 190 \) mg/dL in patients under age 20
- Obtain further family history
- Rule out secondary causes: hypothyroidism, nephrotic syndrome

## Useful LDL-C Cutpoints for FH Diagnosis

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
<th>LDL-C (mg/dL) cutpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>age &lt;20</td>
</tr>
<tr>
<td>1</td>
<td>General population 95\textsuperscript{th} percentile</td>
<td>130</td>
</tr>
<tr>
<td>2</td>
<td>80% have FH in first-degree relatives</td>
<td>150</td>
</tr>
<tr>
<td>3</td>
<td>80% have FH in general population</td>
<td>190</td>
</tr>
<tr>
<td>4</td>
<td>99% have FH in general population</td>
<td>220</td>
</tr>
<tr>
<td>5</td>
<td>99.9% have FH in general population</td>
<td>240</td>
</tr>
</tbody>
</table>

Diagnosis—Physical Signs

• Absence of physical signs does not exclude possibility of FH

• Presence of tendon xanthoma – almost assures diagnosis but insensitive (differential includes beta-sitosterolemia)

• Arcus, xanthelasma, tuberous xanthomas not generally useful.

• Eruptive xanthomas are not part of FH
Heterozygous FH: Physical Findings

Not all patients have physical findings

Corneal arcus: not specific for FH but suspect FH with both upper and lower arcs before age 40
Xanthelasma can be seen in FH but they can also be seen in other disorders.
Prevalence (%) in 346 Utah FH Heterozygotes

Hopkins PN. Current Treatment Options in Cardiovascular Medicine 2002; 4:121
Recognized FH Diagnostic Criteria

• Best characterized clinical diagnostic tools:
  – US MEDPED Program
  – Simon-Broome Registry Group (UK)
  – Dutch Lipid Clinic Network

• DNA evidence:
  A mutation is not always found—estimates anywhere from 20 to 80% of the time
  Useful in patients where there is a known mutation in the family
  Cost is decreasing but still an issue in US with insurance coverage
Problems with Diagnostic Criteria

• They are old
  • Incidence of tendon xanthomas may be less with many people on statins
  • Many clinicians do not try to find or cannot recognize tendon xanthomas
  • Dutch Lipid Clinic criteria are useful but are being used to deny coverage of PCSK9 Mabs (lack of knowing untreated LDL-C, absence of tendon xanthomas can make it hard to get a score greater than 8)
# AHA Statement: the agenda for familial hypercholesterolemia

<table>
<thead>
<tr>
<th><strong>ICD-10 Category</strong></th>
<th><strong>Clinical Criteria</strong></th>
<th><strong>When Genetic Testing Performed</strong></th>
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| Heterozygous Familial Hypercholesterolemia | LDL-C ≥ 160 mg/dl (4 mmol/L) for children  
LDL-C ≥ 190 mg/dl (5 mmol/L) for adults  
**And** with one first degree relative similarly affected  
--or with premature CAD  
--or with positive genetic testing for an LDL-C raising gene defect (LDL receptor, apo B or PCSK9) | Presence of one abnormal LDL-C raising (LDL receptor, apo B or PCSK9) gene defect  
Diagnosed as heterozygous FH if gene raising defect positive and LDL-C < 160 mg/dl (4 mmol/L)  
Occasional heterozygotes will have LDL-C > 400 mg/dl (10 mmol/L), they should be treated similarly to homozygotes  
Presence of both abnormal LDL-C raising (LDL receptor, apo B or PCSK9) gene defect(s) and LDL-C lowering gene defect(s) with LDL-C < 160 mg/dl (4 mmol/L) |

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<td>Homozygous Familial Hypercholesterolemia</td>
<td>LDL-C &gt; 400 mg/dl (10 mmol/L) And one or both parents: a) having clinically diagnosed familial hypercholesterolemia, b) positive genetic testing for an LDL-C raising (LDL receptor, apo B or PCSK9) gene defect or c) autosomal recessive FH</td>
<td>Presence of two identical (true homozygous FH) or non-identical (compound heterozygous FH) abnormal LDL-C raising (LDL receptor, apo B or PCSK9) gene defects; includes the rare autosomal recessive type Occasional homozygotes have LDL-C &lt; 400 mg/dl (10 mmol/L) Sitosterolemia, obstructive liver disease and other causes of secondary hypercholesterolemia should be excluded</td>
</tr>
</tbody>
</table>

If LDL-C > 560 mg/dl (14 mmol/L) or LDL-C > 400 mg/dl (10 mmol/L) with 1) aortic valve disease or 2) xanthomata at less than 20 years of age, homozygous FH highly likely

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<td>Family History of Familial Hypercholesterolemia</td>
<td>LDL-C level not a criteria; presence of a first degree relative with confirmed FH</td>
<td>Genetic testing not performed</td>
</tr>
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</table>

Comments on Case 1

• She meets Dutch Lipid clinic criteria and others: LDL level, family history, tendon xanthomas

• Additional CAD risk factors: brief smoking history, obesity, hypertension

• But treated for a long time and quite responsive to therapy
Case 2

- 60 year old woman with a long history of hypercholesterolemia
- Diagnosed in her early 20’s, noted at that time to have tendon xanthomas, not recognized as such. One resected and on pathology showed cholesterol deposits. Lipid panel done and total cholesterol 617
- Self-referred to Lipid Research Center (in 1973)
- On lipid-lowering therapy most of the time since then
- On a statin since 1987
- Angina symptoms 2014. CABG July 2014—LIMA to LAD, radial artery to first diagonal
Case 2 Continued

• Medical history includes migraines; melanoma resection 2007, one normal pregnancy and delivery

• Social history: never smoked, no alcohol, divorced, stressful job as a facilities manager

• Medications: aspirin, atorvastatin 80 mg, ezetimibe 10 mg, topiramate, vitamin D

• Lipid medication history: tolerance problems with rosvastatin, colesevelam. Became very ill with niacin.
Family history

• Mother died at 39 of MI
• Father remarried and nothing known about maternal side of the family
• Father died from lung cancer at 59—no known CAD
• Paternal grandparents had cancer and diabetes
• Sister 65, hypertension and DM; MI and CABG 2012
• Brother 59; hyperlipidemia CABG 2012
• Brother 62, DM, high triglycerides
• Daughter 30, baseline cholesterol 600
• Nephew hyperlipidemia
Physical Examination

- Height 5’ 2” Weight 125 lb BMI 22.9 B/P 108/71
- WD WN woman
- Bilateral full arcus corneae
- Bilateral Achilles tendon xanthomas
- Rest of exam normal
Lipids

- 1986, no medication
  - Cholesterol 676
  - Triglycerides 73
  - HDL cholesterol 45
  - LDL cholesterol 516
- 2014, atorvastatin 80 mg, ezetimibe 10 mg
  - Cholesterol 342
  - Triglycerides 117
  - HDL cholesterol 47
  - LDL cholesterol 272
Case 2: Genetic Analysis

- **LDLR gene mutation: c.1118_1121dupGTGG**
  - “The c.1118_1121dupGTGG (also known as 1121_1122insGTGG, Gly374insTrpfsX8, and G353insWfsX8) pathogenic mutation, located in coding exon 8 of the LDLR gene, results from a duplication of 4 nucleotides at position 1118, causing a translational frameshift with a predicted alternate stop codon (p.Y375Wfs*7). This duplication has been reported in several individuals presenting with familial hypercholesterolemia.”
  

- **ApoB gene mutation: pR3572Q**
  - “The p.R3527Q pathogenic mutation (also known as c.10580G>A and p.R2500Q), located in coding exon 26 of the APOB gene, results from a G to A substitution at nucleotide position 10580. The arginine at codon 3527 is replaced by glutamine. This alteration was described in 6 probands with familial hypercholesterolemia (FH) and found to segregate with disease in two large families (Soria et al. Proc Natl Acad Sci USA. 1989; 86(2):587-91). This pathogenic mutation has been reported to be responsible for 2-6% of Western European FH cases.”

Homozygous familial hypercholesterolaemia: new insights and guidance for clinicians to improve detection and clinical management. A position paper from the Consensus Panel on Familial Hypercholesterolaemia of the European Atherosclerosis Society


Cuchel M et al. Eur Heart J 2014;eurheartj.ehu274
Familial Hypercholesterolemia

- Autosomal co-dominant high LDL
- Heterozygotes: untreated LDL-C 155 to 500 mg/dL
- 1 in 200 to 500 people, early CAD
- Homozygotes often have untreated LDL-C >500 mg/dL
- CAD onset in childhood and adolescence
- Insufficient response to usual lipid lowering medication, even in combination
- Pre 1990: average age at death 18.4 years, age at first cardiac event 12.8
- Post 1990: average age at death 32.9 years, age first event 28.3 years

Gene Loci of Familial Hypercholesterolemia

Heterozygotes = one abnormal allele

Homozygotes = same mutation in both alleles of the same gene

Compound heterozygotes = different mutations in each allele of the same gene

Double heterozygotes = mutations in two different genes affecting LDL receptor function

Cuchel M et al. Eur Heart J 2014;eurheartj.ehu274
Criteria for the Diagnosis of Homozygous Familial Hypercholesterolemia

Genetic confirmation of two mutant alleles at the \textit{LDLR}, \textit{APOB}, \textit{PCSK9}, or \textit{LDLRAP1} gene locus

\textbf{OR}

An untreated LDL-C $>13$ mmol/L (500 mg/dL) or treated LDL-C $\geq 8$ mmol/L (300 mg/dL)\footnote{Lower LDL-C levels, especially in children or in treated patients, do not exclude HoFH} together with either:
- Cutaneous or tendon xanthoma before age 10 years
- Untreated elevated LDL-C levels consistent with heterozygous FH in both parents (except in ARH)

Cuchel M et al. Eur Heart J 2014;eurheartj.ehu274
Homozygous Familial Hypercholesterolemia:
Phenotypic Variability in Homozygous Familial Hypercholesterolemia

Cuchel M et al. Eur Heart J 2014;eurheartj.ehu274
Low-density lipoprotein-cholesterol levels in homozygous autosomal dominant hypercholesterolemia patients before and after lipid lowering therapy. Plus indicates patients with two null alleles.

LLT = lipid lowering therapy

5 mmol/L = 193 mg/dl
Case 2

- After CABG further discussion of possible therapies
- Start LDL-apheresis every one to two weeks
Comments on Case 2

- LDL cholesterol levels and early onset of tendon xanthomas (very symptomatic by age 19) suggestive
- No clear paternal history of FH
- Treatment for years prior to statins and then on statin as soon as available
- Normotensive, never smoker, always careful with diet
- Likely a somewhat intermediate phenotype given her double heterozygous status
- Her daughter could have the same phenotype since apoB and LDL receptor genes are on separate chromosomes
Risk of Cardiovascular Disease in FH

• Genetic dose effect: homozygosity worse
• Modifying genes
• LDL levels drive risk
• Having FH matters due to lifelong exposure
• Age X LDL = cholesterol-years, an exposure effect
  (Leren TP, Berge KE. PLoS ONE 2011; 6:e16721; doi:10.1371/journal.pone.0016721)
• Risk factors modify the threshold for CVD: age, gender, blood pressure, smoking, diabetes, obesity, lipoprotein (a)

Nordestgaard B G et al. Eur Heart J 2013;eurheartj.eht273
Case 3

- 20 year old man with cholesterol testing done at age 10 due to family history of CAD
- Cholesterol 420 at age 10 and he was started on medication
- Currently taking simvastatin 80 mg and ezetimibe 10 mg daily
- College sophomore, misses at least 2 to 3 doses of medication per week
- Diet is not too bad, does some exercise
- No tobacco use, occasional alcohol
Family History

• Father died of MI at age 38, had been on statin and blood pressure medication, some cigarette use formerly

• Paternal grandfather died of MI at 30

• Mother 39, obese, cholesterol okay

• Maternal grandfather 64, living, diabetes, hypertension, hypercholesterolemia

• Maternal grandmother alive and well at 62

• Half-siblings (same father): sister 23, high LDL and statin started after puberty; half-brother high triglycerides
Physical Exam

• BMI 28.9
• Blood pressure 122/80
• No arcus, no tendon xanthomas
Lipids

• Initial visit
  • Cholesterol 404
  • Triglycerides 160
  • HDL cholesterol 43
  • LDL cholesterol 329
  • Lipoprotein (a) 17 (<30)

• Changed to atorvastatin 80 mg and ezetimibe 10 mg—did not get follow up lab work done

• During high school LDL cholesterol was 135
Case 3: Genetic Testing

• LDLR gene mutation: c.230delG

“The c.230delG pathogenic mutation, located in coding exon 3 of the LDLR gene, results from a deletion of one nucleotide at position 230, causing a translational frameshift with a predicted alternate stop codon. This mutation was first identified in a study of 200 unrelated familial hypercholesterolemia (FH) patients of Japanese origin.

(Yu et al 2002, Atherosclerosis; 165(2):355-42)

This nucleotide position is well conserved in available vertebrate species. Since frameshifts are typically deleterious in nature, this alteration is interpreted as a disease-causing mutation.”

Measure Lipids in Children

- Universally at ages 9-11 years and 17-21 years
- Can measure total cholesterol and HDL-cholesterol or fasting lipid profile
- Measure at > 2 years of age as part of CVD risk assessment
  - e.g. positive family history, other CVD risk factor, high risk condition, obesity

Pediatric Screening Rationale

• Age 9 to 11 identifies individuals at the potential onset of advanced atherosclerosis.

• This age has the best discrimination between those with and without inherited dyslipidemias and avoids confounding by pubertal related changes in lipid levels.

• Identifying dyslipidemia in those with other major CVD risk factors is critical for risk stratification.
Pediatric Evaluation

• LDL-C $\geq 160$ mg/dl consistent with FH
• Non HDL-C $\geq 145$ mg/dl in screening requires a full lipid panel
• History, PE and labs to exclude secondary hypercholesterolemia (hypothyroidism, nephrotic syndrome)
• Repeat lipid profile after diet intervention to confirm diagnosis
• Presence of additional risk factors intensifies risk
Comments on Case 3

• Screening at age 10 led to early diagnosis and treatment

• Starting early helps

• Adolescence can be challenging with regard to medication adherence—continues into college and often after

• The severity of his initial levels would seem to correlate with the type of mutation—but this is not always the case
Clinical Utility of Genetic Testing in FH

- Higher risk with a mutation at same LDL cholesterol levels
- Helpful in diagnosis in relatives—prevent false negatives on the basis of LDL levels
- Possibly improvement in adherence to therapy
- But a mutation is not found in all cases
- Multiple SNPs can cause FH range LDL-C levels
Relationship Between Cumulative LDL-C Exposure and Age

Overlap of Clinical and Mutation Diagnosis of Heterozygous Familial Hypercholesterolemia

Patient: treat LDL
Family: monitor LDL and consider treatment

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

Patient: monitor LDL and consider treatment
Family: monitor LDL and consider treatment

Nordestgaard B G et al. Eur Heart J 2013;34:3478-3490
Summary

• Diagnosis of FH can be challenging
• Patients with mutations in two alleles may not always fit the classic picture of homozygous FH
• Early screening is important
• Multiple factors can affect the development of cardiovascular disease
• Genetic testing can offer valuable insights but can be challenging to accomplish
Thank you for your attention!