THE SEVERE HYPERCHOLESTEROLEMIA PHENOTYPE: GENES AND BEYOND.

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ABSTRACT

Genetic disorders resulting in hypercholesterolemia comprise autosomal dominant hypercholesterolemia (ADH), as well as other rare conditions such as autosomal recessive hypercholesterolemia. All of these disorders cause profound elevations in LDL cholesterol (LDL-C) and, as a result, greatly increased risk of incident cardiovascular disease (CVD). Genetic loci involved in ADH include *LDLR* (which codes for the LDL receptor, whose dysfunction causes Familial Hypercholesterolemia, or FH), *APOB* (which codes for apoB-100, the major protein component of LDL), and *PCSK9* (which codes for PCSK9, the circulatory protein that terminates the lifecycle of the LDLR). Importantly, a large percentage of people with a severe hypercholesterolemic phenotype do not possess a readily identifiable gene defect. Thus, identification of a specific genetic substrate is not a necessary condition for the diagnosis of a genetic hypercholesterolemia. Several formal diagnostic criteria exist for familial hypercholesterolemia; they involve lipid levels, family history, personal history, physical exam findings, and genetic testing. As all individuals with severe hypercholesterolemia are at high risk for CVD, treatment is centered on dietary and lifestyle modifications and early institution of lipid-lowering pharmacotherapy. Treatment should initially be statin-based, but most patients require adjunctive medications such as ezetimibe, niacin, and fibrates. Few patients with extreme and unresponsive elevations in LDL will need invasive therapies such as lipoprotein apheresis. Novel agents for the treatment of severe hypercholesterolemia include microsomal triglyceride transfer protein (MTP) inhibitors, apoB-100 antisense oligonucleotides (ASOs), and monoclonal antibodies against PCSK9. The FDA has just approved two PCSK9 inhibitors, alirocumab and evolocumab, for use in addition to diet and maximally tolerated statin therapy in adult patients with heterozygous familial hypercholesterolemia or those with clinical CAD who require additional LDL-C lowering. For complete coverage of this and all related areas of endocrinology, please see our free web-book, www.Endotext.org.

INTRODUCTION

Genetic disorders resulting in hypercholesterolemia consist of autosomal dominant hypercholesterolemia and rarer conditions inherited as recessive trait. The term "autosomal dominant hypercholesterolemia" (ADH) refers to those patients with dominantly inherited severe hypercholesterolemia (LDL-C greater than 190 mg/dL), who likely carry mutations in genes regulating serum LDL levels. ADH includes "classic" familial hypercholesterolemia (FH), which is a codominant disorder involving aberrations in the LDL receptor (LDLR), as well as other codominant forms of "nonclassical" FH, which involve defects in two other genes that regulate plasma clearance of LDL, *APOB* (which synthesizes the main protein of LDL) and *PCSK9* (which synthesizes a circulatory protein that limits LDLR lifespan).[1] All forms of ADH result in very high levels of LDL-C and increase the risk of early and accelerated coronary artery disease (CAD).[2] While many individuals with severe hypercholesterolemia do not have identifiable defects in any of the aforementioned genes (pointing toward a polygenic, epigenetic, or non-genetic etiology), the present chapter will largely focus on ADH involving these three genetic loci. Also important to remember, however, is the inherited severe hypercholesterolemia that goes by the name of "autosomal recessive hypercholesterolemia", or ARH. It is due to mutations in an adaptor protein, LDLR-RAP, which normally positions the LDLR on the sinusoidal side of the hepatocyte.[3] Although in principle it may seem easy to distinguish ARH from ADH (with the parents of ARH patients being apparently unaffected by elevated LDL), in practice an incomplete or missing family history will make a patient with ARH look identical to one with ADH.

GENETICS

The LDL receptor is an 893-amino acid cell surface glycoprotein that binds and internalizes LDL particles, primarily in the liver. Mutations in LDLR (i.e., "classic" FH) give rise to nearly 90% of cases of clinical FH.[4] Over 1500 such mutations in LDLR have been identified, including deletions, insertions, missense, and nonsense mutations.[2,4] FH patients can be homozygous (as rare as one in a million), carrying mutations in both alleles encoding for LDLR, or heterozygous (as frequent as one in 200), possessing mutations in only one allele.[5] Homozygous FH (HoFH) should be suspected when LDL-C exceeds 400 mg/dL, whereas heterozygous FH (HeFH) should be suspected when LDL-C is greater than 190 mg/dL in adults and 160 mg/dL in children.[2] Patients with HoFH can be true FH homozygotes, with two identical mutations in each allele, versus compound heterozygotes, with a different mutation in each allele. In addition, FH can result in elevated levels of lipoprotein (a) (Lp[a]) through an unclear mechanism, not necessarily linked to the dysfunctional LDLR pathway.[6-9] Elevated Lp(a) contributes to increased risk of incident CAD. There is evidence to suggest that the actual prevalence of FH is much higher than has been previously reported. This may be due, at least in part, to "founder effects" in some populations. Founder effects influencing the type and frequency of mutations causing FH are seen among Afrikaaners, French Canadians, Ashkenazi Jews, Christian Lebanese, and some Tunisian groups. Slimane et al. estimated the prevalence of individuals with HoFH and HeFH in Tunisia to be 1:125,000 and 1:165, respectively.[10]

"Nonclassical" FH, which phenotypically resembles classic FH in presentation and severity, involves dominantly inherited gene defects in *APOB* and *PCSK9*, which code for proteins that modulate ligand-LDLR interaction.[11,12] ApoB is the major protein constituent of LDL and acts as a ligand for LDLR. Mutations in ApoB (most commonly a single base change at a position near amino-acid 3,500) block the binding of LDL containing the apoB-100 to LDLR, resulting in severely elevated levels of LDL-C. This condition was originally defined as "familial defective APOB-100" or FDAB.[13] PCSK9, on the other hand, is a circulating protein that terminates the lifecycle of LDLR by binding to it and targeting it to lysosomal degradation. Gain-of-function mutations in PCSK9 lead to clinical FH, whereas loss-of-function mutations lead to lower LDL-C and protection from coronary atherosclerotic events.[14,15] Absence of circulating PCSK9 has been reported in a few subjects, who were reportedly healthy and had LDL levels around 20 mg/dl. This has spurred a frenzy of targeted research that has led to the development and recent FDA approval of therapeutic antibodies against PCSK9 to reduce LDL levels in individuals with CAD and inappropriate LDL levels on current standard treatment, and in FH patients. The prevalence of ADH resulting from mutations in *APOB* and *PCSK9* has been difficult to estimate, but it is agreed that these are extremely rare.

Finally, an additional ultra-rare recessive genetic disorder causing hypercholesterolemia bears mentioning. It involves a homozygous deletion mutation in the gene *CYP7A1*. This gene codes for the enzyme cholesterol 7α-hydroxylase, which catalyzes the initial step in cholesterol catabolism and bile acid synthesis. The mutation results in loss of enzymatic function and high levels of LDL-C, and was first identified relatively recently in three homozygotes within a single kindred of English and Celtic descent.[16]

An important practical point is that 30-50% of people with the FH phenotype have no readily identifiable defects in any of the genes that have been mentioned here; thus, diagnosing an individual with the FH phenotype does not necessarily means the presence of a monogenic defect in the LDLR pathway.[17] The etiology may be polygenic, due to an as-yet-unidentified genetic error or to polygenic, epigenetic, or non-genetic factors, including co-morbid and environmental modifiers. For this reason, genetic testing is not encouraged, as the absence of a mutation does not modify the cardiovascular risk faced by a patient with life-long inherited hypercholesterolemia, and does not modify therapeutic strategies.[18] On the other hand, the reduced costs and more widespread availability of genetic testing warrant performance of this test to obtain information that can help the physician fine tune genotype-phenotype correlations and to identify subjects that can be studied for the discovery of novel pathways leading to severe hypercholesterolemia.

PATHOPHYSIOLOGY

Most circulating LDL particles end up in the liver. The LDLR pathway is the predominant method for LDL uptake.[19,20] ApoB binds to a specific binding site on LDLR and the

receptor-ligand complex is subsequently internalized from clathrin-coated pits on the cell membrane. The receptor-ligand complex undergoes endocytosis and is targeted to the lysosome, where LDL is released for degradation while the LDLR is recycled back to the cell surface. PCSK9 terminates LDLR lifespan by disallowing its recycling, thus providing a physiologic mechanism of protein removal much different from, and stronger than, that caused by IDOL (inducible degrader of LDL), an E3 ubiquitin ligase.[14,21] There are other nonspecific and constitutively active pathways of LDL clearance as well.[19,22] In HeFH, though transport through the LDLR pathway is reduced by 50%, LDL-C clearance is doubled through these other, non-LDLR pathways. The same holds true for HoFH, where despite a near-absolute reduction in LDLR transport, total LDL-C clearance via non-specific pathways is increased by 4-fold.[23] Excess LDL cholesterol, which accumulates in liver cells, is then re-exported via the apoB system back into the plasma, secreted into bile unchanged or transformed into bile acids. This increased production of LDL adds to inefficient clearance via LDLR to cause elevated serum LDL levels typical of the FH phenotype.[24]

ADH can thus be further classified into subtypes 1, 2, and 3, based on which protein of the LDLR pathway is causative (Figure 1). ADH-1 comprises mutations within *LDLR,* the canonical form of Familial Hypercholesterolemia. There are five major classes of ADH-1, based on the type of mutation, include those that: inhibit synthesis of LDLR; impede exit of mature LDLR from the endoplasmic reticulum; affect the binding site of LDLR to apoB-100, preventing the ligand-receptor interaction; prevent endocytosis of the LDLR-apoB-100 complex; or inhibit recycling of LDLR to the cell surface for further rounds of lipid uptake (not shown). ADH-2 comprises mutations of *apoB* that block the association of apoB-100 to LDLR. ADH-3 is due to gain-of-function mutations of *PCSK9*, which reduce LDLR recycling and accelerate its lysosomal degradation.[12] Some authors have suggested that mutations that affect binding of apoB-100 to LDLR carry a less severe phenotype than those that affect LDLR directly, but in most cases, the two mechanisms result in a clinically indistinguishable picture.[25,26]

Figure 1

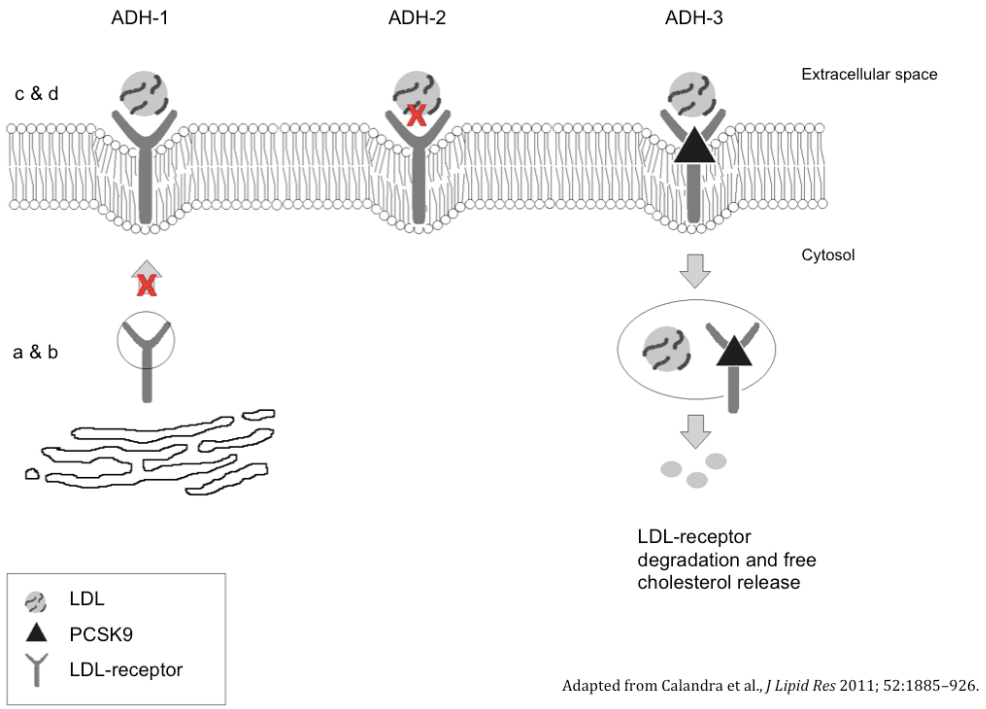

Figure 1: ADH can be classified into subtypes 1, 2, and 3, based on which protein of the LDLR pathway is affected. ADH-1 comprises mutations within LDLR. There are five major classes of ADH-1, affecting: 1) synthesis of LDLR; 2) exit of mature LDLR from the endoplasmic reticulum; 3) binding site of LDLR to apoB-100; 4) endocytosis of LDLR-apoB-100 complex; and recycling of LDLR to the cell surface (not shown). ADH-2 comprises mutations in apoB that block the association of apoB-100 to LDLR. ADH-3 is due to gain-of-function mutations of PCSK9, which reduce LDLR recycling and accelerate its lysosomal degradation

CLINICAL DIAGNOSIS

ADH, despite its different underlying gene abnormalities, leads to severe hypercholesterolemia and a distinct FH phenotype with markedly increased risk of developing CAD. HoFH, for example, can manifest as myocardial infarction and sudden cardiac death within the first decade of life, or with severe aortic stenosis (due to lipid deposition and subsequent calcification on the aortic valve).[27-29] In general, there are five major clinical criteria for diagnosing ADH: a family history of early CAD (less than age 55 in a first-degree relative in men, and less than age 65 in women), early CAD in the index case, elevated LDL (greater than 190 mg/dL), tendon xanthomas (especially in the Achilles and finger extensor tendons), and corneal arcus (which is highly specific in younger patients, but overall an insensitive finding). Mutations in any of the aforementioned genes of the LDLR pathway, when they are identified, are diagnostic. As

has been mentioned in other chapters of this text, when evaluating a patient suspected of having ADH, it is critical to rule out secondary causes of hypercholesterolemia, such as hypothyroidism, nephrotic syndrome, and liver disease. Another extremely rare cause of nonfamilial hypercholesterolemia has been described, involving autoantibodies to LDLR that inhibit receptor-mediated binding and catabolism of LDL.[30]

There are three sets of statistically validated criteria that are most commonly used in the diagnosis of FH: the Dutch Lipid Network criteria, Simon Broome Register criteria, and Make Early Diagnosis to Prevent Early Deaths (MEDPED) criteria.[31,32] These are summarized in Table 1, below:[33]

TABLE 1:

<table>
<tr><th colspan="6">MEDPED Criteria (USA)</th></tr>
<tr><td></td><td colspan="4">Total cholesterol (LDL-C) levels in mg/dL</td><td>Comments</td></tr>
<tr><td>Age</td><td>1st degree relative</td><td>2nd degree relative</td><td>3rd degree relative</td><td>General population</td><td rowspan="6">98% specificity
87% sensitivity</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td><18</td><td>220 (155)</td><td>230 (165)</td><td>240 (170)</td><td>270 (200)</td></tr>
<tr><td>20</td><td>240 (170)</td><td>250 (180)</td><td>260 (185)</td><td>290 (220)</td></tr>
<tr><td>30</td><td>270 (190)</td><td>280 (200)</td><td>290 (210)</td><td>340 (240)</td></tr>
<tr><td>>40</td><td>290 (205)</td><td>300 (215)</td><td>310 (225)</td><td>360 (260)</td></tr>
<tr><th colspan="6">Simon Broome Criteria (UK)</th></tr>
<tr><td rowspan="3">Total cholesterol (LDL-C) 290 (190) mg/dL in adults, or 260 (155) mg/dL in children</td><td rowspan="3">AND</td><td colspan="3">DNA mutation</td><td>Definite FH</td></tr>
<tr><td colspan="3">Tendon xanthomas in patient or 1st or 2nd degree relative</td><td>Probable FH</td></tr>
<tr><td colspan="3">Family history of MI at age <50 in 2nd degree relative or <60 in 1st degree relative
OR
Family history of total cholesterol >290 mg/dL in 1st or 2nd degree relative</td><td>Possible FH</td></tr>
<tr><th colspan="6">Dutch Criteria (Netherlands)</th></tr>
<tr><td>1 point</td><td colspan="4">1st degree relative with premature CVD or LDL-C >95th percentile, OR
Personal history of premature peripheral or cerebrovascular disease, OR
LDL-C 155-189 mg/dL</td><td>Definite FH (8 points or more)</td></tr>
<tr><td>2 points</td><td colspan="4">1st degree relative with tendon xanthoma or corneal arcus, OR
1st degree relative child (<18 yrs) with LDL-C > 95th percentile, OR
Personal history of CAD</td><td>Probable FH (6-7 points)</td></tr>
<tr><td>3 points</td><td colspan="4">LDL-C 190-249 mg/dL</td><td>Possible FH</td></tr>
</table>

4 points	Presence of corneal arcus in patient < 45 years old	(3-5 points)
5 points	LDL-C 250-329 mg/dL	
6 points	Presence of a tendon xanthoma	
8 points	LDL-C >330 mg/dL, OR Functional mutation of the *LDLR* gene	

Adapted from Fahed et al., *Nutrition & Metabolism* 2011, 8:23.

Unlike MEDPED criteria, which use only lipid levels, the Simon Broome and Dutch criteria also use family history, personal history, physical exam findings, and genetic testing to establish an FH diagnosis. Again, however, it should be emphasized that FH should be diagnosed phenotypically, as opposed to genetically—most FH patients are genotype-negative and do not possess a clear genetic substrate for their hyperlipidemic phenotype, but they clearly warrant aggressive intervention.

Patients with ADH often have, in addition to severely elevated LDL-C, normal triglycerides and reduced high-density lipoprotein cholesterol (HDL-C). Low and dysfunctional HDL may contribute to the increased propensity toward atherosclerosis in ADH individuals.[34] While genetic screening is not required for clinical management, lipid screening in family members should be undertaken in all individuals by age 20, starting as early as age 2.[35] Cascade screening—i.e., lipid screening of first-degree relatives of the proband—is infrequently employed, but is recommended as the most economical method of identifying new cases of ADH.[36] It is the responsibility of the examining clinician to attempt identification of other cases when making the diagnosis of ADH in any given patient.

TREATMENT

In genetic disorders causing hypercholesterolemia, aggressive lipid-lowering through lifestyle modification, pharmacologic treatment, and invasive treatments such as apheresis has been shown to decrease angiographically-apparent CAD and reduce cardiovascular events.[37-39] However, traditional risk assessment tools like the Framingham risk score do not apply to ADH patients. Recent guidelines suggest that drug therapy should be initiated when LDL-C is greater than 190 mg/dL in all patients, including children over the age of ten.[35] The National Lipid Association recommends at least 50% reduction in LDL-C with statin therapy as a starting goal, and the European Atherosclerosis Society suggests LDL-C goals of less than 135 mg/dL in pediatric patients, less than 100 mg/dL in adults, and less than 70 mg/dL in adults with known CAD or diabetes mellitus (a goal that is seldom attained in most ADH patients).[40,41]

In patients with HeFH, statins are a mainstay of treatment, despite the dearth of randomized clinical trials of statin efficacy in this special population. The major early statin trials (4S and WOSCOPS) likely had study populations that were enriched with ADH patients, given that mean baseline LDL-C ranged from 189 to 216 mg/dL.[42,43] As monotherapy, statins can reduce LDL-C up to 60%.[44] The vast majority of patients, however, require additional pharmacotherapy in the form of ezetimibe, niacin, fibrates, and bile-acid sequestrants. Combination treatment can lead to an additional 20-30%

reduction in LDL-C.[45-47] Statins may prove ineffective in the treatment of HoFH, however, because the mode of action of these drugs largely depends on the upregulation of functional LDLR in the liver. In HoFH, measurable activity of both copies of the LDL receptor is absent or greatly reduced.[27] As such, therapy for HoFH often requires invasive interventions like lipoprotein apheresis.

The FDA has approved lipoprotein apheresis for subjects with cardiovascular disease (CVD) and LDL>200 mg/dl or without CVD and LDL>300 mg/dL.[48] Recently, this threshold has been moved to 160 mg/dl, thus increasing the target population for cholesterol dialysis at a time when arrival of stronger medications is curtailing patient entry into this therapeutic program. The process, which involves removing apoB-containing lipoproteins from plasma, is usually performed every two weeks and results in a 60-70% reduction of LDL-C and Lp(a) in the immediate post-procedure period. Levels tend to revert to baseline within two weeks.

Novel agents for the treatment of severe hypercholesterolemia include PCSK9 inhibitors, microsomal triglyceride transfer protein (MTP) inhibitors, and apoB-100 antisense oligonucleotides (ASO). MTP is involved in the transfer of lipid droplets to apoB as well as assembly and secretion of apoB-containing lipoproteins in the liver and gut. MTP inhibition thus reduces production and secretion of chylomicrons and VLDL. In one study, 29 patients with HoFH were treated with the MTP inhibitor lomitapide for 26 weeks and were followed until week 78. Average LDL-C reductions were 50% (to 166 mg/dl) at week 26, 44% (to 197 mg/dl) at week 52, and 38% (to 208 mg/dl) at week 78.[49]

ASO molecules bind to specific mRNAs and target them for degradation, reducing protein synthesis in the process. Mipomersen is an ASO that binds to apoB-100 mRNA and thus prevents the formation of apoB-100. Mipomersen results in decreased synthesis of apoB-containing lipoproteins, mostly VLDL, eventually leading to a drastic reduction of LDL-C levels in plasma. In one trial, 51 patients with either genetically-defined HoFH, untreated LDL-C levels of >500 mg/dl plus xanthomas, or evidence of HeFH in both parents were randomized to placebo versus mipomersen for a treatment duration of 26 weeks. In the placebo group, baseline LDL-C was 402 mg/dl and declined to 390 mg/dl; in the treatment group, baseline LDL-C dropped from 440 mg/dl to 324 mg/dl.[50]

Subcutaneous monoclonal antibodies against PCSK9 have been studied in at least eleven clinical trials, which have shown significant reductions in LDL-C, apoB, and Lp(a) levels.[51-61] Most recently, the Food and Drug Administration has approved alirocumab and evolocumab, injectable monoclonal antibodies against PCSK9, for use in addition to diet and maximally tolerated statin therapy in adult patients with HeFH or those with clinical CAD who require additional LDL-C lowering. ODYSSEY LONG TERM was a randomized, double-bind trial involving more than 2300 patients with either HeFH, established CAD, or CAD risk factors and LDL-C greater than 70 mg/dL despite maximally tolerated statin therapy. The primary endpoint was LDL-C at 24 weeks;

alirocumab resulted in a more than 60% reduction in LDL-C compared with placebo. Post-hoc analysis revealed a statistically significant reduction in major cardiovascular events with alirocumab.[62] In another trial, RUTHERFORD-2, more than 300 patients with HeFH were randomized to either high- or low-dose evolocumab versus placebo. At both dosing regimes, evolocumab resulted in significantly reduced LDL-C at 12 weeks compared with placebo.[63]

In patients with HoFH, response to PCSK9 inhibition varies depending on the specific gene defect. In the TESLA-B trial, 49 patients with HoFH were treated with evolocumab or placebo every four weeks for 12 weeks. LDL-C in the evolocumab treatment arm was significantly reduced by almost 31% compared with placebo. In the addition to overall reduction in LDL-C, the trial investigators examined the treatment effect by *LDLR* mutation status. The researchers found that response to evolocumab aligns with the genetic cause of HoFH, with a greater reduction in LDL-C observed in subjects with two LDL receptor-defective mutations (i.e., abnormal receptor functionality in both alleles) when compared with those patients with even just one LDL receptor-negative mutation (i.e., nonexistent receptor functionality in one allele).[64]

Based on these trial results, and with an expected LDL reduction approaching 70%, most patients with ADH will, for the first time, reach levels of LDL low enough to sufficiently control the lipid component of CVD risk. Moreover, these novel agents may, for the first time, direct the conversation to the paradoxical point of safety concerns surrounding the consequences of long-term ultra-low cholesterol in subjects with genetic disorders resulting in severe hypercholesterolemia.

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