# RARE GENETIC DISORDERS ALTERING LIPOPROTEINS

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## ABSTRACT

The aim of this chapter is to examine other, less common genetic disorders including elevations of lipoprotein(a); disorders of high density lipoprotein (familial hypoalphalipoproteinemia, Tangier disease, and LCAT deficiency); familial hypocholesterolemias (familial hypobetalipoproteinemia, abetalipoproteinemia, PCSK9 loss of function mutations, familial combined hypolipidemia, and chylomicron retention disease); ß-sitosterolemia; cerebrotendinous xanthomatosis, and lysosomal acid lipase deficiency. While the prevalence of these individual disorders is quite low, collectively they are important. The busy practitioner will undoubtedly encounter patients with these conditions. Providers need to be familiar with their diagnosis and treatment as they may be associated with high morbidity and mortality. Practical aspects of evaluation and management of these disorders are reviewed. For complete coverage of this area and all of Endocrinology, visit www.endotext.org.

## INTRODUCTION

Common genetic disorders of lipid metabolism have been covered in previous chapters of this text. The purpose of this chapter is to review the experimental, genetic, epidemiologic, and therapeutic data regarding less common genetic disorders. The study of rare disorders has emerged as a very effective way of gaining insight into cellular and molecular biology, and this certainly has been the case in the field of lipidology. Indeed, as will be covered in this chapter, defining the molecular underpinnings of rare lipid disorders has effectively prioritized a number of therapeutic targets, including lipoprotein (a), apolipoprotein B, microsomal triglyceride transport protein, and PCSK9.

**Table 1. Mode of inheritance and prevalence of select rare genetic disorders of lipid metabolism**

| Condition | Mode of inheritance | Prevalence |
|---|---|---|
| Familial hypobetalipoproteinemia | Codominant | 1:1000 – 1:3000 |
| Abetalipoproteinemia | Autosomal recessive | <1:1,000,000 |

| | | |
|---|---|---|
| Familial combined hypolipidemia | Codominant | Very rare |
| Chylomicron retention disease | Autosomal recessive | Very rare |
| Familial hypoalpha-lipoproteinemia | Autosomal dominant | Very rare |
| Tangier disease | Autosomal recessive | Very rare |
| LCAT deficiency | Autosomal recessive | Very rare |
| Familial hyperalpha-lipoproteinemia | Autosomal dominant | 4:100 – 5:100 |
| ß-Sitosterolemia | Autosomal recessive | Very rare |
| Cerebrotendinous xanthomatosis | Autosomal recessive | Very rare |
| Lysosomal acid lipase deficiency | Autosomal recessive | 1:40,000 – 1:300,000 |

**LIPOPROTEIN (a)**

Lipoprotein (a) [Lp(a)] comprises a unique lipoprotein subclass and consists of a low-density lipoprotein (LDL) particle and apolipoprotein(a) [apo(a)]. Apo(a) is covalently bound to apoB on the LDL particle. Elevations in Lp(a) have been associated with premature atherosclerotic cardiovascular disease (ASCVD) and calcific aortic valve stenosis (1). The epidemiological and genetic data are consistent and suggest that Lp(a) is an independent risk factor for the development of ASCVD events (discussed below). The atherogenicity of Lp(a) is likely multifactorial and related to its LDL and apo(a) moieties as well as its enriched concentration of proinflammatory oxidized phospholipids. Additionally, Lp(a) induces the expression of intercellular adhesion molecule-1 with attendant recruitment of monocytes to the subendothelial space (2). Lp(a) also enhances the susceptibility of LDL to oxidative modification (3). Furthermore, Lp(a) inhibits the fibrinolytic system and thereby facilitates atherothrombosis (4).

Serum Lp(a) levels are largely genetically determined and are related to variation found in the gene that codes for Lp(a), *LPA*. Genomic variation at LPA is multifactorial and includes a) polymorphisms at the apo(a) gene locus (isoforms) due to copy number variation (5), b) a pentanucleotide repeat in the LPA promoter that affects gene expression (6), c) variants affecting RNA splicing (5, 7), and d) numerous single-nucleotide polymorphisms (SNPs) within key structural and functional domains (8, 9). These sources of genetic variation contribute to interindividual differences in plasma Lp(a) concentrations, which remain extremely stable within an individual over her or his lifespan.

Interestingly, *LPA* is located on the short arm of chromosome 6 adjacent to the plasminogen gene. *LPA* codes for multiple Kringle structures that are highly homologous to plasminogen. Thus, Lp(a) is not only an atherogenic particle but is prothrombotic as well (10). Interestingly, the plasminogen gene contains five Kringles and an active protease domain while *LPA* consists of Kringles IV, V, and an inactive protease domain. Kringle IV is present in 10 subtypes of which Kringle IV-2 is present in three to more than 40 copies, mediating its size variability (11). This copy number variation explains differences in plasma levels among the two alleles in individual patients and between patients as a whole. Most patients have two different sized alleles, and therefore two different sized circulating Lp(a) particles. There is an inverse correlation between the size of the apo(a) isoform and the Lp(a) plasma concentration (12). There appears to be a relationship between the number of kringle repeats and the processing time of the precursor apo(a) protein. That is, the larger isoforms have a slower rate of production that limits the plasma concentration (13). Experimental data suggest that Lp(a) levels are primarily determined by the rate of synthesis and not affected to any significant extent by the rate of clearance. The mechanism for the clearance of Lp(a) is still not understood, but is not thought to be primarily be related to the LDL receptor (14). It is the absolute level of Lp(a), rather than apo(a) isoform size, that are the main determinant of ASCVD risk (15, 16). There are 2 SNPs in the apo(a) gene locus, rs3798220 and rs10455872, that significantly predict Lp(a) concentrations and ASCVD risk [13]. This association is eliminated when the model includes Lp(a) level and thus supports the direct relationship between plasma Lp(a) levels and ASCVD risk.

The association of Lp(a) with coronary heart disease (CHD) risk was first suggested by small cross-sectional and retrospective studies (17-28). A prospective case-control study reported in 2008 described an adjusted odds ratio for CHD of 1.60 (95% confidence interval, 1.38-1.85) between the upper and lower thirds of baseline Lp(a) levels (29). A large meta-analysis of 36 prospective studies which included 126,634 subjects demonstrated a continuous risk for CHD with elevated Lp(a) levels (30). A 2.4 fold increase in CHD risk associated with Lp(a) levels in the upper tertile relative to the bottom tertile was reported in the Framingham Offspring Study (30). These results are \ consistent with genetic data from a Mendelian randomization study that demonstrated that *LPA* variants 1) largely determine Lp(a) plasma levels and 2) that these variants increase risk of myocardial infarction (31). These findings support a direct causal relationship between high levels of Lp(a) and increased risk of ASCVD, with a 22% increase in myocardial infarction for each doubling of Lp(a) levels. Two recent trials, JUPITER and AIM-HIGH, demonstrated the residual risk associated with elevated Lp(a) despite achievement of low LDL-C during treatment in both primary and secondary prevention, respectively ((32, 33). Taken together, these data support an independent and causative role of Lp(a) in ASCVD.

Recent data indicate that measurement of Lp(a) can improve risk ascertainment when added to global risk assessment (20). Net reclassification improvement by incorporating

Lp(a) in to the evaluation was 39.6% overall, and most useful in subjects at intermediate risk of future ASCVD events. Other individuals to consider screening for Lp(a) are listed in Table 2 (34).

**Table 2. Individuals to consider screening for Lp(a)**

| |
|---|
| Premature ASCVD |
| Family history of premature ASCVD or elevated Lp(a) |
| Recurrent ASCVD events despite effective statin therapy |
| Familial hypercholesterolemia |
| Hypercholesterolemia refractory to therapy with LDL-C lowering therapies (Lp(a) excess may account for significant component of the LDL-C level in some) (35) |
| Individuals at intermediate risk of future ASCVD events |

Lp(a) levels are highly skewed in the general population. The relationship of Lp(a) to ASCVD appears to be curvilinear with significantly increased risk starting at levels that surpass 30 mg/dl (>75nmol/l). The attributable risk of Lp(a) is independent of LDL-C, non-HDL-C, and the presence of other risk factors. Currently, there are no commercially available drugs that selectively decrease Lp(a) and as such, no evidence that lowering Lp(a) in particular results in ASCVD risk reduction. In fact, no endpoint study has been performed in patients recruited on the basis of elevated Lp(a) levels and randomized to a therapy. Niacin, estrogens, PCSK9 inhibitors, and mipomersen reduce Lp(a) levels but also modify other lipids/lipoproteins. Agents in development that reduce Lp(a) include cholesteryl ester transfer protein inhibitors and the antisense oligonucleotide directed to apo(a). In clinical trials PCSK9 inhibitors and CETP inhibitors lowered Lp(a) levels by 20–40% (36, 37).

The most promising potential therapeutic development for Lp(a) lowering is an antisense oligonucleotide (ASO) directed to apolipoprotein(a) [apo(a)]. In phase I and II clinical trials, the ASO directed at apo(a) reduced Lp(a) by approximately 80%, without significantly affecting other lipoproteins (38, 39). In the meantime, guidance issued by the European Atherosclerosis society suggests that statins are indicated to lower LDL-C and that niacin may be considered to lower Lp(a) (by ~30-40%) (40). In the FATS angiographic trial, aggressive LDL-C lowering largely mitigated the risk due to elevated Lp(a) and many experts recommend targeting LDL-C to <70 mg/dl in such patients (38). Apheresis may be considered in individuals with very high levels of Lp(a), especially if they continue to experience ASCVD events despite medical therapy (41, 42). Aspirin therapy is generally recommended for patients with elevated Lp(a) due to the associated prothrombotic effect of Lp(a) (43).

## HYPOCHOLESTEROLEMIA

Genetic mutations leading to very low levels of lipids, individually or in combination, are rare. The principle examples include familial hypobetalipoproteinemia (FHBL), abetalipoproteinemia (ABL), chylomicron retention disease (CMRD), and loss of function

mutations in PCSK9 and angiopoietin-like protein 3 (ANGPTL3). Increased understanding of the genetic and the molecular underpinnings of these disorders has allowed a focused prioritization of therapeutic targets for drug development. Table 3 summarizes genetic, lipid, and clinical features of the major hypolipidemia syndromes.

**Table 3. Characteristics of the hypolipidemia syndromes**

| | **Effected gene** | **Lipids** | **Clinical features** |
|---|---|---|---|
| FHBL | Truncation mutations in apoB | apoB <5th percentile<br>LDL-C 20- 50 mg/dL | Hepatic steatosis<br>Mild elevation of transaminases |
| ABL | MTP | Triglycerides < 30 mg/dl<br>Cholesterol < 30 mg/dl)<br>LDL and apoB undetectable | Hepatic steatosis<br>Malabsorption, steatorrhea, and diarrhea<br>Deficiency of fat-soluble vitamins. |
| PCSK9 | Loss of function mutations in PCSK9 | Heterozygous – mild to moderate reduction in LDL-C<br>Homozygous – LDL-C ~15 mg/dl | Normal health; significantly lower prevalence of ASCVD |
| Familial combined hypolipidemia | ANGPTL3 | Panhypolipidemia | Normal health; significantly lower prevalence of ASCVD |
| CMRD | SAR1B | LDL-C and HDL-C - decreased by 50%<br>Triglycerides - normal | hypocholesterolemia associated with failure to thrive, diarrhea, steatorrhea, and abdominal distension |

**Familial Hypobetalipoproteinemia**

Familial Hypobetalipoproteinemia (FHBL) is most commonly due to truncation mutations in the gene coding for apoB (42). Secondary, non-familial, forms of hypobetalipoproteinemia include strict vegan diet, malnutrition, malignancy, and chronic liver disease. The truncated forms of apoB found in FHBL are generally non-functional (truncation decreases lipidation and secretion) and are catabolized quickly, resulting in markedly reduced levels in the plasma (apoB <5th percentile and LDL-C typically between 20- 50 mg/dL) (44). Although there is one normal allele in heterozygous FHBL, plasma apoB levels are approximately 24% of normal rather than the predicted 50% (45). These lower than expected levels result from a 74% lower secretion rate of VLDL

apoB from the liver, decreased production of LDL apoB, increased catabolism of VLDL, and extremely low secretion of the truncated apoB (46-49). Given the reduced substrate (apoB) for lipid (predominantly triglyceride) loading, fatty liver develops in these patients (50). Hepatic steatosis and mild elevation of liver enzymes are common in heterozygous FHBL. In contrast to non-alcoholic fatty liver disease, FHBL is not associated with hepatic or peripheral insulin resistance (51-53). This observation, however, does not imply that hepatic steatosis associated with FHBL is benign. There are several reports of steatohepatitis, cirrhosis, and hepatocellular carcinoma in patients with FHBL (20, 53-57). While hepatic fat accumulation is the rule, there is generally sufficient chylomicron production to handle dietary fat. However, oral fat intolerance and intestinal fat malabsorption have been reported.

Given the association of FHBL and low LDL-C, apoB has been an attractive target for drug development. Indeed, unraveling the genetic and molecular mechanisms of FHBL provided the motivation to pharmacologically antagonize apoB synthesis for therapeutic gains (58). This culminated in the production of mipomersen, a synthetic single strand anti-sense oligonucleotide to apoB. Essentially, anti-sense oligonucleotides contain approximately ~20 deoxyribonucleic acid (DNA) base pairs complementary to a unique messenger ribonucleic acid (mRNA) sequence. The hybridization of the anti-sense oligonucleotide to the mRNA of interest leads to its catabolism via RNase H1, with markedly reduced mRNA levels and ultimately reduced target protein levels. In this case, mipomersen binds to apoB mRNA leading to reduced production of the protein, and mimicking (albeit to a lesser extent) FHBL. Mipomersen is the first anti-sense oligonucleotide approved by the United States Food and Drug Administration (FDA) and was commercialized in 2013 with a limited indication for adjunctive LDL-C lowering in patients with homozygous familial hypercholesterolemia (HoFH) (59-61). It is an injectable agent administered subcutaneously once a week. In the clinical trials, mipomersen was associated with a reduction of LDL-C by 25% in subjects with HoFH and 28% in subjects with heterozygous familial hypercholesterolemia (HeFH) (62, 63). Interestingly, it was also found to lower Lp(a) by 21% (61). While it is highly efficacious LDL-C lowering, it has side effects, many of which can be predicted based on the experience with FHBL (e.g., hepatic steatosis, elevated liver enzymes). It is also associated with injection site reactions in a considerable number of subjects. Its long-term safety has not been established.

Homozygous hypobetalipoproteinemia (HHBL) is extremely rare. These patients are homozygous or compound heterozygous for mutations in the apoB gene. The clinical manifestations mimic ABL (see below).

**Abetalipoproteinemia**

Abetalipoproteinemia (ABL) is a rare disorder characterized by very low plasma concentrations of triglyceride and cholesterol (under 30 mg/dl) and undetectable levels of LDL and apoB (64). It is due to mutations in the gene that codes for microsomal

triglyceride transfer protein (*MTP*) (60). MTP lipidates nascent apoB in the endoplasmic reticulum to produce VLDL and chylomicrons in the liver and small intestine, respectively. Unlipidated apoB is targeted for proteasomal degradation leading to the absence of apoB containing lipoproteins in the plasma (and thus markedly reduced levels of LDL-C and triglycerides) (65). Similar to FHBL, VLDL production is inhibited. The reduced triglyceride export from the liver leads to hepatic steatosis. Additionally, lack of MTP facilitated lipidation of chylomicrons in the small intestine causes lipid accumulation in enterocytes with associated malabsorption, steatorrhea, and diarrhea. The malabsorption and diarrhea lead to failure to thrive during infancy. An additional issue of importance related to ABL is deficiency of fat-soluble vitamins. Early diagnosis of ABL and HHBL is extremely important as vitamin E deficiency culminates in atypical retinitis pigmentosa, spinocerebellar degeneration with ataxia, and vitamin K deficiency that can lead to a significant bleeding diathesis (66). High dose supplementation with fat soluble vitamins early in life can prevent these devastating complications. Additional treatment measures include a low-fat diet and supplementation with essential fatty acids.

Given the very low level of atherogenic lipoproteins and lipids associated with ABL, there was interest in antagonizing MTP therapeutically (67, 68). Lomitapide is an oral MTP inhibitor that has been developed over the course of many years. In early trials, it was tested at a relatively high dose and the side effect profile was prohibitive (nausea, flatulence, and diarrhea). The more recent clinical trial program tested lower doses with drug titration in subjects with HoFH (69, 70). Lomitapide reduced LDL-C by 50% from baseline to week 26 and remained reduced by 38% at week 78. Interesting, lomitapide reduced Lp(a) modestly (-13%) (70) Lomitapide received the same limited indication as mipomersen for adjunctive treatment of patients with HoFH. Besides the gastrointestinal issues already alluded to, its side effect profile includes hepatic steatosis. Its long-term safety has not been established.

**Proprotein Convertase Subtilisin/ Kexin Type 9 (PCSK9)**

Proprotein convertase subtilisin/ kexin type 9 (PCSK9) belongs to the proprotein convertase class of serine proteases. After synthesis, PCSK9 undergoes autocatalytic cleavage. This step is required for secretion, most likely because the prodomain functions as a chaperone and facilitates folding (71). PCSK9 is associated with LDL particles (~40%) and the LDL-receptor (LDLR) (~60%) (67, 71). In 2003, Abifadel reported the seminal work that mapped PCSK9 as the third locus for autosomal dominant hypercholesterolemia (2). This finding revealed a previously unknown actor involved in cholesterol homeostasis and served to launch a series of investigations into PCSK9 biology. As it turns out, PCSK9 functions as a central regulator of plasma LDL-C concentration. It binds to the LDLR and targets it for destruction in the lysosome (10).

Since the discovery of gain-of-function mutations in PCSK9 as a cause of FH, investigators have also uncovered loss of function mutations of PCSK9. Loss-of-function mutations in PCSK9 are associated with low LDL-C levels and markedly reduced

ASCVD (3, 4, 72, 73). Interestingly, rare individuals homozygous for loss of function mutations in PCSK9 have been reported with extremely low levels of LDL-C (~15 mg/dl), normal health and reproductive capacity, and no evidence of neurologic or cognitive dysfunction (74, 75). Collectively, these observations served as further motivation to pursue antagonism of PCSK9 as a therapeutic target. Theoretically, antagonizing PCSK9 would prolong the lifespan of LDLR, leading to significant reductions in plasma LDL-C levels. There are numerous approaches to inhibiting PCSK9 including humanized monoclonal antibodies (mAbs), gene silencing, and use of small inhibitory peptides. Thus far, approaches utilizing mAbs are the only therapeutic agents that are FDA approved. The two fully human monoclonal antibodies (alirocumab and evolocumab) targeting PCSK9 became commercially available in 2015. Clinical trials of mAbs targeted to PCSK9 have demonstrated remarkable efficacy in LDL-C reduction (~50% reduction in LDL-C as monotherapy and ~65% reduction in LDL-C in combination with a statin) with an excellent short-term safety and tolerability profile (76). Moreover, one large randomized controlled trial (FOURIER) demonstrated incremental improvement with a 15% reduction in the composite primary endpoint of major adverse cardiovascular outcome with addition of evolocumab on top of standard of care in patients with stable vascular disease (77). More recently, the ODYSSEY OUTCOMES trial was presented at the American College of Cardiology Meeting (Presented by Dr. Philippe Steg at the American College of Cardiology Annual Scientific Session (ACC 2018), Orlando, FL, March 10, 2018.) and demonstrated a similar reduction in major adverse cardiovascular events with alirocumab vs. placebo in patients with recent acute coronary syndromes.

**Familial Combined Hypolipidemia**

Familial combined hypolipidemia is due to loss of function mutations in the gene encoding ANGPTL3 (20). ANGPTL3 inhibits various lipases, such as lipoprotein lipase and endothelial lipase. Therefore, loss of function mutations in ANGPTL3 relinquishes this inhibition resulting in more efficient metabolism of VLDL and HDL particles. Clinically, this manifests as panhypolipidemia. Interestingly, heterozygotes for certain nonsense mutations in the first exon of ANGPTL3 have moderately reduced LDL-C and triglyceride levels while compound heterozygotes have significant reductions in HDL-C as well (78). Homozygosity or compound heterozygosity for other loss-of-function mutations in exon 1 of ANGPTL3 have no detectable ANGPTL3 in plasma and striking reductions of atherogenic lipoproteins with HDL particles containing only apo A-I and preß-HDL (78). Individuals who are heterozygous for the loss of function mutations in ANGPTL3 have normal HDL-C levels and significantly reduced LDL-C (<25$^{th}$ percentile).

Recently, a pooled analysis of all reported cases of familial combined hypolipidemia was published (79). One hundred fifteen individuals carrying 13 different mutations in the *ANGPTL3* gene (14 homozygotes, 8 compound heterozygotes, and 93 heterozygotes) and 402 controls were evaluated. Homozygotes and compound heterozygotes (two mutant alleles) had no measurable ANGPTL3 protein. In heterozygotes, ANGPTL3 was reduced by 34-88%, according to genotype. All cases

(homozygotes and heterozygotes) demonstrated significantly lower concentrations of all plasma lipoproteins [except for Lp(a)] as compared to controls. Familial combined hypolipidemia is not associated with any comorbidity. In fact, the prevalence of fatty liver was the same as controls. ASCVD and diabetes were not found amongst homozygotes.

**Chylomicron Retention Disease**

Chylomicron retention disease (CMRD), known also as Anderson's disease for the individual who first described the condition in 1961, is a rare inherited lipid malabsorption syndrome (80). It is due to mutations in the *SAR1B* gene which codes for the protein SAR1b involved in intracellular protein trafficking. This disorder usually presents in young infants with diarrhea, steatorrhea, abdominal distention, and failure to thrive. Patients with CMRD demonstrate a specific autosomal recessive hypocholesterolemia that differs from other familial hypocholesterolemias. CMRD is associated with a 50% reduction in both plasma LDL-C and HDL-C with normal triglyceride levels. Beyond the typical lipid profile, several other findings support the diagnosis, including:

a) absence of secretion of chylomicrons after a fat load

b) white duodenal mucosa on endoscopy

c) cytosolic lipid droplets and lipoprotein-sized particles in enterocytes on intestinal biopsy

d) SAR1B gene mutations

**LOW HDL CONDITIONS**

The inverse relationship between HDL-C and ASCVD risk is well established (81). Furthermore, the risk attributable to low HDL-C is independent of LDL-C levels (82). Isolated low HDL-C levels can occur; however, it is more commonly found in association with hypertriglyceridemia and/or elevated apoB (83). Patients with very low HDL-C (<20 mg/dl) in the absence of severe hypertriglyceridemia are rare These individuals may have rare monogenic disorders associated with marked HDL deficiency, including familial hypoalphalipoproteinemia, Tangier disease, and lecithin acyltransferase (LCAT) deficiency. Table 4 summarizes genetic, lipid, and clinical features of the major low HDL conditions.

**Table 4. Characteristics of the low HDL syndromes**

| | **Effected gene** | **Lipids** | **Clinical features** |
|---|---|---|---|
| Familial hypoalpha-lipoproteinemia | apo A-I/apo C-III/ apo A-IV<br>apo A-I/apo C-III<br>apo A-I | Apo AI undetectable, marked deficiency in HDL-C, low – normal triglycerides, normal LDL-C | Xanthomas<br>Premature ASCVD<br>Corneal manifestations |
| Tangier disease | ABCA1 | HDL species exclusively preß-1 HDL-C <5 mg/dl<br>LDL-C low (half normal) | Hepatosplenomegaly<br>Enlarged tonsils<br>Neuropathy |

| | | | |
|---|---|---|---|
| | | | ASCVD (6-7th decade) |
| LCAT deficiency | LCAT | HDL-C <10 mg/dl<br>apo A-I 20-30 mg/dl<br><36% cholesteryl esters<br>Low LDL-C<br>Presence of Lp-X particles | FLD develop corneal opacities ("fish eye"), normochromic anemia and proteinuric end stage renal disease<br><br>FED only develop corneal opacities |

**Familial Hypoalphalipoproteinemia**

Familial hypoalphalipoproteinemia is a heterogeneous group of apolipoprotein A-I (apo A-I) deficiency states. These various conditions are characterized by the specific apolipoprotein genes that are affected on the apo A-I/C-III/A-IV gene cluster. The genes for these 3 apolipoproteins (apo A-I, apo C-III, and apo A-IV) are grouped together in a cluster on human chromosome 11 (84). In patients with apo A-I/C-III/A-IV deficiency, apoA-1 is undetectable in the plasma and is associated with marked deficiency in HDL-C, low triglyceride levels (due to apo C-III deficiency), and normal LDL-C (85). This condition is associated with aggressive, premature ASCVD. Additionally, there is evidence of mild fat malabsorption. Patients with apo A-I/C-III deficiency have undetectable apo A-I and a similar lipid profile as those with apo A-I/C-III/A-IV deficiency. This condition is also associated with premature ASCVD. It is distinguished from the former by presence of planar xanthomas and absence of fat malabsorption (since apo A-IV is present) (86) . Familial apo A-I deficiency is itself a heterogeneous group of disorders associated with numerous gene (Apo A-I) mutations. Common manifestations include undetectable plasma Apo A-I, marked HDL deficiency with normal LDL-C and triglyceride levels, xanthomas (planar, tendon, and/or tubero-eruptive depending on the specific gene mutation), and premature ASCVD. Some forms of the disease are also associated with corneal manifestations, including corneal arcus and corneal opacification. One of the interesting manifestations of familial apo A-I deficiency is that levels of apo A-IV and apo E containing HDL particles are normal, with preserved electrophoretic mobility and particle size. As such, it appears that there are 3 distinct HDL particles, e.g., apo A-I HDL, apo A-IV HDL, and apo E HDL. The latter 2 species normally make up a minor portion of the HDL population but may have functional significance. Given the increased ASCVD risk associated with Apo A-I deficiency, treatment is directed towards aggressive reduction of LDL-C and non-HDL-C.

**Tangier Disease**

Tangier disease is due to mutations in the gene that codes for ATP-Binding Cassette transporter A1 (ABCA1) and is inherited in an autosomal recessive manner. Fredrickson

first reported this condition in two patients who hailed from Tangier Island in the Chesapeake Bay, for which the disorder is named. ABCA1 facilitates efflux of intracellular cholesterol from peripheral cells to lipid poor A1, the key first step of reverse cholesterol transport. As such, this disorder is characterized by plasma with exclusively preß-1 HDL and severe deficiency of HDL-C (HDL-C <5 mg/dl). The poorly lipidated apo A-I is rapidly catabolized by the kidney. These patients also demonstrate moderate hypertriglyceridemia. Since ABCA1 deficiency impairs free cholesterol efflux from cells, there is accumulation of cholesterol esters in many tissues throughout the body. Classically, patients present with hepatosplenomegaly and enlarged tonsils, however, a wide spectrum of phenotypic manifestations is now appreciated with considerable variability in terms of clinical severity and organ involvement. Tangier disease patients have an increased risk of premature ASCVD, though not as pronounced as those with familial hypoalphalipoproteinemia. CHD generally manifests in the $6^{th}$ and $7^{th}$ decades. Later onset of atherosclerosis in Tangier disease may be due to the fact that LDL-C is generally half that of the normal population.

Individuals who are heterozygous for ABCA1 mutations have HDL-C levels that are 50% of normal with normal levels of preß-1 HDL but deficiency of large α-1 and α-2 HDL particles. Cholesterol efflux capacity in heterozygotes has been reported as ~50% of normal. A mutation in one ABCA1 allele has been associated with increased risk of ASCVD in some studies but no increase in risk in others. This continues to be a significant unanswered question. Recent data links discrete mutations in ABCA1 to varying HDL-C levels. These genotype/phenotype correlations suggest a direct association between ABCA1 function and HDL-C concentration (87). This association may additionally inform ASCVD risk.

While Tangier patients manifest characteristically low HDL-C and apo A-I, this lipid/lipoprotein phenotype is not adequate to make the diagnosis. ABCA1 gene sequence analysis is the preferred test to make the diagnosis of Tangier disease. However, molecular diagnostics are not readily available and are expensive. Alternatively, non-denaturing two-dimensional electrophoresis followed by anti-apo A-I immunoblotting demonstrates only preβ1-HDL (88). Currently, there is no specific treatment for Tangier disease. In fact, HDL-C raising therapies such as niacin and fibrates have proven ineffective in patients with this condition (84). Even HDL infusion and plasma exchange were attempted but not found to be beneficial (89). The major clinical issue in Tangier patients is disabling neuropathy; however, there is no effective intervention to manage this complication.

**LCAT Deficiency**

LCAT is an enzyme that is bound primarily to HDL, with some also found on LDL. It facilitates cholesterol esterification by transferring a fatty acid from phosphatidyl choline to cholesterol. The hydrophobic cholesteryl esters are then sequestered into the core of the lipoprotein particles. LCAT is critical in the maturation of HDL particles. LCAT

deficiency is an autosomal recessive disorder that manifests as either familial LCAT deficiency (FLD) or fish-eye disease (FED). The phenotype of these conditions is dictated by residual LCAT activity, with FLD associated with essentially no enzyme activity and FED associated with some activity, generally on apoB-containing lipoproteins (90).

Individuals with FLD develop corneal opacities ("fish eye"), normochromic anemia (due to cholesterol enrichment of red blood cell membranes), and proteinuric end stage renal disease. Patients with FED generally only manifest the corneal opacities. The lipid and lipoprotein profile demonstrate low HDL-C (<10 mg/dl), low apo A-I (20-30 mg/dl), <36% cholesteryl esters, low LDL-C, and the presence of lipoprotein X (Lp-X) particles. Lp-X is a multilamellar vesicle with an aqueous core. It is primarily composed of free cholesterol and phospholipid with very little protein (albumin in the core and apolipoprotein C on the surface) and cholesteryl ester. Given the association of Lp-X and kidney disease only with FLD (and not FED), some have speculated the role of this particle in the genesis of the renal dysfunction. Lp-X particles accumulate in the mesangial cells in the glomerulus. It is thought that they induce inflammation and breakdown of the basement membrane leading to proteinuria. The current theory posits that Lp-X causes inflammation in the kidney by activating the inflammasome (part of the innate immune system). The cholesterol in the Lp-X partitions into the lysosomal membrane. The vacuolar ATPase in the lysosome is inhibited by this cholesterol deposition and culminates in lysosomal distention and dysfunction.

It is unclear as to whether LCAT deficiency is associated with ASCVD. Sethi et al reported increased pre-beta HDL and decreased LCAT activity in subjects with coronary heart disease (90). Atherosclerosis imaging studies have yielded divergent data. Initial studies demonstrated no increase in carotid intima media thickness. However, a subsequent carotid MRI study of LCAT heterozygotes revealed a ~30-fold increase in plaque volume in FLD compared to controls (91). Current opinion suggests that LCAT deficiency is associated with increased risk of ASCVD however it is likely subtle due to associated low LDL-C.

Current management of FLD focuses on managing the renal dysfunction. The associated kidney disease is traditionally managed with angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and rarely steroids (92). Currently, a human recombinant LCAT is being tested in clinical trials. This treatment is associated with rapid increases in HDL-C and cholesterol esterification as well as increased in vitro cholesterol efflux by plasma (93). Future trials will determine whether it can prevent or reverse renal disease in FLD.

**Approach to the Patient with Low HDL**

When encountering a patient with very low HDL-C, it is important to first exclude hypertriglyceridemia as the etiology. If the extreme depression in HDL-C is not

secondary to hypertriglyceridemia, one must exclude artifactual causes secondary to paraproteinemia. If not artifactual, a detailed medication history needs to be elicited. If the HDL-C drop (to below 20 mg/dl) is sudden, an occult malignancy needs to be excluded. Additionally, severe liver disease or sepsis can cause marked reductions in HDL levels. Finally, certain drugs can markedly decrease HDL resulting in very low HDL levels (for example high dose androgens and idiosyncratic reactions to fibrates and thiazolidinediones). If prior HDL-C levels are normal, this excludes a primary monogenic etiology. To evaluate potential primary causes, a detailed family history, with attention to HDL-C levels, is important. A focused physical examination, with particular attention to the skin, eyes, tonsils, and spleen may point to a specific monogenic disorder. In these cases, plasma apo A-I levels should be obtained. Individuals with apo A-I deficiency have undetectable plasma apo A-I. Patients with Tangier disease demonstrate very low apo A-I levels (<5 mg/dl). LCAT deficiency is associated with apo A-I levels that are low but substantially higher than the other monogenic etiologies. Patients with LCAT deficiency also have a higher ratio of free: total cholesterol in plasma and measurement of plasma free (unesterified) cholesterol can be helpful. Two-dimensional gel electrophoresis of plasma followed by immunoblotting with antibodies specific for apo A-I separates lipid-poor preß-HDL from lipid-rich–HDL and can be used to differentiate these disorders (94) .

## HIGH HDL CONDITIONS (HYPERALPHALIPOPROTEINEMIA)

An elevated concentration of apo A-I and apo A-II is called hyperalphalipoproteinemia (HALP). HALP is a heterogeneous condition caused by a variety of genetic and environmental factors. Given the focus of this chapter, genetic causes of HALP will be reviewed. Familial HALP includes primary HALP, CETP deficiency, familial hepatic lipase deficiency, and selective up-regulation of apo A-I production. Despite the consistent epidemiology that demonstrates an inverse relationship between HDL-C and ASCVD risk, some forms of familial HALP are paradoxically associated with increased cardiovascular risk. The major genetic causes of HALP are summarized in Table 5.

**Table 5. Causes of Familial HALP**

| Condition | Overview |
|---|---|
| Primary HALP | Familial elevated HDL-C levels that are not due to CETP deficiency. Epidemiologic studies have suggested that this syndrome is associated with a decreased risk for ASCVD and with increased longevity |
| CETP deficiency | Caused by low CETP levels. CETP deficiency is the most important and frequent cause of HALP in Japan. It is associated with marked elevations of plasma HDL cholesterol in homozygotes (usually >100 mg/dL). In heterozygotes, the HDL levels are only moderately elevated. CETP deficiency has not yet been demonstrated |

| | |
|---|---|
| | to be associated with a decreased risk for ASCVD with some studies demonstrating an association with longevity in some populations and ASCVD in others. |
| Up-regulation of apo A-I production | Selective up-regulation of apo A-I. Affected individuals have elevated HDL cholesterol and apo A-I levels. Many patients have a reduced risk of ASCVD. |
| Genetic deficiency of hepatic lipase | HALP due to hepatic lipase deficiency. Mutations in the gene coding for hepatic lipase resulting in reduced lipase activity and increased plasma levels of HDL-C are linked to increased risk of ASCVD |

HALP is generally identified incidentally after routine assessment of a lipid profile as it is generally not associated with any signs or symptoms. Rarely it is associated with premature corneal opacities and multiple symmetric lipomatosis (95). Generally, patients are asymptomatic and no medical therapy is required. However, patients with corneal opacity may need an evaluation by an ophthalmologist. Clearly, some forms of HALP are associated with ASCVD and selective screening for such is warranted in some.

**ß-SITOSTEROLEMIA**

ß-Sitosterolemia (also known as phytosterolemia) is an extremely rare (only 100 cases reported in the literature) disorder due to homozygous or compound heterozygous mutations in either one of the two adenosine triphosphate binding cassette transporters genes, ABCG5/ABCG8 (96). These proteins are expressed in the liver and small intestine and facilitate excretion of absorbed plant sterols and cholesterol into the intestinal lumen and bile. Thus, defects in these genes are associated with markedly elevated plasma levels of plant sterols (e.g., sitosterol and campesterol) and normal to moderately elevated plasma levels of cholesterol. While splenomegaly, thrombocytopenia, and hemolytic anemia can complicate the course of sitosterolemia, ASCVD is the most devastating feature of this condition.

Clinical manifestations may include hypercholesterolemia, tendon and tuberous xanthomas, and premature ASCVD. Individuals with sitosterolemia seem to be more susceptible to xanthomatosis than those with similar plasma levels of cholesterol. Even low plasma levels of phytosterols (30-40 mg/dl) are sufficient to cause xanthomas while plasma levels of LDL-C associated with xanthomas in FH are generally greater than 400 mg/dl (97). Patients with sitosterolemia are at risk for ASCVD in early childhood and adulthood, which may be due to elevations in both plasma cholesterol and phytosterols (20, 70, 98). Its presentation is sometimes confused with FH, given the overlapping clinical features (see Table 6). Importantly though, most patients with sitosterolemia demonstrate either normal or only moderately elevated plasma cholesterol levels with very high plasma levels of plant sterols. Xanthelasma and corneal arcus are less common in sitosterolemia as compared to FH. Some sitosterolemic patients also present with pseudo-homozygous FH, which is due to a complete failure of

cholesterol efflux into bile (21, 99, 100). One speculative mechanism to explain the profound hypercholesterolemia in this condition proposes that hepatic retention of phytosterols leads to a reduction in LDLR function mediated by the sterol regulatory element binding protein pathway (101).

The foundation of treatment of sitosterolemia is through dietary means with strict reduction in foods rich in plant sterols and cholesterol (e.g., nuts, seeds, olives, avocados, vegetable oils, shortening, margarine, shellfish, and chocolate). Dietary measures alone, however, do not adequately lower plasma phytosterols levels. Statins may be used to lower LDL-C and reduce ASCVD risk but often do not lower plant sterol levels. Furthermore, sitosterolemic patients generally only have a modest LDL-C lowering response to statins since de novo cholesterol synthesis is already suppressed (99). Bile acid binding resins may be used and reduce total plasma sterols by ~50% in patients with sitosterolemia (102, 103). The introduction of ezetimibe, a sterol absorption inhibitor, has transformed the management of these patients and is considered standard of care. Most patients will require dietary measures coupled with combination medical therapy to adequately lower plasma sterol levels.

**CEREBROTENDINOUS XANTOMATOSIS**

Cerebrotendinous xanthomatosis (CTX) is a rare (only several hundred reported cases) disease caused by a defective sterol 27-hydroxylase enzyme, due to a mutation in the *CYP27A1* gene. This mitochondrial enzyme (a member of the cytochrome P450 system) deficiency causes the accumulation of cholesterol and cholestanol in virtually all tissues, leading to diffuse xanthoma formation, most notably in the central nervous system and tendons.

The fundamental defect in bile acid synthesis is at the core of CTX. Normal cholesterol catabolism involves the synthesis of primary bile acids (cholic acid and chenodeoxycholic acid [CDCA]) by way of several sterol intermediates (104). Due to the disturbance in bile acid synthesis in CTX, feedback regulation on cholesterol 7α-hydroxylase, the rate-limiting enzyme, is disturbed (105). Thus, cholestanol and other bile acid precursors accumulate in tissues resulting in a progressive degenerative systemic and neurologic disorder.

Systemic and neurologic symptoms typical of CTX include intractable diarrhea, premature cataracts, tendon xanthomas, and progressive neurologic disease (104). Chronic diarrhea and bilateral cataracts typically present in early childhood (106, 107). Patients usually develop tendon xanthomas and neurologic symptoms after the second decade of life (108). Besides forming on extensor tendons (particularly the Achilles), xanthomas can form in the brain, bones, and lungs.

ASCVD and non-atherosclerotic cardiovascular disease have been reported with CTX, including premature coronary heart disease, coronary aneurysms, mitral regurgitation,

and lipomatous hypertrophy of the interatrial septum (109-111). The mechanism for the development of atherosclerosis is unclear, especially in light of the relatively low to normal plasma concentrations of LDL-C (112). It is likely related to the uptake of cholestanol within the arterial walls (113).

The disruption of bile acid synthesis in CTX results in a number of laboratory abnormalities. Laboratory findings include elevated plasma levels of cholestanol and bile alcohols. The formation of CDCA is markedly decreased with concomitant diminished concentrations in the bile. Urine concentrations of bile alcohols and bile alcohol glucuronides are increased. Serum and tissue levels of cholestanol are elevated to 5-10 times the normal level whereas serum cholesterol levels are normal or decreased. A presumptive diagnosis is established when typical symptoms (neurologic, cataracts and xanthomas) and lab abnormalities (elevated plasma and bile cholestanol levels, increased urinary excretion of bile alcohol glucuronides associated with diminished biliary concentrations of CDCA) are present. Genetic testing can be done to confirm the diagnosis.

It is imperative to recognize CTX before neurologic deterioration ensues in order to prevent severe mental and neurologic dysfunction and death. The mainstay of treatment for CTX is CDCA (114). It can stabilize or potentially reverse some of the associated symptoms. The neurologic and psychiatric symptoms are the most difficult to treat and may not improve with this therapy (115). Statins have been studied as a treatment for CTX (111). Although data are sparse, statin monotherapy appears to have little or no benefit in this condition. However, statins may be useful for lowering cholestanol levels when combined with CDCA. There is limited evidence that statins provide incremental benefit over CDCA treatment alone. A theoretical concern regarding statin therapy in CTX is the prospect of worsening the condition by increasing LDL uptake secondary enhanced LDLR activity.

FH, sitosterolemia, and CTX share certain similarities in their clinical manifestations. Table 6 compares and contrasts these three conditions.

**Table 6. Characteristic features of FH, Sitosterolemia, and CTX**

| Condition | Genetic Defect | Lab Findings | Clinical Features | Treatment |
|---|---|---|---|---|
| Familial Hypercholesterolemia | Most commonly mutations in LDL-R (can also be due to defective apoB or gain of function mutations in | Elevated plasma LDL-C | ASCVD<br>Tendon and tuberous xanthomas | Cholesterol lowering diet<br>Cholesterol lowering medications<br>LDL apheresis |

| | | | | |
|---|---|---|---|---|
| | PCSK9 | | | |
| Sitosterolemia | Mutations in ABCG5 and ABCG8 | Marked elevations in plasma phytosterol levels<br>Normal or modestly elevated plasma cholesterol | ASCVD<br>Tendon and tuberous xanthomas<br>Thrombocytopenia<br>Hemolytic anemia | Low plant sterol diet<br>Ezetimibe<br>Bile acid binding resins |
| Cerebrotendinous Xanthomatosis | Mutation in CYP27A1 (defective sterol 27-hydroxylase) | Elevated plasma cholestanol and bile alcohols<br>Normal or low plasma cholesterol | Intractable diarrhea<br>Premature cataracts<br>Tendon xanthomas<br>Progressive neurologic disease | Chenodeoxycholic acid |

**LYSOSOMAL ACID LIPASE DEFICIENCY**

Lysosomal acid lipase deficiency (LAL-D) is a rare disorder due to mutations in the *LIPA* gene, which codes for lysosomal acid lipase. The prevalence of this condition is unknown but current estimates range from 1:40,000 – 1:300,000 individuals (116). Normally, LDL particles in plasma bind to the LDLR and then via clathrin-mediated endocytosis gain entrance into the cell and ultimately fuse with the lysosome. Lysosomal acid lipase hydrolyzes the cholesterol esters and, to a lesser extent, triglycerides found within these lipoprotein particles. LAL-D is a lysosomal storage disorder characterized by absent or markedly reduced lysosomal acid lipase activity and thus cholesterol esters and triglycerides accumulate within the lysosomes. As such, the hallmarks of this condition include dyslipidemia, accelerated atherosclerosis, and progressive liver disease. Patients typically presents with severe hypercholesterolemia (with depressed HDL-C), hepatomegaly, elevated transaminases, and/or microvesicular steatosis that can progress to fibrosis and cirrhosis. The disease was initially described as two separate entities; a fulminant, rapidly progressive disease presenting in newborns called Wolman disease and a less severe form typically presenting in childhood or young adulthood called cholesterol ester storage disease. It is now understood that these two conditions are on a spectrum with a common molecular basis resulting from mutations in the *LIPA* gene. Currently, it is thought that the severity and progression of disease is related to the degree of residual enzyme activity. Given its similar manifestations with other cardiovascular and liver diseases, the differential diagnosis of LAL-D can be challenging (Table 7).

| Table 7. Differential Diagnosis of Lysosomal Acid Lipase Deficiency |
|---|
| Familial Hypercholesterolemia |
| Familial Combined Hyperlipidemia |
| Polygenic Hypercholesterolemia |
| Metabolic Syndrome |
| Non-alcoholic Fatty Liver Disease |
| Non-alcoholic Steatohepatitis |
| Glycogen Storage Disease |
| Cryptogenic Cirrhosis |

From a lipid disorders standpoint, LAL-D can mimic HeFH, and in the appropriate setting, an evaluation for LAL-D should ensue (117). As a first line test, lysosomal acid lipase enzyme activity can be measured in dry blood spot using the fluorometric substrate 4-methylumbelliferyl palmitate. If there is very low or absent enzyme activity on dry blood spot testing, this is considered diagnostic. Genetic testing can also confirm the diagnosis of LAL-D.

Until recently, there was no specific therapy available for LAL-D. Supportive therapies, including liver transplant in some, had been the mainstay of treatment. Given the profound hypercholesterolemia that is frequently present in this disorder, statins have been utilized and have been found to lower LDL-C in many cases reported in the literature (118). However, liver disease continues to progress and there is theoretical concern related to increased LDLR activity with statins. While plasma LDL-C is lowered, the higher receptor mediated uptake of LDLR particles may accelerate the lysosomal accumulation of cholesteryl esters and thus lead to further decline in liver and other organ function.

Enzyme replacement with human recombinant lysosomal acid lipase (sebelipase alfa) demonstrated a reduction in multiple disease-related hepatic and lipid abnormalities in children and adults with LAL-D and has subsequently been approved by the FDA for treatment of this condition (119). The initial clinical experience suggests that sebelipase alfa is well tolerated, rapidly decreases serum transaminases, improves the lipid profile, and reduces the hepatic fat fraction (120, 121).

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