**Review**

This Review is part of a thematic series on **High-Density Lipoprotein**, which includes the following articles:

- Regulation of High-Density Lipoprotein Metabolism [Circ Res. 2014;114:143–156]
- **ATP-Binding Cassette Transporters, Atherosclerosis, and Inflammation**
  - High-Density Lipoprotein: Vascular Protective Effects, Dysfunction and Potential as Therapeutic Target
  - MicroRNA Control of High-Density Lipoprotein Metabolism and Function
  - Novel Therapies Focused on the High-Density Lipoprotein Particle
  - High-Density Lipoprotein and Atherosclerosis Regression: Evidence From Preclinical and Clinical Studies
  - Population Genetics
  - Cholesterol Efflux and Reverse Cholesterol Transport
  - Proteomics and Particles

*Alan Tall and Daniel Rader, Editors*

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**ATP-Binding Cassette Transporters, Atherosclerosis, and Inflammation**

Marit Westerterp, Andrea E. Bochem, Laurent Yvan-Charvet, Andrew J. Murphy, Nan Wang, Alan R. Tall

**Abstract:** Although recent genome-wide association studies have called into question the causal relationship between high-density lipoprotein (HDL) cholesterol levels and cardiovascular disease, ongoing research in animals and cells has produced increasing evidence that cholesterol efflux pathways mediated by ATP-binding cassette (ABC) transporters and HDL suppress atherosclerosis. These differing perspectives may be reconciled by a modified HDL theory that emphasizes the antiatherogenic role of cholesterol flux pathways, initiated in cells by ABC transporters. ABCA1 and ABCG1 control the proliferation of hematopoietic stem and multipotential progenitor cells in the bone marrow and hematopoietic stem and multipotential progenitor cell mobilization and extramedullary hematopoiesis in the spleen. Thus, activation of cholesterol efflux pathways by HDL infusions or liver X receptor activation results in suppression of hematopoietic stem and multipotential progenitor cell mobilization and extramedullary hematopoiesis, leading to decreased production of monocytes and neutrophils and suppression of atherosclerosis. In addition, macrophage-specific knockout of transporters has confirmed their role in suppression of inflammatory responses in the arterial wall. Recent studies have also shown that ABCG4, a close relative of ABCG1, controls platelet production, atherosclerosis, and thrombosis. ABCG4 is highly expressed in megakaryocyte progenitors, where it promotes cholesterol efflux to HDL and controls the proliferative responses to thrombopoietin. Reconstituted HDL infusions act in an ABCG4-dependent fashion to limit hypercholesterolemia-driven excessive platelet production, thrombosis, and athrogenesis, as occurs in human myeloproliferative syndromes. Activation of ABC transporter–dependent cholesterol efflux pathways in macrophages, hematopoietic stem and multipotential progenitor cells, or platelet progenitors by reconstituted HDL infusion or liver X receptor activation remain promising approaches to the treatment of human atherothrombotic diseases. (Circ Res. 2014;114:157-170.)

**Key Words:** atherosclerosis ■ blood platelets ■ hematopoietic stem cell ■ lipoproteins, HDL ■ macrophages ■ thrombosis

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Mechanisms and Roles of ABCA1- and ABCG1-Mediated Cholesterol Efflux

ABCA1 is primarily localized in the plasma membrane of cells. ApoA-I and other apolipoproteins such as apoE can bind directly to cell surface ABCA1. Because lipid-free apoA-I binds cholesterol relatively poorly, phospholipid efflux to apoA-I is considered to be essential for cholesterol efflux to apoA-I. The direct interaction of apolipoproteins with ABCA1 is of key importance to ABCA1-mediated cholesterol efflux; however, the detailed molecular mechanisms are still elusive and several models have been proposed.

Using a novel single-molecule fluorescence tracking technique, Nagata et al. have shown that lipid efflux to apoA-I involves the ATPase-dependent conversion of mobile ABCA1 monomers into immobile homodimers in the plasma membrane: the model proposes that ABCA1 monomers translocate lipids at the plasma membrane and form dimers; only the dimers can bind apoA-I, with one molecule of apoA-I binding to each ABCA1 molecule in the dimer. ApoA-I is subsequently lipidated, and a discoidal HDL particle containing 2 apoA-I molecules is formed. The lipiddation of apoA-I promotes its dissociation from the ABCA1 dimer, which facilitates conversion to ABCA1 monomers, that again can translocate lipids.

This model contrasts with earlier models that proposed that the lipid translocating activity of ABCA1 led to an excess of phospholipids and cholesterol in the outer leaflet of the plasma membrane, membrane bulging and interaction with apoA-I to generate a nascent HDL particle. In addition to acting at the cell surface, ABCA1 can be internalized and there is evidence that the internalization and trafficking of ABCA1 is functionally important in mediating cholesterol efflux from intracellular cholesterol pools, especially in cells that ingest large amounts of lipids such as macrophages.

ABCA1-mediated cholesterol efflux to apoA-I is essential for HDL formation. Abca1−/− mice and patients with TD who carry a homozygous mutation for ABCA1 leading to a loss of function show nearly absent plasma HDL levels. Studies in tissue-specific ABCA1 knockout mice showed that hepatic cholesterol efflux is reduced by 80% and apoA-I binding to the liver is decreased by 30% whereas enterocyte deletion resulted in a 15% decrease in HDL levels. In these studies, wild-type mice and not Abca1−/− mice were used as controls, thus a potential role of reduced ABCA1 expression in nontargeted tissues cannot be completely ruled out. ABCA1 expression in macrophages and other hematopoietic cells does not contribute to plasma HDL cholesterol levels. ABCA1 is transcriptionally induced by LXR which is activated in response to cellular oxysterol accumulation. Treatment of mice with LXR agonists leads only to a moderate increase in HDL levels, possibly reflecting upregulation of ABCA1 expression in the intestine.
but not in the liver. Recently, it has also been shown that miR-33 and miR-144 suppress hepatic ABCA1 expression. Activation of the farnesoid X receptor in the liver increases the expression of miR-144, leading to decreased ABCA1 protein and reduced HDL plasma levels. This implies that bile acids regulate plasma HDL levels through a farnesoid X receptor-miR-144-ABCA1 pathway in hepatocytes. Along with the upregulation of scavenger receptor BI by bile salts, the down-regulation of hepatocyte ABCA1 by bile salts/farnesoid X receptor/miR-144 in the postprandial state may lead to an increase in reverse cholesterol transport from the basolateral side of the hepatocyte into bile. A similar concept was originally proposed by Yamamoto et al based on the finding that probucol treatment led to downregulation of hepatocyte ABCA1 but increased reverse cholesterol transport across the hepatocyte. This was proposed as an explanation for antiatherogenic actions of probucol despite lowering of HDL cholesterol levels.

In contrast to ABCA1, ABCG1 mediates cholesterol efflux to HDL particles but not to lipid-free apolipoproteins. In addition, ABCG1 promotes efflux of certain oxysterols such as 7-ketocholesterol from cells to HDL, decreasing their toxic effects on cells, whereas both ABCA1 and ABCG1 can promote efflux of 25-hydroxycholesterol. ABCG1−/− mice showed defective macrophage cholesterol efflux to HDL and, when challenged with a high-fat diet, developed prominent macrophage cholesterol efflux with subsequent transport via the plasma compartment to the liver and feces. The lack of impact of ABCG1 expression on plasma lipoprotein levels may reflect the fact that its expression in hepatocytes is low, with most hepatic expression reflecting contributions of Kupffer and endothelial cells. According to recent studies, there are almost complete disappearance of hepatic ABCG1 expression in mice with macrophage-specific knockout of ABCG1.

ABCG1 also promotes efflux of choline phospholipids, particularly sphingomyelin, from transected cells to HDL. ABCG1-mediated cholesterol efflux to HDL is defective in cells lacking ceramide transferase which transports ceramide from the inner to the outer leaflet of the plasma membrane. The increased availability of sterol on the cytosolic membranes likely promotes rapid diffusion to the ER, leading to suppression of sterol-mediated regulatory events.

In contrast to ABCG4, the authentic cellular localization of ABCG1 is still unknown, reflecting the lack of suitable specific antibodies. Available studies have largely relied on overexpression of tagged versions of ABCG1, an approach that is notoriously prone to artifacts; for example, the localization of caveolin in endosomes was recently shown to be an artifact of overexpression. Wang et al reported localization of ABCG1 to plasma membrane, Golgi, and recycling endosomes in transfected 293 cells. Using biotinylation, trypsin digestion, and Western blotting, ABCG1 was detected in the plasma membrane of macrophages, especially when ABCG1 expression was increased by LXR activation. Macrophase deficiency of ABCG1 led to suppression of Ldlr and Hmgcr expression relative to wild-type cells and increased cholesteryl ester formation by acyl-CoA:cholesterol acyltransferase, even in the absence of acceptors in the media to promote cholesterol efflux. This suggested redistribution of cholesterol from plasma membrane to the ER, leading to suppression of sterol biosynthetic genes, independent of cholesterol efflux. Although several laboratories have reported localization of overexpressed ABCG1 in plasma membrane, Tarling and Edwards could not detect ABCG1 in plasma membrane in their overexpression system and found predominant localization to endosomes. Consistent with the observations of Wang et al, the increased ABCG1 expression led to an increase in the mature form of sterol regulatory element binding protein-2 and upregulation of sterol regulatory element binding protein-2 target genes. It was proposed that ABCG1 may facilitate the movement of sterols away from the ER, thus increasing sterol regulatory element binding protein-2 processing and relieving the sterol-mediated inhibition of sterol regulatory element binding protein-2 processing. Studies using a fluorescent cholesterol derivative cholesterol tetratrienol in Abca1−/−Abcg1−/− macrophages demonstrated that ABCA1 and ABCG1 jointly promote movement of sterol from the inner to the outer leaflet of the plasma membrane. Thus, in the absence of the transporters, there is increased accumulation of sterol on the inner leaflet of the plasma membrane. The increased availability of sterol on the cytosolic surfaces of the plasma membrane or intracellular organelles membranes likely promotes rapid diffusion to the ER, leading to sterol-mediated regulatory events.

Although the precise cellular localization of ABCG1 remains unknown, a unifying hypothesis to explain its cellular effects is that ABCG1 promotes the flopping of sphingomyelin, cholesterol, and certain oxysterols across various cellular membranes, possibly including the Golgi, endosomes, and plasma membrane. The depletion of sterol of the membrane cytosolic surface creates a cholesterol and oxysterol chemical gradient that leads to diffusional removal of sterols from the ER and the anticipated regulatory events. Whether ABCG1 acts directly in the plasma membrane or like ABCG4 acts intracellularly to influence plasma membrane lipid organization and cholesterol availability to HDL, the net result is increased availability of sterols at the cell surface, where they can be picked up by HDL.

Even though mouse and human ABCG1 are highly conserved, a recent study suggested that human ABCG1 does not mediate cholesterol efflux from macrophages to HDL because suppression of ≈80% of ABCG1 accomplished by small
interfering RNA in human macrophages seemed not to reduce cholesteryl efflux. An independent study performed under similar conditions showed that ≈80% decreased expression of ABCG1 decreased cholesteryl efflux to HDL by ≈50%. The reasons for the discrepant results are unclear. However, levels of net cholesteryl efflux were 10-fold lower, and no significant increase in cholesteryl ester in the media (suggesting that HDL did not contain active lecithin-cholesterol acyltransferase) was detected in the first study in contrast to the second study. Moreover, a third group also showed decreased cholesteryl efflux to HDL in the context of specifically decreased ABCG1 expression in human macrophages.

As pointed out above, macrophage Abca1 and Abcg1 expression are transcriptionally regulated by LXR. LXRs are activated by certain oxysterols and also sterols such as desmosterol, a precursor of cholesteryl in the cholesterol biosynthetic pathway. Oxysterols are formed after cells take up sterols through enzymatic reactions with cholesteryl hydroxylases. The most important LXR-activating oxysterols are thought to be 20S-, 22R-, 24S-, 25-, and 26-hydroxycholesterol, thus controlling ABCA1, ABCG1, and HDL suppress inflammatory signaling. Although it is clear that macrophage foam cell formation and macrophage inflammation are both central processes in atherogenesis, the detailed mechanisms linking these processes remain incompletely understood. There is strong evidence that Abca1, Abcg1, and HDL act to suppress inflammatory signaling via Toll-like receptors (TLRs). Mouse Abca1<sup>−/−</sup>, Abcg1<sup>−/−</sup>, and Abca1<sup>−/−</sup>Abcg1<sup>−/−</sup> peritoneal macrophages showed increased expression of inflammatory cytokines and chemokines when challenged with ligands for TLR2, 3, or 4. Compared with wild-type cells, Abca1<sup>−/−</sup> knockout macrophages showed increased cell surface levels of TLR4/myeloid differentiation protein 2 (MD-2) complexes and increased signaling via the MyD88 pathway in response to lipopolysaccharide (Figure 1A). The increased cell surface level of TLR4/MD-2 may reflect decreased internalization in response to lipopolysaccharide. Transporter deficiency was associated with increased plasma membrane cholesteryl toxin B binding. Effects of transporter deficiency were exaggerated by cholesteryl loading and abrogated by cholesteryl removal.

A key property of TLR inflammatory signaling is transpression of the expression of LXR target genes, mediated by LXR and ATP-binding cassette (ABC) transporter expression in macrophages. A. Minimally modified low-density lipoprotein (mmLDL) or lipopolysaccharide (LPS) activates TLR4, leading to (1) interferon regulatory factor 3 (IRF-3)–mediated transpression of LXR and (2) MyD88-mediated nuclear factor (NF)κB activation in the nucleus (white circle). As a consequence, ABCA1/G1 expression is reduced and cholesteryl (shown as black dots) accumulates in lipid rafts in the membrane, which enhances TLR4 surface expression, thus amplifying TLR4 signaling and increasing inflammatory gene expression. B, LXR is activated, thus activating mRNA transcription of ABCA1 and ABCG1, which mediate cholesteryl efflux to high-density lipoprotein (HDL). As a consequence, less cholesteryl accumulates in the membrane, decreasing TLR4 surface expression. LXR also transrepresses NFκB target gene activation. Both processes reduce inflammatory gene expression.

Splenic Abca1<sup>−/−</sup>Abcg1<sup>−/−</sup> macrophages was observed, in particular of M-csf and Mcp-1, contributing to increased monocyte chemoattractant protein 1 (MCP-1) and macrophage colony-stimulating factor plasma levels. Surprisingly, in a recent study from the Glass laboratory, in vivo cholesterol loading of peritoneal macrophages was associated with suppression of inflammatory gene expression. This was linked to concomitant accumulation of desmosterol, an LXR activator, and induction of LXR target genes including Abca1 and Abcg1. The authors speculated that in the milieu of the atherosclerotic plaque, factors exogenous to macrophage foam cells must induce inflammation, overcoming the anti-inflammatory effects of LXR activation. Earlier studies provide strong clues that relevant exogenous factors likely include modified forms of LDL acting via pattern recognition receptors, such as TLR4, 6, and CD36, to activate inflammatory signaling.

A key property of TLR inflammatory signaling is transpression of the expression of LXR target genes, mediated by interferon regulatory factor 3 (Figure 1A). Thus, it is likely that in the normal inflammatory plaque milieu, expression of Abca1 and Abcg1 is relatively suppressed. Indeed while performing atherosclerosis regression studies, the Fisher laboratory noted that plaque macrophage Abca1 expression is initially low but becomes rapidly induced when the atherosclerotic segment is transplanted into a low plasma cholesterol environment. Although there is limited information, one study suggested low expression of ABCA1 in human atherosclerotic plaques. Overall, there may be a balance between...
activity of TLRs and LXRs within macrophages of atherosclerotic plaques; the levels of expression of ABCA1 and ABCG1 may have a central role in suppressing the activation of TLRs by modified LDL and other factors (Figure 1A and 1B). Based on this model, one successful therapeutic approach would be to decrease TLR responses most obviously by decreasing plasma LDL levels or perhaps LDL modifications that induce TLR signaling. In addition, targeting specific aspects of the inflammatory response could be beneficial, such as interleukin (IL)-1 antagonism or antagonism of signaling downstream of the IL-6 receptor, which has been implicated in coronary heart disease in a recent meta-analysis. However, a challenge to these latter approaches is redundancy in inflammatory pathways and potential immunosuppression. Macrophage-specific targeting of LXR/retinoid X receptor would seem to be an ideal approach leading to induction of cholesterol efflux pathways and suppression of inflammatory responses (Figure 1B).

HDL is also able to suppress innate inflammatory responses mediated by TLR signaling. In part, this may be mediated by promotion of cholesterol efflux via ABCA1/G1 (Figure 1B). However, higher concentrations of HDL are still able to suppress inflammation potently even in macrophages lacking ABCA1 and ABCG1. A particular role of HDL in suppressing type 1 interferon responses mediated by Toll/IL-1 receptor domain-containing adaptor inducing interfero (TRIF) signaling from endosomes has been suggested. Thus, various strategies to increase HDL may have benefit on plaque inflammation, in part, by promotion of cholesterol efflux via ABCA1/G1, but also likely by incompletely understood mechanisms that may operate independently of cholesterol efflux.

TD and Cardiovascular Risk

Patients with TD are homozygous ABCA1 mutation carriers who display a loss of function of the ABCA1 protein and near absent HDL levels. Heterozygous ABCA1 mutation carriers have ≈50% decreased HDL levels. Based on their HDL phenotype, increased CVD was expected in ABCA1 mutation carriers. However, the reports on CVD in patients with TD are variable. Whereas some individuals with TD display striking premature atherosclerotic CVD, other patients with TD seem to be spared. The contradictory findings on the association of TD with atherosclerosis could be explained by reduced plasma LDL levels in patients with TD, as well as a compensatory increase in expression of ABCG1 and modification of the complex atherogenic response by other genetic and environmental factors. Mechanisms reported to underlie the increased atherosclerosis in patients with TD include decreased ABCA1-mediated cholesterol efflux to a residual level of ≈20% to 30% and monocyte and neutrophil activation as assessed by expression levels of CD11b and without effects on blood monocyte/neutrophil levels and endothelial dysfunction. In heterozygous ABCA1 carriers, either increased CVD or no CVD phenotype has been reported. It has been suggested that the lack of CVD phenotype in some heterozygotes is attributable to a higher level of residual cholesterol efflux compared with the heterozygotes with increased CVD. This is supported by a study in ABCA1 heterozygotes in which there was an inverse correlation between cholesterol efflux and carotid atherosclerotic plaque burden as assessed by carotid intima-media thickness. A recent study showed increased atherosclerosis in ABCA1 heterozygotes as assessed by carotid MRI, which is a more specific method for measuring atherosclerotic plaque burden than carotid intima-media thickness, thus further corroborating the increased atherosclerosis in ABCA1 heterozygotes.

Conflicting results have been reported for the correlation of CVD effects with ABCA1 missense variants associated with partial loss of function and moderate effects on cholesterol efflux and HDL levels. In a Mendelian randomization approach in a prospective cohort comprising ≈9000 individuals, heterozygosity for the ABCA1 mutation K776N led to a 2-to-3 times higher risk of ischemic heart disease. Furthermore, 5 single-nucleotide polymorphisms (SNPs) in ABCA1 (V771M, V825I, I883M, E1172D, R1587K) were shown to predict risk of ischemic heart disease in a cohort of 9259 individuals. However, the same group reported more recently that heterozygosity for 4 loss-of-function mutations (P1065S, G1216V, N1800H, R2144X) was not associated with a higher risk of ischemic heart disease in 3 prospective cohorts comprising 56886 individuals. It must be noted, however, that only small decreases in HDL, of ≈28% as opposed to ≈50% in previously reported ABCA1 heterozygotes, were observed. Also, the residual cholesterol efflux was substantial (74%–79% for P1065S and G1216V and 48%–49% for N1800H and R2144X for homozygous mutations compared with controls), whereas in patients with TD there was only 20% to 30% residual cholesterol efflux. In addition, LDL levels were reduced by ≈25%, probably offsetting the effects of reduced HDL on CVD. Thus, the conflicting results in these studies could be related to inclusion of relatively mild ABCA1 mutations as well as offsetting effects of reduced LDL cholesterol levels.

In a meta-analysis of genome-wide association studies, SNPs near the ABCA1 gene have been associated with HDL and total cholesterol levels, but not with cardiovascular risk. Although these studies have the benefit of huge statistical power, some caution is merited in the interpretation of findings. The effects of SNPs on HDL is often small, possibly resulting in an underestimation of the association of the SNPs with cardiovascular risk. Most SNPs result in modest changes of the HDL distribution, which are typically under-represented in genome-wide association studies. Also, the small effect sizes of SNPs on HDL levels introduce the possibility of confounding effects related to lifestyle, medication, or ethnicity.

There is much less known concerning the association of ABCG1 with cardiovascular risk in humans. One ABCG1 variant (g.376C→T) leading to a partial loss of function (≈40%) has been identified, which was associated with an increased risk for myocardial infarction and ischemic heart disease in a combined cohort from the Copenhagen Ischemic Heart Disease and the Copenhagen City Heart Study.

Role of ABCA1 and ABCG1 in Atherosclerosis: Studies in Mouse Models

Studies on the roles of ABCA1 and ABCG1 in atherosclerosis are summarized in the Table and illustrated in Figure 2. Whole-body ABCA1 deficiency in mice on a proatherogenic background of a high-cholesterol diet leads to greater lesion size and earlier lesion formation in the aortas of ABCA1-deficient mice compared with wild-type controls. Mutations in ABCG1 contribute to atherosclerosis in mice. Mutations in ABCG1 contribute to atherosclerosis in mice. Mutations in ABCG1 contribute to atherosclerosis in mice.
Apoe<sup>−/−</sup> or Ldlr<sup>−/−</sup> background does not increase atherosclerotic lesion area, probably attributable to the markedly decreased (≈65%–73%) LDL cholesterol levels. Hepatic ABCA1 deficiency, leading to ≈50% decreased HDL levels and only ≈30% decrease in LDL levels, increases atherosclerosis ≈75% in Apoe<sup>−/−</sup> mice.

### Table. Atherosclerosis Studies in Mouse Models Deficient in Abca1 or Abcg1 Expression or Both

<table>
<thead>
<tr>
<th>Authors</th>
<th>Mouse Model and Genetic Background</th>
<th>Diet and Time on Diet</th>
<th>(V)LDL Cholesterol</th>
<th>HDL Cholesterol</th>
<th>Lesion Area</th>
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<tr>
<td>Aiello et al&lt;sup&gt;115&lt;/sup&gt;</td>
<td>Apoe&lt;sup&gt;−/−&lt;/sup&gt;Abca1&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Chow, 12 wk</td>
<td>≈50%↓</td>
<td>100%↓</td>
<td>nc</td>
</tr>
<tr>
<td>Aiello et al&lt;sup&gt;115&lt;/sup&gt;</td>
<td>Apoe&lt;sup&gt;−/−&lt;/sup&gt;Abca1&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>0.15% chol, 20% fat, 17 wk</td>
<td>≈65%↓</td>
<td>100%↓</td>
<td>nc</td>
</tr>
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<td>Aiello et al&lt;sup&gt;115&lt;/sup&gt;</td>
<td>Ldlr&lt;sup&gt;−/−&lt;/sup&gt;Abca1&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Chow, 20 wk</td>
<td>≈50%↓</td>
<td>100%↓</td>
<td>nc</td>
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<td>Ldlr&lt;sup&gt;−/−&lt;/sup&gt;Abca1&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>0.15% chol, 20% fat, 20 wk</td>
<td>≈73%↓</td>
<td>100%↓</td>
<td>nc</td>
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<td>Brunham et al&lt;sup&gt;45&lt;/sup&gt;</td>
<td>Apoe&lt;sup&gt;−/−&lt;/sup&gt;AlbCreAbca1&lt;sup&gt;fl/fl&lt;/sup&gt;</td>
<td>Chow, 12 wk</td>
<td>≈30%↓</td>
<td>≈50%↓</td>
<td>≈75%†</td>
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<tr>
<td>Aiello et al&lt;sup&gt;115&lt;/sup&gt;</td>
<td>Apoe&lt;sup&gt;−/−&lt;/sup&gt;Abca1&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Chow, 12 wk</td>
<td>nc</td>
<td>nc</td>
<td>≈50%†</td>
</tr>
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<td>van Eck et al&lt;sup&gt;116&lt;/sup&gt;</td>
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<tr>
<td>Baldán et al&lt;sup&gt;117&lt;/sup&gt;</td>
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<td>nc</td>
<td>≈40%↓</td>
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<tr>
<td>Ranalette et al&lt;sup&gt;118&lt;/sup&gt;</td>
<td>Abcg1&lt;sup&gt;−/−&lt;/sup&gt;BMM→Ldlr&lt;sup&gt;−/−&lt;/sup&gt;</td>
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<td>nc</td>
<td>≈24%↓</td>
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<td>nc</td>
<td>≈20%↓</td>
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<td>Westerterp et al&lt;sup&gt;122&lt;/sup&gt;</td>
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<td>nc</td>
<td>nc</td>
<td>≈2.2-fold†</td>
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<td>Yvan-Charvet et al&lt;sup&gt;123&lt;/sup&gt;</td>
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<td>1.25% chol, 7.5% fat, 0.5% cholate, 12 wk</td>
<td>nc</td>
<td>nc</td>
<td>≈19-fold†, Il-1, Il-6, Mcp1, Mip1α mRNA↑</td>
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<td>Out et al&lt;sup&gt;120&lt;/sup&gt;</td>
<td>Abca1&lt;sup&gt;−/−&lt;/sup&gt;Abcg1&lt;sup&gt;−/−&lt;/sup&gt;BMM→Ldlr&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>0.25% chol, 15% fat, 6 wk</td>
<td>≈75%↓</td>
<td>nc</td>
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<td>Westerterp et al&lt;sup&gt;124&lt;/sup&gt;</td>
<td>Abca1&lt;sup&gt;−/−&lt;/sup&gt;Abcg1&lt;sup&gt;−/−&lt;/sup&gt;BMM→Ldlr&lt;sup&gt;−/−&lt;/sup&gt;</td>
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<td>nc</td>
<td>nc</td>
<td>≈2.7-fold†</td>
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<td>LysmCreAbca1&lt;sup&gt;fl/fl&lt;/sup&gt;Abcg1&lt;sup&gt;−/−&lt;/sup&gt;BMM→Ldlr&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Chow, 20 wk</td>
<td>nc</td>
<td>nc</td>
<td>≈73%†</td>
</tr>
<tr>
<td>Westerterp et al&lt;sup&gt;124&lt;/sup&gt;</td>
<td>LysmCreAbca1&lt;sup&gt;fl/fl&lt;/sup&gt;Abcg1&lt;sup&gt;−/−&lt;/sup&gt;BMM→Ldlr&lt;sup&gt;−/−&lt;/sup&gt;</td>
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<td>nc</td>
<td>nc, Mcp1, Mip1α mRNA↑</td>
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BM indicates bone marrow; chol, cholesterol; HDL, high-density lipoprotein; (V)LDL, very-low-density lipoprotein; and nc, not changed.
Bone marrow (BM) transplantation studies were performed to study the role of hematopoietic ABCA1 in atherogenesis. BM Abca1 deficiency moderately increased atherosclerosis in Apoe−/− or Ldlr−/− mice. Although these studies were interpreted as showing that macrophage ABCA1 deficiency was proatherogenic, a macrophage-specific ABCA1 knockout showed no effect on atherosclerotic lesion area. These findings suggested that BM Abca1 deficiency in nonmacrophage hematopoietic cells could be contributing to accelerated atherosclerosis.

The role of hematopoietic Abcg1 in atherogenesis was investigated in 3 different studies in Ldlr−/− mice transplanted with Abcg1−/− BM on a Western-type diet. Whereas 1 study showed increased atherosclerosis in mice transplanted with Abcg1−/− BM, BM Abcg1 deficiency decreased atherosclerosis in the 2 other studies. The reason for the discrepancy in the outcomes of these studies is still not completely clear. A later study by the same group found no effect of BM Abcg1 deficiency in atherosclerosis, leading to the proposal that different results could be related to the time of the diet feeding. The decreases in atherosclerosis were attributed to increased oxidized LDL (oxLDL)−induced Abcg1−/− macrophage apoptosis and increased expression of ABCA1 and increased apoE secretion in Abcg1−/− macrophages. A subsequent study suggested that the oxLDL-induced apoptosis in Abcg1−/− macrophages was because of the accumulation of oxysterols such as 7-ketocholesterol. 7-Ketocholesterol is a major component of oxLDL and is found in atherosclerotic plaques. ABCG1 mediated the efflux of 7-ketocholesterol to HDL, protecting macrophages from oxLDL-induced apoptosis. The combined deficiency of Abcg1 and Apoe in hematopoietic tissues was associated with reduced atherosclerosis compared with Apoe−/− controls, increased susceptibility of macrophages to oxysterol-induced apoptosis, and a marked increase in macrophage apoptosis in lesions.

ABCG1 is also highly expressed in human aortic endothelial cells and human umbilical vein endothelial cells, where it mediates cholesterol and 7-ketocholesterol efflux to HDL. Human aortic endothelial cells and human umbilical vein endothelial cells show almost no expression of ABCA1 and no cholesterol efflux to lipid-free apoA-I. However, studies in aortic endothelial cells subjected to laminar shear flow conditions showed upregulation of LXRα expression and induction of its target genes Abca1 and Abcg1, thus suggesting that under conditions that simulate the in vivo environment of endothelial cells, both ABCA1 and ABCG1 may be highly expressed. Overexpression of human ABCA1 in mouse endothelium decreased atherosclerosis, concomitant with decreased mRNA expression of proatherogenic CXC motif ligand 1 and tumor necrosis factor superfamily 10 in the aorta. Surprisingly, HDL levels were increased by ±40% in these mice, potentially caused by a 2.6-fold increase in endothelial cell cholesterol efflux to apoA-I compared with controls.

In contrast to the results with hematopoietic Abcg1 deficiency, vascular Abcg1 deficiency resulting from transplantation of wild-type BM in Abcg1−/− Ldlr−/− mice resulted in accelerated atherosclerosis compared with Ldlr−/− controls transplanted with wild-type BM. Vascular Abcg1 deficiency was associated with decreased endothelium-dependent vasorelaxation. The increased atherosclerosis was thus likely attributable at least in part to decreased NO bioavailability attributable to endothelial Abcg1 deficiency. NO has an atheroprotective role in endothelial cells in part by decreasing the expression of adhesion molecules and proinflammatory cytokines that enhance monocyte adhesion. Abcg1−/− endothelial cells have been shown to exhibit increased secretion of MCP-1 and IL-6 as well as increased surface expression of intracellular adhesion molecule-1 and E-selectin concomitant with a 4-fold increase in monocyte adhesion (Figure 2, step D). The decreased endothelial NO synthase (eNOS) activity on Abcg1 deficiency may have been attributable in part to accumulation of oxysterols and cholesterol. 7-Ketocholesterol accumulation in endothelial cells was shown to generate reactive oxygen species that combined with NO to form peroxynitrite, which disrupts eNOS dimers that are required for its activity. Cholesterol accumulation enhanced the inhibitory interaction between eNOS and caveolin-1, leading to decreased eNOS activity.
Although endothelial ABCG1 thus seemed to have a major role in preserving eNOS activity, endothelium-dependent vasorelaxation was also mildly decreased in Abca1−/− mice on a cholesterol-rich diet and further decreased in Abca1−/−Abcg1−/− mice compared with Abcg1−/− mice on the same diet, thus suggesting that endothelial ABCA1 may also play a role in preserving endothelial function and have an atheroprotective role in addition to endothelial ABCG1.

**Studies in Mice With Combined Abca1/g1 Deficiency**

**Atherosclerosis Studies**

In general, Abca1−/−Abcg1−/− mice display more dramatic phenotypes than Abca1−/− or Abcg1−/− mice, reflecting the fact that ABCA1 and ABCG1 have overlapping functions and display mutual compensation. To study the role of ABCA1/G1 in atherogenesis in vivo, Ldlr−/− mice were transplanted with Abca1−/−Abcg1−/− BM. On a high-cholesterol, bile salt diet, mice with Abca1−/−Abcg1−/− BM deficiency displayed markedly increased atherosclerosis, compared with mice transplanted with wild-type, Abca1−/−, or Abcg1−/− BM. Abca1−/−Abcg1−/− macrophages showed a ≈70% decrease in macrophage cholesterol efflux to HDL, increased inflammatory gene expression, and increased free cholesterol or oxLDL-induced apoptosis.

In another transplantation study of Abca1−/−Abcg1−/− BM into Ldlr−/− mice and WTD feeding, combined BM Abca1/g1 deficiency did not lead to an increase in atherosclerosis compared with the control group. However, in contrast to the earlier study, cholesterol levels were decreased by ≈75% in the Abca1−/−Abcg1−/− BM recipients, suggesting susceptibility to atherosclerosis at a lower threshold of plasma cholesterol levels than seen in control mice. Because these mice were homozygously deficient for the Ldlr, in contrast to heterozygous Ldlr mice in the previous study, less very-low-density lipoprotein/LDL cholesterol was being cleared by the liver than in the Ldlr−/− mice, and the very-low-density lipoprotein/LDL may have been taken up by macrophages and monocytes deficient in Abca1/g1.

Interestingly, Abca1−/−Abcg1−/− mice exhibited a dramatic ≈5-fold increase in blood monocyte and neutrophil counts, as well as infiltration of the spleen, lung, liver, and small intestine with myeloid cells including macrophage foam cells and neutrophils, a phenotype suggestive of a myeloproliferative syndrome. The increased blood leukocyte counts reflected a 5-fold expansion of the HSPC population in the BM. This expansion was caused by enhanced proliferation probably attributable to increased cell surface expression of the common β subunit that is shared by IL-3, IL-5, and granulocyte-macrophage colony-stimulating factor receptors. Thus, the increased atherosclerosis in mice transplanted with Abca1−/−Abcg1−/− BM may have been partly caused by HSPC expansion and the associated increased numbers of blood monocytes and neutrophils (Figure 2, step A). Increased monocyte and neutrophil counts are well known to be associated with increased CVD in humans. Expression of the human APOA1 transgene reversed the accelerated atherosclerosis in Ldlr−/− mice transplanted with Abca1−/−Abcg1−/− BM concomitant with decreased expression of the common β subunit, diminished proliferation of HSPCs, and reversal of monocytosis to the level of the control group. This suggested that markedly elevated apoA-I and HDL levels could act independent of ABCA1 or ABCG1 to promote cholesterol efflux from HSPCs and macrophages, presumably via passive diffusion or scavenger receptor BI facilitated efflux. Thus, in the setting of hypercholesterolemia, cholesterol efflux pathways mediated by ABCA1, ABCG1, and HDL act to suppress the proliferation of HSPCs and the resultant monocytosis and neutrophilia.

Apoε−/− mice were shown to have increased blood monocyte and neutrophil counts, especially when fed a high-fat, high-cholesterol diet. This was associated with expansion and proliferation of the HSPC population. ApoE is highly expressed on the surface of HSPCs, where it acts in an ABCA1/G1-dependent fashion to promote cholesterol efflux. Apoε−/− mice also have increased expression of the common β subunit on the surface of HSPCs, promoting HSPC proliferation, monocytosis, and increased entry of monocytes into atherosclerotic plaques.

To assess the role of cholesterol efflux pathways in different populations of myeloid cells, we developed Abca1fl/flAbcg1fl/fl mice. When crossed with the LysmCre strain, these mice displayed efficient (≈95%) deletion of ABCA1 and ABCG1 in macrophages but no deletion in HSPCs. Atherosclerosis was increased by 73% in chow-fed Ldlr−/− mice transplanted with LysmCreAbca1fl/flAbcg1fl/fl BM, in the absence of monocytosis or HSPC expansion. This result established a role for macrophage cholesterol efflux mediated by ABCA1/G1 in suppressing atherosclerosis. Analysis of lesional inflammatory gene expression by laser capture microdissection revealed increased expression of Mcp-1 and other inflammatory genes (Figure 2, step E), similar to observations in Abca1−/−Abcg1−/− peritoneal macrophages treated with TLR4 ligands. In a parallel experiment, Ldlr−/− mice transplanted with Abca1−/−Abcg1−/− BM displayed a more pronounced 2.7-fold increase in atherosclerosis, in association with HSPC expansion and monocytosis.

The more dramatic atherosclerosis phenotype in these mice suggested a role of cholesterol efflux pathways in HSPCs as well as in macrophages in the suppression of atherosclerosis. When Ldlr−/− mice transplanted with LysmCreAbca1fl/flAbcg1fl/fl BM were fed a high-fat, high-cholesterol diet, they showed ≈2-fold increases in monocytes and neutrophils, concomitant with increased expression of Mcs-f, Mcp-1, and G-csf in splenic Abca1−/−Abcg1−/− macrophages and increased macrophage colony-stimulating factor, MCP-1, and granulocyte colony-stimulating factor plasma levels. Macrophage colony-stimulating factor and granulocyte colony-stimulating factor stimulate granulocyte macrophage progenitor–mediated monocyte and neutrophil production, respectively (Figure 2, step C). Increased HDL levels achieved by expression of the human APOA1 transgene reversed the increased macrophage colony-stimulating factor, MCP-1, and granulocyte colony-stimulating factor and the associated monocytosis and neutrophilia, indicating that HDL also suppresses inflammation independent of the ABC transporters. Thus, macrophage cholesterol efflux pathways mediated by ABCA1, ABCG1, and HDL suppress inflammation and the resulting monocytosis and neutrophilia.
Extramedullary Hematopoiesis

Extramedullary hematopoiesis involves mobilization of HSPCs from the BM via the blood into the spleen and other organs. In Apoe−/− mice, extramedullary hematopoiesis involving proliferation of granulocyte macrophage progenitors has been shown to produce monocytes that infiltrate atherosclerotic plaques, thus promoting lesion progression. Abca1−/−Abcg1−/− mice also exhibited splenomegaly and extramedullary hematopoiesis; Abca1−/−Abcg1−/− and Apoe−/− mice displayed increased HSPC mobilization from the BM to the spleen (Figure 2, step B). Cell-specific knockout models revealed that the mechanism underlying this phenomenon increased IL-23 secretion from Abca1−/−Abcg1−/− macrophages and dendritic cells as a result of upregulation of the TLR4 and TLR3 pathways in these cells. Whereas splenic Abca1−/−Abcg1−/− macrophages and dendritic cells were shown to have a major contribution to IL-23 secretion, other Abca1−/−Abcg1−/− peripheral macrophages or dendritic cells, such as those in the adipose tissue, also may have contributed to the increased IL-23 levels in these cell-specific knockout models. IL-23 secretion is also regulated by granulocyte-macrophage colony-stimulating factor that signals through the common β subunit which is increased on Abca1−/− Abcg1 deficiency. IL-23 is known to initiate a signaling cascade leading to enhanced production of IL-17 by Th17 cells and granulocyte colony-stimulating factor by BM stromal cells, thus directing granulocyte macrophage progenitors in the BM toward neutrophil production rather than monocyte/macrocyte production. This subsequently decreases the abundance of osteoblasts and nestin mesenchymal stem cells that express CXCL12, which is a key retention ligand for CXCR4 on HSPCs. Thus, the BM niche is altered, decreasing its ability to retain HSPCs and HSPCs are mobilized to organs, including the spleen. Increasing HDL via the human APOA1 transgene, or by infusion of reconstituted HDL (rHDL), suppressed HSPC mobilization in several different mouse models including Apoe−/− as well as mouse models of acute myeloid leukemia. The suppression of HSPC mobilization and extramedullary hematopoiesis represent additional potential therapeutic effects resulting from the activation of cholesterol efflux pathways. These may be particularly relevant in the setting of acute coronary syndromes, where sympathetic nervous system activation leads to mobilization of HSPCs, contributing to extramedullary hematopoiesis and atherogenesis.

ABCG4 in Thrombosis and Atherosclerosis

In contrast to the extensive studies on ABCA1 and ABCG1, relatively little is known about the function of ABCG4, a transporter highly homologous to ABCG1. Earlier studies demonstrate that ABCG4, like ABCG1, promotes cholesterol efflux to HDL when overexpressed in cultured cells. Both ABCG1 and ABCG4 are highly expressed in brain and promote efflux of cholesterol and other sterols to lipid poor discoidal HDL particles. Combined ABCG1 and ABCG4 deficiency results in increased levels of several oxysterols in the brain, in association with decreased cholesterol biosynthesis and repressed expression of several cholesterol response genes such as 3-hydroxy-3-methylglutaryl coenzyme A reductase and the LDL receptor. Deficits in memory have been reported in Abcg4−/− mice. However, ABCG4 is not expressed in macrophages and ABCG4 deficiency does not affect macrophage cholesterol efflux.

In a recent study, it was found that ABCG4 was selectively expressed in MkPs, type a progenitor cell in megakaryocyte/platelet lineage. Little ABCA1 or ABCG1 was expressed in these cells. In MkPs, ABCG4 staining colocalized with trans-Golgi markers. ABCG4-deficient MkPs showed defective cholesterol efflux to HDL and increased free cholesterol accumulation, with prominent accumulation in plasma membrane. Thus, even though localized in the Golgi, ABCG4 deficiency resulted in defective cholesterol efflux to HDL and an increase in cell cholesterol content including in the plasma membrane, consistent with studies suggesting segregation of sterol-rich plasma membrane domains in the trans-Golgi.

BM ABCG4 deficiency led to accelerated atherosclerosis and arterial thrombosis in hypercholesterolemic Ldlr−/− mice, in association with increased platelet counts, increased reticulated platelets, platelet/leukocyte complexes, and platelet-derived microparticles, with proven proatherosclerotic and prothrombotic properties. Abcg4−/− MkPs showed increased proliferation in response to thrombopoietin (TPO), the most important growth factor regulating megakaryocyte/platlet lineage development in vivo, and increased numbers of megakaryocytes in the BM and spleen. There were increased levels of c-MPL, the TPO receptor, on the surface of Abcg4−/− MkPs and markedly enhanced increases in platelet counts in response to TPO injection.

The increased cell surface c-MPL levels in Abcg4−/− MkPs were because of blunting of the negative feedback regulation of c-MPL in response to TPO and involved a defective activation of Lyn kinase and c-CBL E3 ligase. Lyn kinase, a palmitoylated membrane protein, seems to act as a membrane cholesterol sensor. Increased membrane cholesterol in Abcg4−/− MkPs may increase Lyn association with the membrane and decrease its tyrosine kinase activity in response to TPO, causing defective phosphorylation of c-CBL. This disrupts the negative feedback regulation of c-MPL and leads to increased platelet production.

Infusion of rHDL reduced MkP proliferation and platelet counts in wild-type mice but not in Abcg4−/− mice. The therapeutic potential of rHDL infusions in the control of platelet overproduction was exemplified by the finding that in a mouse model of essential thrombocythemia induced by BM cell expression of a mutant form of c-MPL found in human subjects with essential thrombocythemia, rHDL reduced the platelet count in mice receiving Abcg4−/− but not Abcg4−/− BM cells. These studies link increased platelet production, initiated from its lineage progenitor cells, to accelerated atherosclerosis and arterial thrombosis.

Human genome-wide association studies have linked SNPs in or near the c-CBL gene to platelet count. Interestingly, ABCG4 is in tight linkage disequilibrium with c-CBL, and the SNPs associated with platelet counts could be influenced by expression of c-CBL and ABCG4. Together, these findings strongly support the human relevance of ABCG4 and the related mechanisms identified in mouse studies in regulation of megakaryopoiesis and platelet production. Increased platelet production is associated with an increased risk of arterial and...
venous thrombosis and atherothrombosis in myeloproliferative syndromes such as essential thrombocytosis and myelofibrosis. In addition, there is some evidence that increased platelet production may precede the onset of acute coronary syndromes. These studies suggest that rHDL infusions or platelet production may play a role in the suppression of platelet overproduction in these settings.

Summary and Implications

Since the discovery that mutations in ABCA1 were responsible for TD, there has been a proliferation of studies demonstrating the role of cholesterol efflux pathways mediated by ABCA1, ABCG1, ABCG4, and scavenger receptor BI in atherogenesis. While confirming the importance of cholesterol efflux pathways in macrophage foam cell formation and inflammation, new roles for ABCA1/G1 in the control of HSPC and megakaryocyte progenitor proliferation, HSPC mobilization and extramedullary hematopoiesis have been discovered. These pathways control the production of monocytes, neutrophils, and platelets. Although studies have been largely done in mouse models, monocytopoiesis, neutrophilia, and parameters of platelet function have been associated with human atherothrombotic disease, suggesting translational relevance. In contrast, human genome-wide association studies have called into question the causal relationship between SNPs associated with HDL-influencing genes and coronary heart disease, including for ABCA1. Although it is undoubtedly true that not all interventions to raise HDL cholesterol levels in humans will be associated with protection, we suggest some caution in the interpretation of these studies and conclude that genetic deficiency of ABCA1 likely is associated with premature atherosclerosis. Thus, future therapies may be directed at cholesterol efflux pathways. One approach could be direct upregulation of Abca1/g1 by LXR activators. LXR agonists have been shown to have atheroprotective effects, either directly in the vessel wall or by regulating HSPC proliferation and the associated monocyte and neutrophil levels. These effects seem to be dependent, at least in part, on Abca1/g1 expression. Thus, LXR agonists could constitute a potential antiatherogenic therapy provided that their adverse effects on liver triglyceride metabolism, for example, hepatic steatosis, could be circumvented. Another approach could be infusions of rHDL. It was shown recently that injections of pegylated rHDL particles that have a prolonged circulation time improve atherosclerotic lesions in mice, concomitant with reducing HSPC proliferation and monocytosis. Another study showed that injections of HDL particles suppressed platelet counts, which was mediated by ABCG4. Elucidation of the pathway regulating the effects of ABCG4 on MpTPO receptor expression has indicated that Lyn kinase activators could be an alternative method to suppress platelet production especially in myeloproliferative neoplasms where atherothrombotic risk is greatly increased. In the future, intervention trials for HDL-directed therapies may take on a personalized medicine approach in which instead of taking all-comers who have been optimally treated with statins, individuals with persistent low HDL, high levels of atherogenic lipoproteins, and patients with myeloproliferative neoplasms or an adverse genetic risk score may be targeted for treatment.

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