Mendelian disorders refer to diseases caused by mutations in single genes that are inherited following a simple pattern. When considering high-density lipoprotein (HDL) metabolism, 3 such disorders can be distinguished. These are APOAI, LCAT, and ABCA1 (encoding apolipoprotein AI [apoA-I], lecithin:cholesterol acyltransferase [LCAT], and ATP-binding cassette transporter A1 [ABCA1], respectively) deficiency, which all cause a loss of the capacity to produce or mature HDL. All 3 are inherited in an autosomal-recessive manner. Although homozygotes and compound heterozygotes for loss-of-function mutations in these genes mostly have clinical complications,1 heterozygotes are generally without clinical symptoms. In this context, HDL cholesterol (HDL-C) levels can be considered a Mendelian trait with levels depending on the action of single gene products. On the contrary, this trait is genetically also heterogeneous because >40 different genes are currently reported to affect HDL-C levels. In the majority of cases, only heterozygotes of gene variants are known, and HDL cholesterol as a trait is inherited in an autosomal-dominant manner. Only 3 Mendelian disorders of HDL metabolism are currently known, which are inherited in an autosomal-recessive mode. (Circ Res. 2014;114:124-142.)

Key Words: cholesterol • lipoproteins, high-density lipoprotein

Mendelian Disorders of High-Density Lipoprotein Metabolism

Federico Oldoni, Richard J. Sinke, Jan Albert Kuivenhoven

Abstract: High-density lipoproteins (HDLs) are a highly heterogeneous and dynamic group of the smallest and densest lipoproteins present in the circulation. This review provides the current molecular insight into HDL metabolism led by articles describing mutations in genes that have a large affect on HDL cholesterol levels through their roles in HDL and triglyceride metabolism. Using this information from both human and animal studies, it is discussed how HDL is produced, remodeled in the circulation, affected by factors that control the metabolism of triglyceride-rich lipoproteins, how it helps maintain cellular cholesterol homeostasis, and, finally, how it is catabolized. It can be concluded that HDL cholesterol as a trait is genetically heterogeneous, with as many as 40 genes involved. In most cases, only heterozygotes of gene variants are known, and HDL cholesterol as a trait is inherited in an autosomal-dominant manner. Only 3 Mendelian disorders of HDL metabolism are currently known, which are inherited in an autosomal-recessive mode. (Circ Res. 2014;114:124-142.)

Key Words: cholesterol • lipoproteins, high-density lipoprotein

Mendelian disorders refer to diseases caused by mutations in single genes that are inherited following a simple pattern. When considering high-density lipoprotein (HDL) metabolism, 3 such disorders can be distinguished. These are APOAI, LCAT, and ABCA1 (encoding apolipoprotein AI [apoA-I], lecithin:cholesterol acyltransferase [LCAT], and ATP-binding cassette transporter A1 [ABCA1], respectively) deficiency, which all cause a loss of the capacity to produce or mature HDL. All 3 are inherited in an autosomal-recessive manner. Although homozygotes and compound heterozygotes for loss-of-function mutations in these genes mostly have clinical complications,1 heterozygotes are generally without clinical symptoms. In this context, HDL cholesterol (HDL-C) levels can be considered a Mendelian trait with levels depending on the action of single gene products. On the contrary, this trait is genetically also heterogeneous because >40 different genes are currently reported to affect HDL-C levels. In the majority of cases, only heterozygotes for loss-of-function mutations are known and, despite their effect on HDL-C levels, they are again not reported to cause disease.

This review is an attempt to discuss the genes for which there exists clear evidence that they play important roles in regulating HDL-C levels in humans and mice. The Figure gives
an overview of the knowledge that has been obtained through mutations (or targeted disruption) of these genes and illustrates the current molecular details of HDL anabolism, conversion, and catabolism. The Table summarizes how mutations in these genes may affect atherosclerosis.

### Terminology

**Definitions of HDL and HDL-C**

The mobilization of cellular cholesterol by HDL, the transport of cholesterol by HDL in the circulation, and the hepatic uptake of HDL-C are generally considered as key to the function of this lipoprotein class. HDLs are characterized by several distinct subpopulations. The dynamic macromolecular HDL complexes range from 70 to 100 Å in diameter and from 200,000 to 400,000 daltons in mass, are rich in protein (50%), and transport triglycerides and CE packaged in a monolayer of phospholipids and apolipoproteins. ApoA-I and apoA-II are the major structural components of HDL, and many other amphipathic apolipoproteins are isolated in HDL preparations. Adding to the complexity, many HDL-associated bioactive lipid species are thought to play important roles in various processes.

<table>
<thead>
<tr>
<th>Nonstandard Abbreviations and Acronyms</th>
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<tbody>
<tr>
<td>ABCA1</td>
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<td>ABCG1</td>
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<td>ANGPTL3</td>
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<td>apoA-I</td>
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<tr>
<td>apoM</td>
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<td>CAD</td>
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<td>CE</td>
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<td>CETP</td>
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<td>CVD</td>
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<td>FC</td>
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<td>GWAS</td>
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<td>HDL</td>
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<td>HDL-C</td>
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<td>LCAT</td>
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<td>LDL-C</td>
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<td>LIPC</td>
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<td>LIPG</td>
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<td>LPL</td>
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<tr>
<td>ORPs</td>
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<tr>
<td>PLTP</td>
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<tr>
<td>SNP</td>
</tr>
<tr>
<td>sPLA2</td>
</tr>
<tr>
<td>SR-B1</td>
</tr>
<tr>
<td>TRIB1</td>
</tr>
<tr>
<td>TRL</td>
</tr>
<tr>
<td>VLDL</td>
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</table>

both animal and human studies. Finally, the terms hypoalphalipoproteinemia and hyperalphalipoproteinemia are used when HDL-C concentrations are <10th percentile or >90th percentile for age and sex, respectively.

### Reverse Cholesterol Transport and Cellular Cholesterol Homeostasis

Reverse cholesterol transport is generally used to describe the transport of cholesterol by HDL from the vascular wall to the liver for excretion into bile as neutral sterol or bile acid. Despite ≈50 years of research, there is, as of yet, little evidence that HDL can transport cholesterol from the vessel wall to the liver for catabolism. The overall key scheme presented in the Figure is thus not meant to illustrate the routing of cholesterol that may be mediated through HDL but rather to illustrate the main HDL pathways known to date.

### Structure and Composition of HDL

HDLs are characterized by several distinct subpopulations. By ultracentrifugation, one can distinguish 2 main subfractions, namely the larger HDL₂ and the smaller and denser HDL₃. The dynamic macromolecular HDL complexes range from 70 to 100 Å in diameter and from 200,000 to 400,000 daltons in mass, are rich in protein (50%), and transport triglycerides and CE packaged in a monolayer of phospholipids and apolipoproteins. ApoA-I and apoA-II are the major structural components of HDL, and many other amphipathic apolipoproteins are isolated in HDL preparations. Adding to the complexity, many HDL-associated bioactive lipid species are thought to play important roles in various processes.

### Main Determinants of HDL-C Levels

#### Genes

Family and twin studies have shown that circulating levels of HDL-C have a strong inherited basis, with heritability estimates ranging from 40% to 80%. Accordingly, numerous genes affecting HDL metabolism have been described in humans or mice or both. Recently, data have emerged that suggest that extreme levels of HDL-C in families can have a polygenic origin, and that common genetic variation can explain a large proportion to the heritability of HDL-C levels.

One can generally distinguish between genes that directly affect de novo HDL genesis and those affecting HDL more indirectly through, for example, affecting hepatic triglyceride output or affecting the lipolysis of triglyceride-rich lipoproteins (TRL). This latter process likely explains the generally tight inverse relationship between plasma levels of HDL-C and triglycerides. Increased plasma triglyceride lipolysis can increase HDL-C levels, but, on the contrary, this process cannot recapitulate HDL-C levels in case the de novo production of HDL is completely disrupted as, for example, in APO-AI deficiency.

#### Lifestyle and Disease States

Age and sex belong to the nonmodifiable risk factors influencing plasma HDL-C levels, with age being positively correlated with HDL-C levels and male sex being associated with lower HDL-C. On the contrary, obesity, diet, physical
activity, smoking, alcohol, and drugs are part of modifiable risk factors.\textsuperscript{107,114} It is furthermore well-acknowledged that disease-related states, such as type 2 diabetes mellitus, metabolic syndrome, and kidney disorders, are all associated with reduced HDL-C levels. In addition to an increased turnover and remodeling of large HDL, these conditions all feature dense and small HDL, suggestive of an impaired conversion from small to large HDL.\textsuperscript{115,116} Alcohol intake increases HDL-C in a dose-dependent fashion,\textsuperscript{117} whereas smoking is associated with low circulating levels of HDL-C,\textsuperscript{118} and several classes of drugs affect HDL metabolism.\textsuperscript{28} Recent studies have also shown that HDL-C levels are low in patients with liver failure and even reflect its severity.\textsuperscript{119} Low plasma HDL-C levels are associated not only with an increased risk of cardiovascular disease (CVD) but also with the rate and incidence of cancer,\textsuperscript{120} as well as neurological disorders.\textsuperscript{121} Combined, the data suggest that HDL-C levels can be considered as a general biomarker for compromised health. Thus, plasma HDL-C levels are an outcome measure of genetics, lifestyle, and possible disease states.

**HDL and CVD**

**Epidemiology and Pharmaceutical Modulation**

Epidemiological studies have indisputably shown that low circulating levels of HDL-C represent a significant, robust, and independent predictor of CVD.\textsuperscript{122,123} This association was first reported by Barr et al\textsuperscript{124} and was ignored until attention was given by the Framingham Heart Study. HDL-C has since been used as an important risk factor to assess cardiovascular risk.
<table>
<thead>
<tr>
<th>Gene (Chr. Position)</th>
<th>Complete Gene Loss in Humans</th>
<th>No. of Mutations and Individuals</th>
<th>Coronary Artery Disease</th>
<th>Genetic Association Studies</th>
<th>Knockout and Silencing Studies</th>
<th>Transgenic Mice and Overexpression</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ABCA1 (9q31.1)</strong></td>
<td>Tangier disease, hypoalphalipoproteinemia</td>
<td>Total 175</td>
<td>Variable effect on atherosclerosis</td>
<td>Common variants are variably associated with HDL-C levels and CAD risk</td>
<td>Tangier-like phenotype, no reduced cholesterol excretion</td>
<td>Increased HDL-C, increased or decreased atherosclerosis.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>−/− 102</td>
<td></td>
<td></td>
<td>No increased atherosclerosis</td>
<td>Increased apolipoprotein levels and accelerated atherosclerosis on LDLr−/− background.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+/− 232</td>
<td></td>
<td></td>
<td>siRNA: reduction of HDL-C, HDL-associated ApoA-I, ApoE in mice.</td>
<td>Decreased atherosclerosis following transplantation of bone marrow from ABCA1 transgenics into LDLr−/− mice but no effects on lipid profile.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Comp +/- 36</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td><strong>ABCG1 (21q22.3)</strong></td>
<td>apoA-I deficiency</td>
<td>Total 63</td>
<td>Increased risk</td>
<td>Both rare and common variants are associated with low or high HDL-C levels</td>
<td>Normal HDL-C levels but increased atherosclerosis in mice lacking LDLr</td>
<td>Stimulation of macrophage-specific reverse cholesterol transport and decreased atherosclerosis when crossed on different atherogenic backgrounds.</td>
</tr>
<tr>
<td></td>
<td>Hypoalphalipoproteinemia</td>
<td>−/− 24</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>+/− 219</td>
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<td></td>
<td></td>
<td>Comp +/- 6</td>
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</tr>
<tr>
<td><strong>APOAI (11q23-q24)</strong></td>
<td>apoA-I deficiency</td>
<td>Total 38</td>
<td>Few reports with increased risk</td>
<td>Most common variants associated with decreased levels of HDL-C, increased levels of TG, and increased CAD risk</td>
<td>Disruption of TRL metabolism; Increased TG levels, no significant changes in plasma HDL-C levels</td>
<td>Decreased plasma TG levels but no significant changes in plasma HDL-C levels.</td>
</tr>
<tr>
<td></td>
<td>Hypoalphalipoproteinemia</td>
<td>−/− 7</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>+/− 43</td>
<td></td>
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<td></td>
<td></td>
<td>Comp +/- 1</td>
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<tr>
<td><strong>APOAV (11q23)</strong></td>
<td>apoAV deficiency</td>
<td>Total 18</td>
<td>A case-control study suggests an increased risk</td>
<td>Rare and common variants associated with high TG</td>
<td>No data on HDL-C levels.</td>
<td>Marked hypertriglyceridemia with accumulation of triglyceride-enriched VLDL; HDL-C is minimally decreased.</td>
</tr>
<tr>
<td></td>
<td>Hypertriglyceridemia</td>
<td>−/− 30</td>
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<tr>
<td></td>
<td>Hypercholesterolemia</td>
<td>+/− 26</td>
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<tr>
<td></td>
<td>Hypoalphalipoproteinemia</td>
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<tr>
<td></td>
<td></td>
<td>Comp +/- —</td>
<td></td>
<td></td>
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<tr>
<td><strong>APOCIII (19q13.2)</strong></td>
<td>apoCIII deficiency</td>
<td>Total 12</td>
<td>One study showed reduced risk</td>
<td>Variants associated with high HDL-C levels and decreased atherosclerosis</td>
<td>Enhanced uptake of TG-derived free fatty acids by adipose tissue; no effect on the VLDL-TG production</td>
<td>Increased plasma TG.</td>
</tr>
<tr>
<td></td>
<td>Chylomicronemia</td>
<td>−/− 6</td>
<td></td>
<td></td>
<td></td>
<td>Increased risk of atherosclerosis because of enhanced endothelial dysfunction.</td>
</tr>
<tr>
<td></td>
<td>Hypertriglyceridemia</td>
<td>+/− 76</td>
<td></td>
<td></td>
<td>Hypotriglyceridemia and protection from postprandial hypertriglyceridemia.</td>
<td>Increased HDL-C and reduced atherogenesis.</td>
</tr>
<tr>
<td></td>
<td>Hypercholesterolemia</td>
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<tr>
<td></td>
<td>Hypoalphalipoproteinemia</td>
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<td></td>
<td></td>
<td>Comp +/- —</td>
<td></td>
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<tr>
<td><strong>ApoM (6p2.1.33)</strong></td>
<td>apoM</td>
<td>Total 39</td>
<td>One study suggesting no effect</td>
<td></td>
<td>Reduced conversion of HDL to preβ-HDL on LDLr−/− background; knockdown leads to reduction of preβ-HDL in mice</td>
<td>Increased HDL-C and reduced atherogenesis.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>−/− 97</td>
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<td></td>
<td></td>
<td>+/− 403</td>
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<td></td>
<td>Comp +/- 32</td>
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<tr>
<td><strong>CETP (16q21)</strong></td>
<td>CETP deficiency</td>
<td>Total 39</td>
<td>Pro- and antiatherogenic effects</td>
<td>Common genetic variation is associated with HDL-C and CAD risk</td>
<td>Mice are naturally CETP deficient.</td>
<td>Reduced HDL-C and apoA-I levels.</td>
</tr>
<tr>
<td></td>
<td>Hyperalphalipoproteinemia</td>
<td>−/− 97</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td>+/− 403</td>
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<td></td>
<td></td>
<td>Comp +/- 32</td>
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<thead>
<tr>
<th>Gene (Chr. Position)</th>
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</thead>
<tbody>
<tr>
<td>GALNT2(1q41-q42)</td>
<td></td>
<td></td>
<td></td>
<td>Rare variants associated with increased HDL-C&lt;sup&gt;69,60&lt;/sup&gt;</td>
<td>Higher HDL-C levels following knockdown in mice&lt;sup&gt;61&lt;/sup&gt;</td>
<td>Reduced HDL-C levels&lt;sup&gt;61&lt;/sup&gt;.</td>
</tr>
<tr>
<td>LCAT(16q22.1)</td>
<td>Familial LCAT deficiency Fish-eye disease Hypoalphalipoproteinemia</td>
<td>Total 94</td>
<td>Increased and decreased carotid intima-media thickness&lt;sup&gt;56-64&lt;/sup&gt;</td>
<td>One variant associated with increased HDL-C but not with risk of MI&lt;sup&gt;65&lt;/sup&gt;</td>
<td>HDL deficiency&lt;sup&gt;60&lt;/sup&gt; Increased atherosclerosis in LDLr&lt;sup&gt;−/−&lt;/sup&gt; and apoE&lt;sup&gt;−/−&lt;/sup&gt; mice&lt;sup&gt;67&lt;/sup&gt;</td>
<td>No effect on atherosclerosis in wild-type mice&lt;sup&gt;65&lt;/sup&gt;. Increased HDL-C and atherosclerosis&lt;sup&gt;60&lt;/sup&gt;. Both increased or reduced atherosclerosis on apoE&lt;sup&gt;−/−&lt;/sup&gt; and LDLr&lt;sup&gt;−/−&lt;/sup&gt; background&lt;sup&gt;67,68&lt;/sup&gt;. Gene therapy decreases plaque volume in LDLr&lt;sup&gt;−/−&lt;/sup&gt; and ob/ob mice&lt;sup&gt;71&lt;/sup&gt;.</td>
</tr>
<tr>
<td>LIPC (15q21-q23)</td>
<td>LIPC deficiency Hyperalphalipoproteinemia</td>
<td>Total 19</td>
<td>Effect on CAD unclear&lt;sup&gt;72&lt;/sup&gt;</td>
<td>Common variants associated with increased HDL-C and CAD risk&lt;sup&gt;67,74&lt;/sup&gt;</td>
<td>Increased HDL-C and reduced atherosclerosis in apoE&lt;sup&gt;−/−&lt;/sup&gt; mice&lt;sup&gt;70&lt;/sup&gt;</td>
<td>Reduced HDL-C and apoAI levels&lt;sup&gt;72&lt;/sup&gt; and atherosclerosis in double-knockout (LIPC/ apoE) mice&lt;sup&gt;75&lt;/sup&gt;.</td>
</tr>
<tr>
<td>LIPG (18q21.1)</td>
<td>LIPG deficiency Hyperalphalipoproteinemia</td>
<td>Total 17</td>
<td>One case report of atheroprotection&lt;sup&gt;79&lt;/sup&gt;</td>
<td>Common variants associated with increased and decreased HDL-C&lt;sup&gt;55,60,75&lt;/sup&gt;</td>
<td>Increased HDL-C&lt;sup&gt;70&lt;/sup&gt; Reduced atherosclerosis in apoE&lt;sup&gt;−/−&lt;/sup&gt; mice&lt;sup&gt;75&lt;/sup&gt;</td>
<td>Reduced HDL-C&lt;sup&gt;75,72&lt;/sup&gt;.</td>
</tr>
<tr>
<td>LPL (15p22)</td>
<td>LPL deficiency Hypertriglyceridemia Chylomicronemia Hypoalphalipoproteinemia</td>
<td>Total 161</td>
<td>Variable effect on atherosclerosis&lt;sup&gt;73,74&lt;/sup&gt;</td>
<td>Loss-of-function and gain-of-function variants associated with increased and decreased HDL-C, respectively&lt;sup&gt;52,65&lt;/sup&gt;</td>
<td>Reduced HDL-C levels&lt;sup&gt;66&lt;/sup&gt; siRNA: reduction of intracellular lipid levels in 3T3-L1 adipocytes,&lt;sup&gt;67&lt;/sup&gt; increased free cholesterol&lt;sup&gt;79&lt;/sup&gt;.</td>
<td>Increased HDL-C&lt;sup&gt;66&lt;/sup&gt;.</td>
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<tr>
<td>PLTP (20q12-q13.1)</td>
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<td>...</td>
<td>One report of decreased risk&lt;sup&gt;69&lt;/sup&gt;</td>
<td>Common variants associated with increased number of HDL particles, smaller HDL size, and decreased CAD risk&lt;sup&gt;99&lt;/sup&gt;</td>
<td>Reduced HDL-C and ApoAI levels and increased atherosclerosis&lt;sup&gt;96,98&lt;/sup&gt;</td>
<td>Increased HDL/non-HDL cholesterol ratio&lt;sup&gt;92&lt;/sup&gt;. Increased atherogenesis&lt;sup&gt;53&lt;/sup&gt; on LDLr&lt;sup&gt;−/−&lt;/sup&gt; background.&lt;sup&gt;94&lt;/sup&gt;</td>
</tr>
<tr>
<td>SCARB1 (12q24.31)</td>
<td></td>
<td></td>
<td></td>
<td>Common variants associated with increased HDL-C but sex-dependent&lt;sup&gt;95-97&lt;/sup&gt;</td>
<td>High HDL-C&lt;sup&gt;66&lt;/sup&gt;; increased atherosclerosis&lt;sup&gt;90&lt;/sup&gt;</td>
<td>Severe atherosclerosis in apoE&lt;sup&gt;−/−&lt;/sup&gt;&lt;sup&gt;100&lt;/sup&gt;. siRNA: increased cholesterol uptake and decreased cholesterol efflux in CaCo-2 cells&lt;sup&gt;101&lt;/sup&gt;.</td>
</tr>
</tbody>
</table>

Individuals: −/−, homozygotes; +/− heterozygotes; comp−/+ compound heterozygotes. Mutations retrieved from HGMD professional (Human Gene Mutation Database, last accessed March 2013). apoA-I indicates apolipoprotein A I; CAD, coronary artery disease; CETP, cholesteryl ester transfer protein; Chr., chromosome; HDL-C, high-density lipoprotein cholesterol; HepG2 human liver hepatocellular carcinoma cell line LCAT, lecithin:cholesterol acyltransferase; LDLr, low density lipoprotein receptor; LIPC, hepatic lipase; LIPC, endothelial lipase; LPL, lipoprotein lipase; MI, myocardial infarction; siRNA, small interfering RNA; TG, triglycerides; THP, human acute monocytic leukemia cell line; TRL, triglyceride-rich lipoprotein; and VLDL, very-low density lipoprotein cholesterol.
In light of these observational findings, clinical trials have been performed to study whether intervention to increase HDL-C would result in reduced risk of CVD but, so far, no trials have proved to be effective. Details of these studies and those that are ongoing are discussed elsewhere in this review series.

**Complete Loss of Function of Major HDL Genes in Humans**

The number of reported families with severe HDL disorders is small and, as a consequence, it is hazardous to speculate on the risk of CVD. In the Table, we have tried summarizing the current data. Most of the mutations in **APOAI** are associated with increased CVD. However, heterozygotes of the apoA-I Milano variant exhibit low HDL-C levels but reduced premature coronary artery disease (CAD), whereas carriers of the apoA-I Paris variant have also been reported to be protected against CAD onset. Carriers of mutations in **LCAT** that cause HDL deficiency and 40% reductions of HDL-C in homozygotes and heterozygotes, respectively, have been reported to be at increased risk and decreased risk of atherosclerosis. Also, when it comes to HDL deficiency caused by mutations in **ABCA1**, evidence supporting an increased risk of CVD is unequivocal. On the contrary, cholesteryl ester transfer protein (CETP) deficiency causes strong increases in HDL-C levels. Although genetic CETP deficiency was first considered to be associated with low morbidity from CAD and longevity, this was subsequently the subject of debate. To date, only a few patients with hepatic lipase (LIPC) deficiency (encoding LIPC) have been described, whereas LIPC promoter variants are associated with elevated plasma levels of HDL-C and paradoxically increased cardiovascular risk. Because most family studies originate from index patients who were referred to the clinic, one may ask the question whether mutations in any of these genes are associated with altered CVD risk in the general population.

**Genetic Population Studies**

Because this is a topic of another review in this series, we only briefly address this topic. There is evidence that both rare and common alleles with major phenotypic effects contribute significantly to low plasma HDL-C levels in the general population. Recent whole-genome sequencing and analysis suggested that common variation contributes more to heritability of HDL-C levels than rare variation. However, it has become clear that genetic variation causing either increased or decreased levels of HDL-C is generally not associated with the anticipated low risk and higher risk of atherosclerosis. Also, results from genome-wide association studies (GWAS) have shown that variation in genes associated with HDL-C levels are not associated with CVD, whereas by contrast this is the case for variation in genes associated with low-density lipoprotein cholesterol (LDL-C) levels.

**Mendelian Randomization Studies**

This approach has been recently used to investigate whether genetically altered HDL-C levels associate with the estimated risk of cardiovascular events: genetic information is used to test for associations between intermediate phenotypes, such as HDL-C levels, and disease outcome. In 2 such studies, several single nucleotide polymorphisms (SNPs) consistently associated with high HDL-C levels were not found to be associated with cardiovascular events.

Clinical and genetic studies to date have shown that changes in HDL-C concentration are generally not associated with the anticipated outcome. Reconsidering these recent outcomes, many investigators point to the notion that the plasma level of HDL-C does not account for beneficial functions associated with HDL. This is true, but none of the HDL function parameters or biomarkers have yet provided answers why increasing HDL-C did not provide the anticipated atheroprotection. It should also be kept in mind that epidemiological studies show that it is the level of HDL-C in plasma that has prospective value. Clearly, new tools and approaches are needed to unravel how HDL and HDL-C relate to pathogenesis.

**HDL Metabolism From a Genetic Perspective**

The Figure illustrates the roles of most of the major genes involved in the genesis, conversion, and catabolism of HDL. We describe the genes involved in the biogenesis of the nascent HDL and its maturation. In the Table, we have summarized the main findings in both humans and mice.

**Biogenesis of Nascent HDL and Its Early Maturation**

**Apolipoprotein A1**

The de novo synthesis of HDL involves the secretion of apoA-I by the liver and small intestine into the circulation, followed by a largely extracellular acquisition of phospholipids (PL) and cholesterol leading to the formation of nascent HDL (step 1 in Figure). The gene is located on chromosome 11q23 and encompasses 4 exons encoding a primary transcript of 267 amino acids. Gene deletion results in extremely low levels of HDL-C in both humans and mice, respectively. Intracellularly, an 18-amino-acid pre-peptide is cleaved at the endoplasmatic reticulum by a signal peptidase, whereas an intermediate pro–apoA-I with a hexapeptide extension at its amino (N-) terminus is secreted into extracellular fluids and plasma. Subsequent cleavage of the hexapeptide produces the mature 243-amino-acid protein, which is necessary for the assembly of small disc-shaped native HDL. In vitro studies have shown that the latter reaction is mediated by bone morphogenetic protein-1. Recent studies in knockout (KO) mice have also highlighted a key role for procollagen C-proteinase enhancer-2 protein in mediating this last step of apoA-I maturation. The data suggest the mandatory involvement of a ternary complex composed of pro–apoA-I, bone morphogenetic protein-1, and procollagen C-proteinase enhancer-2 protein. Multiple other HDLs can be formed in the absence of apoA-I with roles for apoA-II, apoE, and apoA-IV but without apoA-I present HDL-C levels are generally low.

To date, 63 mutations have been identified in APOAI with >70% variants directly implicated in hypoalphalipoproteinemia. ApoA-I deficiency is a rare disorder that is characterized by the total absence of apoA-I in the circulation along with a low or absent HDL-C, whereas LDL-C and triglyceride levels are not affected. Typical clinical symptoms...
of apoA-I–deficient patients are xanthomas and mild-to-moderate corneal opacification. Heterozygote individuals present with ≈50% of normal HDL-C and apoA-I levels without explicit clinical symptoms. Although the impact of APOAI mutations is variable, they seem to cause the utmost elevation in cardiovascular risk compared with mutations in other genes implicated in HDL metabolism.

**ATP-Binding Cassette Transporter A1**

The early apoA-I lipidation with FC and phosphatidylcholine (PC) occurs on its critical interaction with ABCA1 and results in the formation of discoidal pre–β-HDL particles (step 2). Its major role in HDL biogenesis was recognized after the discovery in 1999 that lack of ABCA1 (9q31.1) causes HDL deficiency in Tangier disease. An impaired FC efflux from Tangier cells leads to intracellular accumulation of CE visible in the characteristic deposits of lipids in lymphoid organs, such as the tonsils, and can be accompanied by other clinical features including peripheral neuropathy, hepatosplenomegaly, corneal opacities, thromboctoypenia, premature myocardial infarction, or stroke. This disorder has since been diagnosed in ≈100 patients worldwide, and >170 mutations have been reported. In patients with Tangier disease, plasma levels of apoA-I are only 3% of that of controls, whereas triglyceride levels (>200 mg/dL) are increased along with reduced LDL-C (50% of normal). Heterozygotes for deleterious mutations present with half-normal levels of HDL-C and apoA-I but without apparent clinical symptoms. When ABCA1 function is impaired, apoA-I cannot be lipidated, leading to its rapid clearance from the plasma circulation, resulting in significantly reduced levels of apoA-I and the presence of only small pre–β-HDL particles. The importance of ABCA1 to maintain normal HDL-C levels in mice was illustrated by liver-specific ABCA1 KO mice showing an 80% decrease in HDL-C levels.

**Apolipoprotein M**

ApoM is an HDL-associated apolipoprotein that affects HDL biogenesis by asfeting nascent pre–β-HDL assembly through ABCA1 (step 3). Wolfrum et al demonstrated that apoM KO mice have impaired HDL interconversion and store cholesterol in large HDL. ApoM deficiency decreases plasma HDL-C concentrations by ≈25%. Furthermore, RNA-mediated knockdown of ApoM in vivo causes a reduction in pre–β-HDL. It has been shown that variation in the promoter region of ApoM gene is associated with plasma cholesterol levels, but this was not replicated. Interestingly, recently showed a specific role of apoM as a carrier of the sphingosine 1 phosphate. This sphingolipid activates 5 different G-protein–coupled receptors that affect numerous vascular functions. Recently, reported that plasma levels of sphingosine 1 phosphate in apoM KO and transgenic mice were reduced by 30% and increased by 270%, respectively. In addition, mutations in APOAI, ABCA1, or LCAT in humans reduced plasma levels of HDL-C and apoA-I as well as sphingosine 1 phosphate in an apparent gene–dose-dependent fashion. In contrast, mutations that increase plasma concentrations of both HDL-C and apoA-I did not affect sphingosine 1 phosphate levels.

**Lecithin:Cholesterol Acyltransferase**

In the circulation, nascent disc-shaped HDL is, under normal conditions, thought to mature into larger spherical HDL. This process entails acquisition of CE in its hydrophilic lipid core, a step made possible through LCAT (step 4). The gene is localized in the q21-22 region of chromosome 16 and encodes a 416-amino-acid glycoprotein that is (as apoA-I and ABCA1) expressed in the liver and small intestine where it is secreted into plasma and where it mostly associates with discoidal HDL. This enzyme hydrolyses fatty acids from PC and subsequently transfers and esterifies these to the free hydroxyl group of FC. The acquisition of CE converts disc-shaped HDL into spherical HDL that are predominant in human plasma. Through the esterification of FC, LCAT is thought to maintain a cholesterol gradient that promotes cholesterol efflux from peripheral cells to HDL. The identification of patients with HDL deficiency and abnormal cholesterol and phospholipid tissue deposition have elucidated the fundamental role that LCAT plays in human HDL metabolism.

**CTP:Phosphocholine Cytidyltransferase**

Jacobs et al showed that CTP:phosphocholine cytidyltransferase (encoded by Pcyt1a,b) regulates plasma levels of HDL-C and very-LDL (VLDL) in liver-specific cytidyltransferase alpha (α isoform) KO mice. It concerns a key enzyme in the cytidine diphosphate-choline pathway for the biosynthesis of phosphatidycholine, a vital component for the structural integrity of mammalian membranes and the primary phospholipid in plasma lipoproteins. Plasma HDL (PC, cholesterol, and apoA-I) was 50% lower in the KO mice than in the control mice, indicating that hepatic PC supply from CTα is vital for plasma HDL.

In conclusion, APOAI, ABCA1, and LCAT are key regulators of HDL metabolism. Remarkably, the loss of a single allele of any of the 3 genes cannot be compensated because all cause similar reductions of HDL-C levels. Apparently, apoA-I production, apoA-I lipidation, and CE acquisition by nascent HDL are equally important to steady-state levels of plasma HDL-C. The roles of ApoM and particularly PCTY1 in the
biogenesis of HDL have primarily been studied in mice, and there are, to our knowledge, no reports on the effects of variation in these genes on human metabolism to date.

Remodeling of HDL in the Circulation
In the circulation, several proteins and enzymes modulate HDL. In humans, these include CETP, phospholipid transfer protein (PLTP), LIPC, endothelial lipase (LIPG), and secreted phospholipase A2 (sPLA2). Mice lack CETP and sPLA2. Loss-of-function mutations in these genes can underlie either hyperalphalipoproteinemia (CETP, LIPC, sPLA2) or hypoalphalipoproteinemia (PLTP). Whereas CETP and PLTP are lipid transfer proteins without catalytic activity, the remaining players discussed in this section all exert enzymatic, that is, lipolytic functions that are thought to affect apoA-I turnover.

Cholesteryl Ester Transfer Protein
This protein accommodates the transfer of CE from HDL to apoB-containing lipoproteins in exchange for triglycerides (step 5). Once CEs are conveyed to apoB-containing lipoproteins, they are made available for uptake of LDL via hepatic receptors.7 The evidence that CETP is essential for human HDL metabolism came about with the discovery of human CETP deficiency,137,187 with 2-fold to 3-fold increases of HDL-C levels and remarkably large HDL. Heterozygous CETP deficiency results in less significant increases in HDL-C levels ranging between 10% and 35%.105,188 To date, 39 CETP (16q21) variants have been reported with most data retrieved from Japanese families. Despite the presence of frequent CETP variants with significant effects on HDL-C, the role of CETP in atherogenesis remains controversial.52,53,189 In mice, which naturally lack CETP, the introduction of the human CETP transgene decreases HDL-C and apoA-I levels,190 whereas overexpression can either increase or decrease atherosclerosis, depending on the introduction of other human genes.57,191

Phospholipid Transfer Protein
PLTP is crucial to HDL particle remodeling. As shown in step 6, PLTP facilitates the transfer of PL from TRL to HDL with the formation of both larger and smaller particles,192 whereas it can also induce fusion of smaller HDL.193 PLTP KO mice show decreased HDL-C and apoA-I levels.90,91 The role of PLTP in the transfer and exchange of PL between TRL and HDL has also been tested in animals overexpressing human PLTP. A 29% increase of PLTP activity promoted net phospholipid movement into HDL and, as a result, HDL phospholipid and FC were significantly increased.194 Thus far, studies of PLTP (20q12-q13.1) in humans are restricted to association studies showing that variation in the PLTP gene is associated with HDL-C levels89,195 but no cases of human PLTP deficiency have been described.

Hepatic Lipase
Located on chromosome 15q21, this gene encoding hepatic lipase (HL) is involved in breaking-down HDL-TG and PL, thereby reducing HDL size and enhancing the dissociation of lipid-free/lipid-poor apoA-I from larger HDL (step 7).196 Anchored to cell surface proteoglycans in humans (while circulating in mice), HL also has a bridging function promoting receptor-mediated uptake of lipoproteins.197 Complete LIPC deficiency constitutes a rare metabolic condition genetically transmitted in an autosomal recessive pattern, resulting in increased HDL-C levels attributable to decelerated HDL catabolism.199 To date, >60 individuals (8 homozygotes) have been reported worldwide. All affected individuals present with increased plasma cholesterol (>90th percentile) and TG levels and accumulation of large triglyceride-rich HDL and LDL particles. HL has been proposed to be both proatherogenic and antiatherogenic after studies in mice. Subjects with absent HL activity have been shown to have premature CAD.196

Endothelial Lipase
This gene on chromosome 18 encodes for endothelial lipase (EL) a second lipolytic enzyme. It is expressed in the liver, lung, kidney, and scalp. The enzyme has shown to exhibit more phospholipase activity than TG lipase activity with a major preference for HDL instead of TRL (step 8). It was first described in 1999 through in vitro expression studies in cells of human origin and through in vivo injection of adenovirus encoding human EL in mice. Overexpression of LIPG in mice leads to a reduction of HDL-C79,82 and apoA-I levels. In contrast, loss of EL in mice leads to significant increase in plasma HDL-C79,82 and reduced atherosclerosis.80 Like HL, EL has also been shown to be capable of bridging HDL and other lipoproteins with cell surface proteoglycans.202 An association between LIPG variation and HDL-C levels has been confirmed through GWAS77,201 and several studies suggest that mechanisms underlying the associations between the LIPG SNPs and HDL metabolism may involve loss of function203 as well as impaired secretion of EL, both resulting in elevated levels of HDL-C.205 Singaraja et al206 identified and functionally characterized several partial and complete loss-of-function LIPG mutations. Their impact on HDL-C is directly related to their effect on loss of EL function, supporting the hypothesis that antagonism of EL function would provide cardioprotection.206

Secreted Phospholipase A2
Encoding for the sPLA2 is highly expressed in the liver, particularly during acute and chronic inflammatory states.207 This enzyme hydrolyzes the sn-2 ester bond of phospholipids to release a lysophospholipid and a nonesterified free fatty acid. Overexpression of human group IIa sPLA2 in mice (naturally sPLA2-IIA deficient) results in a reduction of HDL-C levels, HDL size, and increased HDL catabolism.209 Webb et al208 recently showed that sPLA2-IIa can contribute to atherosclerotic lesion development in mice through a mechanism that is independent of systemic lipoprotein metabolism. Recently, 2 sPLA2-IIA noncoding SNPs have been shown to be functional, making them valuable tools to assess whether the relationship between sPLA2-IIA and coronary heart disease is causal.210 To our knowledge, there are no reports on mutations in sPLA2 in humans.

The genes discussed in this section all markedly affect HDL-C levels either through facilitating the transfer of neutral and phospholipids (CETP and PLTP, respectively) between HDL (and among HDL) and apoB-containing lipoproteins or by lipolysis of HDL phospholipids and triglycerides (EL and HL, respectively).
The combined local or systemic actions of these factors and those already discussed, however, do not ultimately determine the actual level of HDL-C in plasma. In this regard, it may be noted that all reports discussed to date have merely studied HDL and other lipids under fasting conditions, whereas for a large portion of the human population worldwide this has become a scarce situation. We will continue with studies describing how the catabolism of TRL affects HDL and HDL-C, although these data are, again, mainly obtained after fasting.

**Interaction of HDL With TRLs**

This section focuses on proteins and enzymes that affect HDL metabolism through their impact on plasma triglyceride lipolysis. These mostly affect the activity of lipoprotein lipase (LPL), the sole enzyme capable of hydrolyzing plasma triglycerides in plasma TRL. LPL is synthesized and secreted by parenchymal cells in metabolically active muscle and adipose tissue. At these sites, surface lipid (FC and PL) and apolipoproteins resulting from TRL hydrolysis are conveyed from TRL to HDL (step 9).

**Lipoprotein Lipase**

The LPL gene is located on chromosome 8p22 and >160 mutations have been reported. LPL deficiency is an autosomal-recessive disorder characterized by severe hypertriglyceridemia (because of the accumulation of chylomicrons) and marked decreases of HDL-C and LDL-C levels. Although homozygote patients can present with severe pancreatitis, heterozygotes do not have clinical complications and show normal-to-elevated triglyceride levels and decreased HDL-C. LPL KO mice display hypertriglyceridemia and low HDL-C levels, whereas overexpression of LPL causes an increase in HDL-C levels. Several common coding SNPs in the LPL gene have been reported to have a significant impact on HDL-C levels, and these associations are confirmed by meta-analysis and are consistent with findings from recent GWAS.

**Determinants of LPL Activity**

**Apolipoprotein CII**

For its catalytic activity, LPL needs apoC-II as cofactor, a small protein of 79 amino acids present on TRLs and HDL. Human APOCII deficiency (2 kindreds reported worldwide) is like LPL deficiency associated with chylomicronemia and low HDL-C. All defects in APOCII (19q13.2) concern nonsense mutations. Heterozygote individuals usually present with normal plasma triglyceride levels. In APOCII-deficient patients, the mature HDL subfractions have been reported to be reduced or lacking.

**Apolipoprotein AV**

ApoA-V can be considered as a modulator of LPL activity. APOAV (11q23) is expressed in the liver and the protein is secreted into plasma, where it associates with VLDL, chylomicrons, and HDL. It seems to be a key modulator of plasma TG homeostasis but the molecular mechanisms are not fully understood. ApoA-V may act by increasing LPL activity in a fashion similar to that of apoC-II, although other studies do not support this. Individuals with complete apoA-V deficiency may present with hypertriglyceridemia and low HDL-C, but the penetrance often depends on other deleterious parameters. Heterozygote individuals have normal or moderately elevated plasma TG. Remarkably, APOAV gene polymorphisms display the most significant associations with HDL-C levels when compared with genes encoding for other apolipoproteins. It may be noted, however, that APOAV is the protein of the AI-CII-AIV gene cluster that is highly polymorphic, and genetic variation may also affect the transcription of these genes. Accordingly, this gene cluster is significantly associated with both triglyceride and HDL-C levels in recent GWAS. Of note, in this regard GWAS have identified APOAI as a gene with TG as main lipid trait.

**GPI-Anchored HDL-Binding Protein-1**

The GPIHBP1 is located on chromosome 8q24.3 and encodes the glycosylphosphatidylinositol (GPI)-anchored HDL-binding protein-1 and was originally identified as an HDL-binding protein, but the finding that GPIHBP1 knockout mice have severe hypertyglyceridemia revealed an essential role for the protein in the action of LPL in capillary endothelium. In these mice, the majority of the triglycerides and cholesterol are present in large lipoproteins, whereas HDL-C levels are low. GPIhbp1 is produced in cardiac muscle, skeletal muscle, and adipose tissue, and has been suggested to facilitate LPL trafficking over the endothelium and to operate as a scaffold for LPL and its substrates at the luminal side of these cells. To date, a few point mutations and 1 large deletion in GPIHBP1 have been reported in patients who present with severe hypertriglyceridemia and low HDL-C.

**Inhibitors of the Catalytic Activity of LPL**

The LPL reaction is regulated in a spatiotemporal fashion by several inhibitory factors encoded by APOCII, angiopoietin-like 3 (ANGPTL3), and ANGPTL4, which all affect HDL metabolism.

**Apolipoprotein CIII**

APOCIII secreted from the liver and, to a lesser extent, by the intestine is a component of both HDL and TRL. Loss-of-function mutations have been associated with higher levels of HDL-C and lower levels of LDL-C and TGs. To date, 12 mutations have been described in APOCIII (11q23.3) associated with apparent cardioprotection. Overexpression of human apoC-III in mice results in hypertriglyceridemia, whereas targeted disruption of Apoc3 results in a reduction of plasma triglyceride and protection from postprandial hypertriglyceridemia. It has also been suggested that apoC-III increases the catabolism of HDL and is involved in other relevant lipid metabolic functions.

**GALNT2**

UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 2 (GalNAc-T2)-encoding polypeptide N-acetylgalactosaminyltransferase 2 has been reported to affect HDL and TG metabolism through glycosylation of apoC-III. This enzyme is involved in the regulation of the O-linked glycosylation of proteins. Common SNPs in this gene through GWAS were shown to be associated with HDL-C and TG levels. Overexpression in mouse liver reduces...
HDL-C levels, whereas silencing hepatic gene expression leads to an increased HDL-C. In 2 families, it was reported that a functional GALNT2 mutation affects HDL metabolism by accelerating postprandial TG clearance.

Angiopoietin-Like 3 and Angiopoietin-Like 4

Although ANGPTL3 (1p31.3) is secreted exclusively from the liver, ANGPTL4 (19p13.2) is primarily found in those tissues that also express LPL. Both encoded proteins can act as inhibitors of LPL activity by promoting, in different ways, the dissociation of the active LPL homodimer into inactive monomers.

Angptl4 KO mice exhibit increased LPL activity, 65% to 90% lower TG levels, slightly lower total cholesterol levels, lower HDL-C, and circulating VLDL, whereas transgenic mice have reduced postheparin plasma LPL activity and elevated plasma triglycerides.

Angptl3 KO mice also show lower plasma triglyceride and cholesterol levels. Interestingly, these mice display a counterintuitive 50% reduction in plasma levels of HDL-C. This has been explained by evidence that ANGPTL3 also inhibits EL. Thus, a resulting increase of EL activity would reduce plasma levels of HDL-C. Angptl3-deficient mice showed a significant decrease in HDL-PLs and cholesterol, which could be restored through reintroducing ANGPTL3.

Double Angptl3/Angptl4 KO mice die before birth or 2 months after showing almost undetectable low cholesterol and TG levels, therefore proving the pivotal role of Angptl3/4 in lipoprotein metabolism. Recently, it was shown that rare ANGPTL3,4 gene variants are associated with low plasma TG levels in humans and with the ability of the ANGPTL protein to inhibit LPL. In contrast to the mouse studies, loss-of-function mutations in ANGPTL3 in humans were not associated with a decrease in plasma levels of HDL-C. On the contrary, Musunuru et al found that complete ANGPTL3 deficiency in humans results in extremely low plasma levels of LDL-C, HDL-C, and TG.

Other Modulators of HDL and TG Metabolism

TRIB1 and glucuronic acid epimerase (GLCE) have also been shown to affect HDL metabolism in both human and mouse studies.

Tribbles Homolog 1

Tribbles homolog 1 is a member of the recently identified tribbles protein family, mapping within 8q24 locus and with suggested function as adaptor or scaffold protein. Minor alleles in TRIB1 SNPs have been found to be associated with lower TG, LDL-C, and higher HDL-C, and also with a significantly reduced risk of CAD. Studies in the general population highlighted the strong association between TRIB1 variation and HDL-C and a less strong, but still significant, association with TG. The mechanism by which TRIB1 affects lipid metabolism is unknown. It may be mediated through mitogen-activated protein kinase pathway, which is directly controlled by TRIB1. Burkhard et al provided evidence that TRIB1 is implicated in regulation of hepatic lipogenesis and VLDL production in mice: hepatic-specific overexpression of Trib1 reduces levels of plasma TG and VLDL, LDL-C, and HDL-C by decreasing VLDL production. Conversely, Trib1-KO mice showed elevated levels of plasma TG, VLDL, and LDL-C because of increased VLDL production, whereas HDL-C was not significantly affected. These TRIB1 studies illustrate that HDL-C is not always inversely related to TG levels in the circulation.

GLCE

GLCE is another genetic locus in the 15q21–23 region (which includes LIPC), which was recently linked to HDL-C levels in Turkish families. The gene encodes a glucuronic acid epimerase and is critically important for the biosynthesis of heparan sulfate proteoglycan, which in turn plays a major role in clearing TRLs from the plasma along with apoE. Moreover, analyses of plasma lipids in Glce−/− mice on the Apoe−/− background support the involvement of Glce in lipid metabolism.

From this section, it is clear that many factors impact HDL metabolism either through directly affecting the hydrolysis of plasma triglycerides or through modulating hepatic VLDL secretion. These observations seem to have thus far received little attention in the HDL and TG research fields, which may need to change when considering, for example, diabetic dyslipidemia characterized by decreased HDL-C and increased plasma TG.

HDL and Cellular Cholesterol Homeostasis

HDL is known for its important role in acting as an acceptor of cellular cholesterol in which major genes encoding ABCA1, ATP-binding cassette transporter G1 (ABCG1), and scavenger receptor class B type I (SR-B1) play key roles. This is discussed in detail in another review in this series. The genetics of ABCA1 have already been addressed, and this section we address studies of defects in ABCG1, SR-B1, ORPs (oxysterol-binding protein–related proteins), and lysosomal storage disorders that affect plasma HDL-C levels.

ATP-Binding Cassette Transporter G1

ABCG1 has been shown to play a fundamental role in the regulation of cellular cholesterol homeostasis through actively mediating cholesterol transport to matured HDL. The gene is located at 21q22.3, with the highest expression in the macrophages, adrenal glands, heart, lung, and spleen. Feeding ABCG1 KO mice a cholesterol-rich, high-fat diet markedly reduces plasma HDL-C levels and increases biliary cholesterol secretion. Little is known about the role of ABCG1 in human metabolism. Schou et al reported a functional ABCG1 promoter variant that associates with increased risk of myocardial infarction and ischemic heart disease in the general population but without affecting levels of HDL-C or other lipids or lipoproteins. However, described a significant association between promoter variant and HDL-C levels. More recently, interactions of ABCG1 gene variants with diet were proposed.

Scavenger Receptor Class B Member 1

The SCARB1 gene (12q24.31) encoding for the main HDL receptor is expressed mainly in steroidogenic tissues and the liver, where it controls the selective uptake of CE from HDL. In contrast to ABCA1 and G1, it mediates bidirectional flux...
of un-CE between cells and HDL,27,245,250 SR-B1 KO mice display a 2-fold increase in plasma HDL-C,28 accelerated atherogenesis, and disruption of cholesterol transport to the liver.99,251 SR-B1 overexpression in mice reduces plasma HDL-C levels.252 In mice, HDL delivers cholesterol to the adrenal gland for steroid production.253,254 Consistently, mice lacking SR-B1 show an impaired adrenal glucocorticoid stress response.255

Genetic association studies in humans show sex-dependent association with HDL-C and LDL-C levels.256,257 Several rare point mutations in SR-B1 in patients with high HDL-C levels have been functionally characterized.95–97 In one case, carriers of a functional mutation displayed augmented HDL-C levels, reduced cholesterol efflux from macrophages, and mild adrenal insufficiency.95 In a recent study, it was reported that basal, but not stimulated, corticosteroid metabolism is lassened in carriers of individuals with mutations in LCAT or ABCA1, supporting a role for HDL as a cholesterol donor for basal adrenal steroidogenesis in humans.258

Oxysterol-Binding Protein–Related Protein 8

Another gene (12q14) that has been shown to play a role in HDL metabolism is OSBPL8, a member of the ORPs family that is known to be implicated as intracellular sterol sensors that regulate cellular functions ranging from sterol, sphingolipid, and neutral lipid metabolism to vesicle transport and cell signaling.259–261 In previous studies, ORP8 has shown to affect the expression of ABCA1 and cellular cholesterol efflux,262 and with ORP8 knockdown leading to several alterations in the cellular lipidome, including increased levels of both FC and CE.263 Recently, the first Osbp8 KO mouse was generated, and Osbp8 deficiency was found to cause a significant elevation of HDL-C, choline phospholipids, and sex-specific alterations of lipid metabolism.264

Glucocerebrosidase

Gaucher disease is the most common of the lysosomal storage disorders, characterized by deficiency of the glucocerebrosidase (encoded by GBA) and resulting in accumulation of glucocerebroside in macrophages. This cellular metabolic abnormality leads to chronic systemic inflammation and a heterogeneous, multisystemic phenotype including hepato-splenomegaly, skeletal disease, and cytopenia, in addition to an abnormal cholesterol profile (HDL-C <50 mg/dL).265,266 Type 1 Gaucher disease is the most prevalent form, with >50 mutations reported to date.157 Interestingly, although carriers of one GBA mutation do not exhibit any Gaucher symptoms, significantly lower HDL-C levels have been reported.265,266

Lysosomal Acid Lipase

Lysosomal acid lipase, encoded by LIPA (10q23.2–q23.3), is a lysosomal enzyme that hydrolyzes CE and TG an dis internalized via receptor-mediated endocytosis of plasma lipoproteins. At present, 47 mutations have been reported that are responsible for Wolman disease or cholesteryl ester storage disease, characterized by low HDL-C, hyperlipidemia, hepatic fibrosis, and premature atherosclerosis.269 The mechanism responsible for low plasma HDL-C is currently unknown but is likely attributable to the reduced FC transported to the plasma membrane, which could affect ABCA1-mediated cholesterol efflux from the cell membrane to extracellular acceptors, such as lipid-poor apoA-I particles.151,270

HDL Catabolism

SR-B1, as the main high-affinity receptor for HDL, enables the selective uptake of CE from circulating HDL via apoA-I recognition.271 This occurs, however, without mediating the degradation of HDL, as is the case for LDL. In humans, plasma levels of HDL-C and apoA-I are inversely related to the catabolism of apoA-I,272 which takes place in the kidney, where lipid-poor apoA-I is initially filtered at the level of the glomerulus and subsequently is catabolized by proximal renal tubular epithelial cells. Chronic kidney disease is associated with marked reductions of plasma HDL-C.273 However, only little is known about the molecular mechanisms. A protein involved in this process is cubilin (CUBN; 10p12.31), an extracellular protein synthesized by proximal renal tubular cells and expressed at the apical surface.274 It has the capability of binding HDL and apoA-I with high affinity and interacting with a coreceptor named megalin or LDL-related protein 2 (4q35.1), a member of LDLR gene family, which facilitates uptake and degradation of apoA-I.275 Studies of cubulin deficiency in animals or humans, however, have not shown marked changes in plasma HDL-C or apoA-I levels.276

It is currently thought that the rate of renal apoA-I catabolism is determined by both apoA-I lipidation (ABCA1, LCAT) and apoA-I delipidation processes (EL, HL) as described.151

Conclusions

The unraveling of the causes of severe hypoalphalipoproteinemia and hyperalphalipoproteinemia in humans and mice and the use of candidate gene approaches have helped in discovering the major HDL pathways in the past century. These included those relating to the 3 Mendelian disorders of HDL metabolism (APOAI, ABCA1, and LCAT deficiency). These key findings have helped to develop novel therapeutic intervention methods, some of which are still undergoing study.126,277 Since 2008, GWAS have subsequently rediscovered the known genes but also have identified many additional candidate genes or genomic regions that are associated with HDL-C levels. Follow-up reports are discussed in this review. GWAS of lipid metabolism have underscored that HDL-C and TG levels in plasma can barely be considered as independent traits. We have discussed mutations (or targeted disruptions) in genes affecting either or both traits in an attempt to provide a complete picture. This review has used the genetic handholds to describe the major players in HDL anabolism and catabolism, for which studies in both humans and mice were considered. In summary, the de novo synthesis of HDL is dependent on 3 major players, respectively, APOAI, ABCA1, and LCAT, each of which confer severe HDL deficiency in case of a total gene loss. For the generation of pre–β-HDL, roles
for \textit{PCYT1}, \textit{ApoM}, and \textit{OSBPL8} are also recognized. HDL is further modulated in the circulation through lipid transfer proteins (\textit{CETP}, \textit{PLTP}) and lipolytic enzymes (encoded by \textit{LIPC}, \textit{LIPG}, \textit{sPLA2}) that affect apoA-I turnover, and mutations in these genes all markedly affect HDL-C levels. The genes that have an impact on HDL metabolism through their effect on plasma TG lipolysis in TRL and modulating hepatic VLDL secretion are, respectively, those affecting/stimulating LPL function (\textit{APOCII}, \textit{APOA-V}, and \textit{GPIHBP1}) or inhibiting LPL (\textit{APOCIII} and \textit{ANGPTL3,4}) and, finally, those for which it is currently not known what the molecular mechanisms are through which they operate (\textit{TRIB1} and \textit{GLCE}). In addition, we describe the roles of other players in the field, including \textit{OSBPL8}, \textit{GBA}, and \textit{LAL}, that affect cellular but also systemic HDL-C homeostasis. Finally, it is recognized that early lipidation of apoA-I and the lipolysis of HDL-TG and HDL-PC are the apparent major determinants of HDL/apoA-I clearance by the kidney.

**Perspectives**

During the past 14 years, HDL gene finding and candidate gene studies have not delivered major breakthroughs that may relate to the notion that there are no other major HDL genes left to be found. This fits with the fact that the molecular defects responsible for extreme HDL-C phenotypes in patients with clear clinical symptoms have, to our knowledge, all been elucidated. Another point is that several studies have now provided evidence that even in cases of extreme hypoalphalipoproteinemia or hyperalphalipoproteinemia in humans, multiple mutations combined can be responsible for these phenotypes. In other words, the HDL-C trait can be polygenic in even these extreme cases. In the respective studies, only the coding regions of a few, \( \leq 197 \), genes\(^{109}\) were investigated. As discussed in this review, \( \geq 40 \) genes are now reported to be significantly associated with plasma levels of HDL-C and this list is likely to grow, as we previously reported.\(^{105}\) However, the integration of the effects of multiple rare and common gene variants has only just begun. A recent whole-genome sequencing study provided evidence that common DNA variations can explain most of the heritability of HDL-C levels in a general population sample, whereas most of these variants were found in intergenic regions.\(^{110}\) The question is whether the genetic HDL picture is nearly complete. This is an intriguing question for especially geneticists. For the HDL scientist, it may be interesting to unravel the molecular mechanisms by which (new) candidate genes affect HDL-C. But where does one start? It is evident that, for example, the effect size of genetic variation identified through GWAS on plasma HDL-C levels is not necessarily related to the potential importance of a candidate gene. For instance, variation in the LCAT gene was indicated by GWAS as being associated with HDL-C levels but only when \( >100,000 \) individuals were studied, whereas loss of LCAT function results in HDL deficiency. This means that every candidate gene or regulating entities in intergenic regions could be relevant to the field. What complicates matters is that with the advance of genome sequencing, we are faced with hundreds of putatively functional mutations in DNA in each individual. To help prioritizing, new tools to select the most promising mutations for functional genetic studies are much needed. Coexpression analyses\(^{79}\) and metabolic profiling\(^{279}\) may give handholds to further dissect HDL metabolism.

Finally, to improve the understanding of how plasma HDL (and HDL-C) and TG relate to atherogenesis, there is, in our opinion, a need to integrate insights from both fields of research. It may help in the understanding of the pathogenesis of diabetic dyslipidemia (as seen in patients with the metabolic syndrome) characterized by high TG levels and low HDL-C. Integrating knowledge obtained through studies under fasting and nonfasting conditions with a focus on the key candidate genes may probably be a first step to take. Maybe this will help us obtain insight into which parameters determine plasma lipid fluxes that will ultimately lead to a better understanding of which pharmaceutical strategy may reduce the risk of CVD.

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**Disclosures**

None.

**References**


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