ATP-Binding Cassette Cholesterol Transporters and Cardiovascular Disease

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Abstract—A hallmark of atherosclerotic cardiovascular disease (CVD) is the accumulation of cholesterol in arterial macrophages. Factors that modulate circulating and tissue cholesterol levels have major impacts on initiation, progression, and regression of CVD. Four members of the ATP-binding cassette (ABC) transporter family play important roles in this modulation. ABCA1 and ABCG1 export excess cellular cholesterol into the HDL pathway and reduce cholesterol accumulation in macrophages. ABCG5 and ABCG8 form heterodimers that limit absorption of dietary sterols in the intestine and promote cholesterol elimination from the body through hepatobiliary secretion. All 4 transporters are induced by the same sterol-sensing nuclear receptor system. ABCA1 expression and activity are also highly regulated posttranscriptionally by diverse processes. ABCA1 mutations can cause a severe HDL-deficiency syndrome characterized by cholesterol deposition in tissue macrophages and prevalent atherosclerosis. ABCG5 or ABCG8 mutations can cause sitosterolemia, in which patients accumulate cholesterol and plant sterols in the circulation and develop premature CVD. Disrupting Abca1 or Abcg1 in mice promotes accumulation of excess cholesterol in macrophages, and manipulating mouse macrophage ABCA1 expression affects atherogenesis. Overexpressing ABCG5 and ABCG8 in mice attenuates diet-induced atherosclerosis in association with reduced circulating and liver cholesterol. Metabolites elevated in individuals with the metabolic syndrome and diabetes destabilize ABCA1 protein and inhibit transcription of all 4 transporters. Thus, impaired ABC cholesterol transporters might contribute to the enhanced atherogenesis associated with common inflammatory and metabolic disorders. Their beneficial effects on cholesterol homeostasis have made these transporters important new therapeutic targets for preventing and reversing CVD. (Circ Res. 2006;99:1031-1043.)

Key Words: atherosclerosis ■ cholesterol homeostasis ■ HDL cholesterol ■ lipoproteins ■ ABC transporters

Cholesterol is an abundant metabolite in mammalian tissues that is essential for several biological systems. Membrane fluidity of all cells is tightly modulated by the ordered packing of cholesterol between phospholipid molecules. Cholesterol also concentrates in sphingolipid-rich domains of the plasma membrane called rafts that contain a variety of important signaling molecules.1 Cholesterol covalently links to sonic hedgehog,2 a cell morphogen required for normal embryonic development and cell differentiation. In addition to its structural functions, cholesterol is a substrate for steroid production. Thus, an inadequate supply of cholesterol would have devastating effects on cell function, tissue development, and whole-body physiology.

Too much cholesterol, however, can also have pathological consequences. Cholesterol is a waxy substance that is poorly soluble in water. An uncontrolled buildup of cholesterol in cells can disrupt membranes and promote apoptosis. Accumulation of excess cholesterol causes atherosclerotic cardiovascular disease (CVD)3 and might contribute to the early onset of Alzheimer’s disease4 and renal dysfunction.5,6
Because of this need for strict maintenance of tissue cholesterol levels, the body relies on a complex homeostatic network to modulate the availability of cholesterol for cells and tissues. This network operates within both cells and the plasma compartment.

**Lipoprotein Metabolism**

**Sources of Cholesterol**

Cholesterol is derived from both exogenous dietary sources and endogenous biosynthetic pathways. Intestinal enterocytes absorb dietary cholesterol and package most of it into triglyceride-rich lipoproteins called chylomicrons (Figure 1).\(^7\),\(^8\) Endothelial-bound lipoprotein lipase hydrolyzes triglycerides in circulating chylomicrons to generate chylomicron remnants, which are cleared rapidly by the liver (Figure 1).\(^7\)

The intrahepatic cholesterol can be repackaged and secreted along with triglycerides in very-low-density lipoproteins (VLDLs), which are also substrates for lipoprotein lipase. Most of the cholesterol secreted from the liver is synthesized by hepatocytes rather than derived from dietary sources (Figure 1).

The remnants formed by hydrolysis of VLDL triglycerides are either taken up by the liver or converted to a cholesteryl-rich lipoprotein subclass called low-density lipoprotein (LDL) (Figure 1).\(^7\),\(^8\) which carries approximately two-thirds of the cholesterol in human plasma. LDL provides a source of cholesterol for steroidogenesis and cellular membranes. This occurs through the interaction of LDL with a cell surface receptor that mediates internalization and degradation of the lipoprotein particles. Most of the cholesterol secreted from the liver is synthesized by hepatocytes rather than derived from dietary sources (Figure 1).

Cells other than those in steroidogenic tissues and the liver cannot metabolize cholesterol. Instead they modulate their membrane cholesterol content by a feedback system that controls the rate of cholesterol uptake by the LDL receptor and de novo biosynthesis.\(^10\) With most cell types, this system is sufficient to provide enough cholesterol for membrane integrity and other functions without overloading them.\(^11\) Some cells, particularly macrophages, can ingest cholesterol by endocytic and phagocytic pathways that are not feedback regulated by cholesterol.\(^12\) These cells must either store this excess cholesterol as esters or secrete it.

**Reverse Cholesterol Transport**

High-density lipoproteins (HDLs), which carry approximately one-third of the cholesterol in human plasma, are involved in the removal of excess cholesterol from cells.\(^13\),\(^14\) HDL is a multifunctional and heterogeneous class of particles that transports a variety of lipids and lipophilic molecules between tissues and lipoproteins. One of the major functions of HDL is to transport cholesterol from peripheral tissues to the liver for elimination in the bile. This occurs by a pathway called reverse cholesterol transport, which involves the coordinate action of multiple cellular and plasma proteins.\(^14\),\(^15\)

Although HDL particles contain a variety of proteins, \(\approx 70\%\) of its total protein cargo is apolipoprotein A-I (apoA-I). The liver and intestine synthesize and secrete apoA-I into the circulation as a lipid-free or poorly lipidated protein (Figure 2). This apoA-I rapidly acquires phospholipids and cholesterol from cells to become partially lipidated. Most of this lipidation appears to involve the interaction of apoA-I with liver cells, but a fraction of the newly secreted or partially lipidated apoA-I may circulate to the periphery, where it interacts with other cholesterol-loaded cells, particularly macrophages. These nascent HDL particles acquire additional phospholipids and cholesterol through cellular cholesterol efflux processes (Figure 2) and by transfer from the surface of other lipoproteins, such as chylomicrons and VLDL, during lipolysis of their triglycerides (not shown).

Most of these nascent HDL particles are disc-shaped because their cholesterol is in an unesterified (free) form that incorporates between phospholipid molecules (Figure 2). In the circulation, an enzyme (lysolecithin cholesterol acyltransferase [LCAT]) esterifies the free cholesterol in these particles, converting it to cholesteryl ester lipid droplets that comprise the core of the mature spherical HDL particles (Figure 2).\(^8\)

Mature HDL particles are remodeled by plasma proteins that transfer their core cholesteryl esters and surface phospholipid to other lipoproteins and hydrolyze their phospho-
lipids. These particles also selectively deliver their core cholesteryl esters into cells, particularly those of the liver and steroidogenic tissues, through their interaction with a receptor called scavenger receptor B1 (SR-B1) (Figure 2). These HDL-remodeling processes are thought to regenerate lipid-poor apoA-I that dissociates from the particles and recycles through the reverse cholesterol transport pathway (Figures 2 and 4).

ATP-Binding Cassette Cholesterol Transporters

Different steps that control the delivery and disposal of cholesterol are regulated by membrane transporters of the ATP-binding cassette (ABC) superfamily. The human genome contains 48 distinct ABC transporters that are grouped into 7 subclasses labeled ABCA through ABCG. Mutations in ABC genes cause a variety of diseases, including cystic fibrosis, Stargardt’s macular degeneration, and disturbances in lipid and lipoprotein metabolism. All ABC transporters use ATP to generate the energy needed to transport metabolites across membranes. Structurally, ABCs fall into 2 groups: whole transporters having 2 similar structural units joined covalently and half-transporters of single structural units that form active heterodimers or homodimers (Figure 3).

Although cholesterol has been implicated as a direct or indirect substrate for at least 7 ABC transporters, 4 members of this family have been shown to have a major impact on lipoprotein metabolism and cell cholesterol biology: ABCA1, a whole transporter that mediates export of cellular cholesterol, phospholipids, and other metabolites to lipid-poor HDL apolipoproteins; ABCG1, a homodimeric half-transporter that mediates cellular cholesterol export to lipiddated lipoprotein particles; and ABCG5 and ABCG8, which form heterodimers that restrict intestinal absorption and promote biliary excretion of sterols. These 4 transporters have common modes of regulation and act in a coordinated fashion to rid tissues and the plasma compartment of excess cholesterol.

ATP-Binding Cassette A1

Structure and Function

ABCA1 is a 2261 amino acid integral membrane protein that comprises 2 halves of similar structure. Each half has a transmembrane domain containing 6 helices and a nucleotide binding domain (NBD) containing 2 conserved peptide motifs known as Walker A and Walker B, which are present in many proteins that use ATP. The Walker C signature unique to ABC transporters (Figure 3A). ABCA1 is predicted to have an N terminus oriented into the cytosol and 2 large extracellular loops that are highly glycosylated and linked by 1 or more cysteine bonds (Figure 3A).

ABCA1 mediates the transport of cholesterol, phospholipids, and other lipophilic molecules across cellular membranes, where they are removed from cells by lipid-poor HDL apolipoproteins. It is likely that ABCA1 forms a channel in the membrane that promotes “flopping” of lipids from the inner to outer membrane leaflet by an ATPase-dependent process. Although still controversial, most data support the idea that ABCA1 simultaneously translocates cholesterol and phospholipids to the outer membrane leaflet to generate lipid domains that are accessible for solubilization by apolipoproteins. ABCA1 localizes to the plasma membrane and intracellular compartments, where it could potentially facilitate transport of lipids to either cell surface–bound or internalized apolipoproteins.

ABCA1 appears to target specific membrane domains for lipid secretion. These are likely to be regions that are sensitive to accumulation of cholesterol. This same source of cholesterol feeds into intracellular compartments that are the preferred substrate for the esterifying enzyme acyl-coenzyme A (acyl-CoA):cholesterol acyltransferase (Figure 4). Because ABCA1 exports only free cholesterol, preformed cholesteryl esters are hydrolyzed by neutral hydrolases before they are depleted by this pathway (Figure 4). Thus, ABCA1 removes cholesterol that would otherwise accumulate as cytosolic cholesteryl ester lipid droplets.

Two models have been proposed to account for the ability of ABCA1 to target lipid domains. The first model suggests that ABCA1 generates lipid domains in the plasma membrane that are subsequently removed by apolipoproteins that bind to cell surface ABCA1. A second model suggests that ABCA1- and apolipoprotein-containing vesicles endocytose to intracellular lipid deposits, where ABCA1 pumps lipids
into the vesicle lumen for release by exocytosis.\textsuperscript{29,30} There is evidence that both mechanisms can operate in the same cell.\textsuperscript{22} 

ABCA1 might have functions independent of lipid export into the HDL pathway. There is evidence that ABCA1 promotes engulfment of apoptotic cells by macrophages\textsuperscript{31} and generates microparticles that bleed from plasma membranes,\textsuperscript{32-34} both processes requiring translocation of phosphatidylserine to the cell surface.\textsuperscript{35}

The ABCA1 pathway has broad specificity for multiple lipid-poor HDL apolipoproteins, including apoA-I, -A-II, -E, -H, -C-I, -C-II, -C-III, and -A-IV.\textsuperscript{36} These apolipoproteins contain 11 to 22 amino acid repeats of amphipathic \( \alpha \) helices.\textsuperscript{37} In this type of helix, the charged amino acids align along 1 face of the long axis, whereas the hydrophobic residues align along the other face. A synthetic 18 amino acid peptide that is an analog of class A amphipathic \( \alpha \) helices, and its dimer can mimic apoA-I in removing cholesterol and phospholipids by the ABCA1 pathway.\textsuperscript{35,38-42} These studies imply that the amphipathic \( \alpha \) helix is the major structural motif required for removing ABCA1-transported lipids. Interestingly, the D-isomer of the 18-mer \( \alpha \) helix is just as active as the L-isomer,\textsuperscript{39,41,42} indicating that there are no stereoselective requirements for these peptides to interact with ABCA1.

Covalent crosslinking studies revealed that apolipoproteins bind directly to ABCA1 with saturability and high-affinity (\( K_d <10^{-7} \) mol/L).\textsuperscript{40,43} These ABCA1 binding sites have a broad specificity for multiple HDL apolipoproteins, including apoA-I, -A-II, -C-I, -C-II, and -C-III.\textsuperscript{44} These sites also recognize the 18-mer synthetic amphipathic \( \alpha \) helix.\textsuperscript{45} Thus, the broad specificity of the ABCA1 binding sites closely mirrors the ABCA1-dependent lipid transport activity of the acceptors. These binding sites have not yet been identified, but their loose specificity for amphipathic \( \alpha \) helices suggests that they might be lipophilic patches of amino acids.

**Regulation**

ABCA1 protein levels and activity are highly regulated by multiple transcriptional and posttranscriptional processes (reviewed previously\textsuperscript{21}). These regulatory steps are likely to coordinate the overall activity of ABCA1 in a cell-specific manner in response to different environmental stimuli. As discussed below, several of these processes are likely to play important roles in lipoprotein metabolism and atherogenesis.

As expected for a transporter that mediates secretion of excess cellular cholesterol, transcription of ABCA1 is markedly induced by overloadings cells with cholesterol.\textsuperscript{45,46} This occurs exclusively through the nuclear receptors liver X receptor (LXR\( \alpha \) and/or LXR\( \beta \)) and retinoid X receptor (RXR).\textsuperscript{47,48} LXRs and RXRs form obligate heterodimers that preferentially bind to response elements within the ABCA1 gene promoter and the first intron. LXRs and RXRs bind to and are activated by oxysterols and retinoic acid, respectively.\textsuperscript{49} Treatment of cells with either an oxysterol or 9-cis-retinoic acid induces ABCA1, but their combined treatment has synergistic effects.\textsuperscript{47,48}

Excess intracellular cholesterol is converted to a “second messenger” oxysterol, which regulates ABCA1 and other cholesterol homeostatic processes through the LXR system.\textsuperscript{50,51} Most oxysterols are generated by cytochrome P450 enzymes that are particularly prevalent in the liver and play a role in bile acid metabolism. One of these enzymes, sterol 27-hydroxylase (Cyp27), is broadly distributed in various tissues and cell types, including macrophages, suggesting that 27-hydroxycholesterol is a major LXR ligand in macrophages and other peripheral cells.\textsuperscript{51}

One study showed a high degree of discordance between ABCA1 mRNA and protein levels in mouse tissues,\textsuperscript{52} implying that ABCA1 is highly regulated posttranscriptionally. Most of this regulation appears to occur at the level of protein stability. Under basal conditions in cultured cells, ABCA1 protein is highly unstable (half-life of 1 to 2 hours).\textsuperscript{53-55} The interaction of apolipoproteins with ABCA1-expressing cells dramatically reduces the rate of ABCA1 protein degradation by inhibiting ABCA1 proteolysis by calpain\textsuperscript{54} and by activating other signaling events.\textsuperscript{56} This regulation acts as a feedback mechanism to sustain ABCA1 levels when acceptors for cellular lipids are available.

Metabolites associated with common metabolic disorders, such as inflammation and diabetes, can destabilize ABCA1 in cultured macrophages. Unsaturated free fatty acids, which are elevated in diabetes and the metabolic syndrome, directly destabilize ABCA1 by a signaling pathway involving activation of phospholipase D2 and phosphorylation of ABCA1 serines.\textsuperscript{55,57-59} The reactive carbonyls glyoxal and glycoaldehyde acutely and severely impair the ABCA1 pathway, presumably by directly damaging ABCA1 protein.\textsuperscript{60} These carbonyls are generated during glucose metabolism and protein glycoxidation and form advanced glycated end products (AGEs) on tissue proteins.\textsuperscript{61-65} AGEs accumulate with aging, and their formation is enhanced by diabetes. These and other metabolic factors could promote accumulation of cholesterol in cells by reducing ABCA1 levels.

**ABCA1 In Vivo**

ABCA1 is widely expressed throughout animal tissues, where it may have multiple and diverse functions. In humans and baboons, ABCA1 mRNA was reported to be most abundant in liver, placenta, small intestine, and lung.\textsuperscript{64,65} In mice, ABCA1 mRNA was reported to be most abundant in liver, kidney, adrenal, heart, bladder, testis, and brain.\textsuperscript{52} Because of extensive posttranscriptional regulation of expression and function, mRNA levels may not truly reflect the actual activity of the ABCA1 pathway in vivo.

Based on cell culture studies, macrophage-rich tissues should have relatively high levels of ABCA1. As scavenger cells, macrophages ingest modified lipoproteins and damaged cell membranes and thus can accumulate large amounts of cholesterol and oxysterols, which are ABCA1 inducers. ABCA1 is also highly expressed in the liver, where it is upregulated when mice are fed a high-cholesterol diet.\textsuperscript{53} It is therefore expected that hepatic ABCA1 would make an important contribution to whole-body lipoprotein metabolism.

Human and animal model studies have confirmed that ABCA1 is a major determinant of plasma HDL levels and macrophage cholesterol content. Mutations in human ABCA1 can cause a genetic disorder called Tangier disease,\textsuperscript{46,66-68} which is characterized by very low levels of plasma HDL and

\[ ABCA1 \]
deposition of sterols in macrophages. The low levels of plasma HDL probably reflect an inability of lipid-poor apoA-I to acquire lipids, leading to a rapid clearance of apoA-I from the plasma (Figure 2). Targeted disruption of the Abca1 gene in mice produces a phenotype similar to that of Tangier disease, and overexpressing ABCA1 in mice elevates plasma HDL levels. Tissue-specific expression or disruption of Abca1 revealed that the hepatic ABCA1 pathway is responsible for generating most of the plasma HDL in mice (Figure 2), presumably because of the large flux of cholesterol through the liver when compared with peripheral tissues.

**Genetic Variations**

More than 70 mutations in ABCA1 have been identified from family studies and population screens for single-nucleotide polymorphisms (SNPs), nearly a third of which are missense mutations. Most of the ABCA1 mutations were present in subjects with low plasma HDL levels, implying that they play a causative role in lowering HDL levels. Some mutations, however, were more frequent in subjects with the highest HDL levels, consistent with an enhancement of the ABCA1 pathway. Although these mutations occur throughout the gene, they tend to cluster in the extracellular loops, the NBD domains, and the C-terminal region (Figure 3). SNP analyses have also identified more than 20 common polymorphisms (>1% allelic frequency) in the coding, promoter, and 5’ untranslated regions of ABCA1, many of which are associated with either low or high plasma HDL levels.

The functional impact of a small subset of the missense mutations has been studied in cultured cells. When overexpressed in cells, most of these mutants appear in the plasma membrane but have severely impaired lipid transport and apolipoprotein binding activities. ABCA1 proteins with missense mutations in the first and second extracellular loop and in the ninth membrane spanning domain have been reported to impair ABCA1 trafficking to the plasma membrane. Only 1 mutant has been described that has near normal apolipoprotein binding activity but defective lipid transport.

**ABCA1 and CVD**

There is an inverse relationship between plasma HDL levels and CVD risk, implying that HDL protects against atherosclerosis. Although there are multiple mechanisms by which HDL can be cardioprotective, the relative activity of ABCA1 plays a major role. Because accumulation of sterol-laden macrophage “foam cells” in the artery wall is an early event in formation of atherosclerotic lesions, the relative activity of ABCA1 in arterial macrophages would be expected to have a major impact on atherogenesis. Studies of human genetic HDL deficiencies and mouse models support this assumption.

Tangier disease homozygotes and compound heterozygotes who are more than 30 years of age have a 6-fold higher than normal incidence of CVD. The low levels of LDL in these subjects may partially protect them from atherogenesis. Studies of heterozygotes, who tend to have more normal levels of LDL, showed a significant inverse correlation between ABCA1 activity in their cultured skin fibroblasts and the prevalence and severity of CVD. In general, premature CVD is associated with ABCA1 mutations that impair function. Some polymorphisms in ABCA1 are associated with either increased or decreased CVD. Interestingly, many of these variants show an altered severity of atherosclerosis without changes in plasma lipid levels.

Mouse studies have also provided support for the cardio- protective effects of macrophage ABCA1. Some but not all reports showed that overexpression of human ABCA1 in mice significantly reduced diet-induced atherosclerosis in association with a favorable change in lipoprotein profile. Reciprocal bone marrow transplantation studies using wild-type and Abca1-null mice have shown that selectively over- or underexpressing ABCA1 in macrophages decreases or increases atherosclerosis, respectively. In contrast, selective overexpression of ABCA1 in the liver of mice lacking LDL receptors increases the plasma levels of more atherogenic apoB-containing particles and enhances atherosclerosis. Taken together, these studies clearly show that macrophage ABCA1 is a cardioprotective factor. Hepatic ABCA1 may override some of these protective effects by increasing atherogenic apoB-containing lipoproteins.

**ATP-Binding Cassette G1**

**Structure and Function**

ABCG1 belongs to the ABCG family of reverse half-transporters (Figure 3). Because an active transporter needs 2 nucleotide-binding domains and 2 transmembrane bundles, members of the ABCG family have to form either hetero- or homodimers to gain function. ABCG1 is believed to act as a homodimer in macrophages. Although earlier studies suggested a shorter gene, it now appears that the full-length human gene has 23 exons spanning 98 kb of genome. Multiple potential transcripts of mouse and human ABCG1 have been identified that use alternate exons or promoters. These are predicted to encode proteins that differ in their N-terminal end. However, there appears to be only one active transcript in mice and humans.

As with ABCA1, ABCG1 is highly expressed in cholesterol-loaded macrophages, consistent with it playing a role in ridding these cells of excess cholesterol. ABCG1 is expressed both on the cell surface and within intracellular compartments. ABCG1 promotes cholesterol efflux from cells to HDL and other lipoprotein particles but not to lipid-free apoA-I. Both ABCA1 and ABCG1 appear to have the intrinsic ability to translocate cholesterol to cell surface domains accessible to extracellular molecules, suggesting that they directly or indirectly operate as cholesterol floppases. In contrast to ABCA1-dependent lipid export, which requires apolipoprotein binding, ABCG1-dependent cholesterol efflux does not appear to require the direct interaction of lipoproteins. It is likely that ABCG1 forms cell surface lipid domains that allow cholesterol to desorb into the surrounding fluid and be picked up by lipoproteins (Figure 4).

In contrast to ABCA1, which transports phospholipids and other lipophilic compounds as well as cholesterol, ABCG1 is largely a cholesterol transporter.
the acceptors that remove ABCG1-transported lipids contain phospholipids, which can readily incorporate cholesterol. It has been reported, however, that ABCG1 promotes some efflux of phospholipids, particularly sphingomyelin.105

ABCA1 and ABCG1 can act synergistically to remove cholesterol from cells (Figure 4).104,106 ABCA1 converts lipid-poor apoA-I to partially lipidated “nascent” lipoproteins that are effective acceptors for cholesterol exported by ABCG1. This is probably because ABCA1 transports phospholipids to apoA-I. These findings raise the interesting possibility that ABCA1 and ABCG1 coordinate the removal of excess cholesterol from macrophages by using diverse types of lipid acceptor particles (Figure 4).

Regulation

Additional evidence that ABCA1 and ABCG1 cooperate to export cholesterol from cells is that they are both regulated by the LXR/RXR nuclear receptor system.100,101,107,108 Thus the conversion of cholesterol to the oxysterol ligands for LXRs drives expression of both ABCA1 and ABCG1 in cholesterol-loaded cells. In macrophages, LXR agonists stimulate the movement of ABCG1 from intracellular compartments to the plasma membrane, which enhances the subsequent efflux of cholesterol to HDL.103 Thus, the LXR system performs the dual function of increasing ABCG1 expression and its translocation to the plasma membrane.

In Vivo Activity

ABCG1 was shown to be highly expressed in mouse and human brain, thymus, lung, adrenals, and spleen.109,110 ABCG1 is expressed in both the gray and white matter in mouse brain.111 In the cerebellar astroglia, which are macrophage-like cells, expression of ABCG1 rather than ABCA1 is correlated with cholesterol release,112 suggesting that ABCG1 plays a greater role than ABCA1 in exporting lipids from these cells in the brain. ABCG1 is also expressed in the intestine, suggesting that it may play a role in transport of dietary lipids. In CaCo-2 human intestinal cells, induction of ABCG1 by LXR agonists increased HDL-mediated cholesterol efflux from the basolateral membrane,113 consistent with the possibility that ABCG1 is involved in formation and maturation of intestinal HDL.

Studies of ABCG1−/− mice have demonstrated the importance of this transporter in macrophase and liver lipid transport. The targeted disruption of Abcg1 in mice had no effect on plasma lipid levels but did result in massive accumulation of both neutral lipids and phospholipids in hepatocytes and in macrophages within multiple tissues following administration of a high-fat and -cholesterol diet.97 In contrast, overexpression of human ABCG1 protected tissues from dietary fat–induced lipid accumulation.95 The results imply that ABCG1 is essential for cholesterol homeostasis in macrophages and is needed to remove excess cholesterol from these cells. The elevated triglycerides in cells from ABCG−/− mice also suggest that ABCG1 may also play a role in modulating metabolism of this class of lipids in vivo.

The accumulation of lipids in the liver of ABCG1−/− mice raises the possibility that this transporter is involved in hepatic lipid metabolism or transport. ABCG1 expression is relatively low in the liver,97 especially when compared with ABCA1 expression. The lack of change in plasma lipoproteins with ablation or overexpression of ABCG1 implies that this transporter has little effect on hepatic lipoprotein production or clearance. It is possible, however, that ABCG1 plays some role in modulating intrahepatic lipid trafficking or biliary secretion of cholesterol.114

Role in CVD

To date, no genetic variations in human ABCG1 have been identified that link it to any inheritable disease. The search for functionally significant rare mutations and polymorphism in this gene may be complicated by the likelihood that ABCG1 contributes little to plasma lipid or lipoprotein levels, thus limiting large-scale screening based on abnormal plasma lipids. If dysfunctional ABCG1 causes CVD in humans, it is likely to be in a patient population with relatively normal levels of lipoprotein cholesterol.

The macrophage cholesterol export activity of ABCG1 predicts that this transporter should be cardioprotective. Surprisingly, 3 studies showed that transplantation of bone marrow from ABCG1−/− mice into atherogenic mouse models caused only a moderate increase or actually decreased atherosclerotic lesions.115–117 The decreased atherosclerosis was associated with increased macrophage apoptosis and enhanced expression of both apoE and ABCA1. Thus, the potential harmful effects of impaired ABCG1 may be overridden by the beneficial effects on clearing apoptotic cells and increasing other compensatory cholesterol efflux pathways. It remains to be determined whether ABCG1 is cardioprotective in humans.

ABCG5 and ABCG8

Structure and Function

ABCG5 and ABCG8 are half-transporters that form heterodimers to become functional (Figure 3).118–120 ABCG5 and ABCG8 each contain 13 exons and are arranged in a head-to-head configuration on chromosome 2p21, with only 140 bases separating them.121

In cultured hepatocytes, coexpression of the transporters caused a translocation of the functional heterodimer from the endoplasmic reticulum to the canalicular membrane of the apical surface of the cell.118–120 Thus, ABCG5 and ABCG8 form an obligate heterodimer that traffics to the surface of the cell membrane, where it presumably performs its sterol export function. It has not yet been shown, however, that ABCG5 and ABCG8 actually promote sterol export from cultured cells.

Studies of human disease and mouse models have shown that ABCG5 and ABCG8 regulate the whole-body retention of plant sterols and biliary secretion of cholesterol (Figure 2).122–126 There is evidence that these transporters promote flopping of sterols from the inner to outer leaflets of the plasma membrane.120 Thus, ABCG5 and ABCG8 heterodimers probably function in a similar manner as other ABC cholesterol exporters.

The observation that patients with mutations in either ABCG5 or ABCG8 accumulate plant and shellfish sterols in
their plasma is consistent with the idea that these transporters are selective for noncholesterol sterols. Mouse model studies, however, have shown that these transporters also promote hepatobiliary secretion of cholesterol. The major plant sterols are sitosterol, campesterol, stigmasterol, and avenasterol. ABCG5/8 also transports plant stanols, such as campestanol and sitostanol. Plant sterols differ from cholesterol by the addition of methyl or ethyl groups or a double bond in the carbon-24 acyl side chain. Stanols differ from cholesterol by the saturation of the Δ5 double bond. It is unknown whether ABCGS/8 also transports phospholipids. Because it pumps sterol into the intestinal lumen, the presumed acceptors of ABCG5/8-transported sterols are complexes of bile acids and phospholipids.

**Regulation**

ABCG5 and ABCG8 belong to the family of ABC transporters that are induced by the LXR/RXR nuclear receptors. Both cholesteryl feeding and the addition of a LXR agonist to the mouse diet increased ABCG5 and ABCG8 levels in the liver and intestine. Activation of LXRs was associated with an increased biliary cholesterol secretion, decreased fractional cholesterol absorption, and increased fecal neutral sterol excretion.

**In Vivo Activity**

Studies of human genetic disorders and mouse models have shown that intestinal ABCG5/8 functions to limit absorption of dietary plant sterols. The daily average Western diet contains 250 to 500 mg of cholesterol and 200 to 400 mg of noncholesterol sterols, the majority of which are plant sterols. Typically, 50% to 60% of this cholesterol is absorbed in the intestine, which is in stark contrast to only 1% to 5% of the plant sterols. A major entry point for the absorption of dietary sterols is through the Niemann–Pick C1 like 1 (NPC1L1) protein, which is expressed on the brush border membrane of enterocytes. It is believed that a small fraction of plant sterols enter the circulation because those taken up by NPC1L1 are secreted back into the lumen by enterocyte ABCG5/8.

These human and mouse studies have shown that ABCG5/8 is also expressed in hepatocytes, where it mediates the biliary secretion of sterols. This secretion appears to require another ABC transporter, MDR2 (ABCB4), which mediates biliary secretion of phospholipids and, secondarily, cholesterol. These studies also suggested that the decreased intestinal absorption of plant sterols in mice overexpressing ABCG5 and ABCG8 is primarily attributable to the increased biliary secretion of cholesterol, which could compete for the uptake of noncholesterol sterols by enterocytes. This implies that the ability of enterocytes to secrete sterols through ABCG5/8 plays a minor role in decreasing fractional absorption of plant sterols.

In humans, the hepatic ABCG5/8 system may play the biggest role in controlling dietary sterol absorption. This idea is supported by a study showing that a liver transplant in a patient with genetically nonfunctional ABCG5/8 normalized plant sterol levels despite the fact that intestinal ABCG5/8 was still impaired. It is possible that the enterocyte ABCG5/8 pathway is the initial defense against an acute supply of dietary plant sterols but that the hepatic pathway has a more sustained effect.

**Sitosterolemia**

Mutations in either ABCG5 or ABCG8 can cause a rare genetic disorder called sitosterolemia. The disease is characterized by markedly elevated plasma levels of plant sterols and modest increases in plasma cholesterol, which is attributable to the hyperabsorption of both cholesterol and plant sterols from the intestine and a low level of excretion into the bile. Because of these abnormal sterol levels, patients with sitosterolemia develop tuberous xanthomas and premature CVD.

Multiple mutations in ABCG5 and ABCG8 have been identified that cause sitosterolemia. SNP analyses have also uncovered multiple polymorphisms, most of which are in ABCG8. Many of these polymorphisms are in highly conserved regions, suggesting that they have functional impacts. A lack of an in vitro lipid export assay has limited the study of the functional effects of these genetic variations. All of the missense mutations in either ABCG5 or ABCG8 studied to date either prevent formation of the obligate heterodimer or block the efficient trafficking of the heterodimer to the plasma membrane.

**Role in CVD**

Sitosterolemic patients are highly sensitive to dietary cholesterol and become markedly hypercholesterolemic when fed a high-cholesterol diet. The high plasma cholesterol levels are associated with severe premature atherosclerosis. The elevated plant sterols may also contribute to CVD by disrupting normal cholesterol homeostasis. These observations suggest that interventions to enhance the ABCG5/8 pathway could protect against CVD in hypercholesterolemic subjects by eliminating more cholesterol in the bile and reducing plasma cholesterol levels. This concept was supported by a study showing that overexpressing ABCG5 and ABCG8 in an athrogenic mouse model attenuates diet-induced atherosclerosis in association with reduced liver and plasma cholesterol levels. Therefore, the role of ABCG5 and ABCG8 in CVD relates to their abilities to modulate plasma cholesterol levels.

**ABC Cholesterol Transporters and Atherogenic Complications of Diabetes**

The prevalence of diabetes has risen dramatically in the last 20 years worldwide. Patients with a constellation of CVD risk factors called the metabolic syndrome are predisposed to both diabetes and CVD. As defined by the Adult Treatment Panel III, the criteria for the metabolic syndrome are 3 of the following risk factors: obesity, hypertriglyceridemia, low HDL levels, hypertension, and hyperglycemia.
CVD is the major cause of morbidity and mortality in both types 1 and 2 diabetes. There is a large body of evidence suggesting that both the hyperglycemia and dyslipidemia accompanying diabetes and the metabolic syndrome contribute to this enhanced atherogenesis. Low plasma HDL is among the CVD risk factors associated with type 2 diabetes and the metabolic syndrome. Moreover, the HDL particle distribution is abnormal in both types 1 and 2 diabetes, with decreases in the relative fraction of the large HDL particles believed to be cardioprotective. These results raise the possibility that abnormal HDL metabolism contributes to the increased CVD caused by diabetes and the metabolic syndrome.

There is emerging evidence that impaired ABC cholesterol transporters contribute to the dyslipidemia and enhanced CVD in these metabolic disorders. Factors that destabilize ABCA1, such as reactive carbonyls and free fatty acids, are elevated in diabetes, and unsaturated fatty acids were shown to suppress ABCA1 and ABCG1 transcription by antagonizing the effects of sterols on LXR activation. Peritoneal macrophages isolated from type 2 diabetic mice were reported to have above normal levels of cholesteryl esters in association with suppressed expression of ABCG1. Transcription of this transporter was severely reduced by chronic exposure of cultured mouse macrophages to high-glucose levels. In rats, inducing type 1 diabetes led to a decreased expression of hepatic and intestinal ABCA5 and ABCG8 associated with altered sterol fluxes.

The metabolic syndrome and diabetes are associated with increased chronic inflammation. Several studies have shown that the inflammatory enzyme myeloperoxidase chlorinates and oxidizes amino acids in apoA-I, which severely reduces its ability to interact with ABCA1. Thus, diabetes and inflammation can impair these ABC pathways by reducing the cell content of transporters and damaging the apoA-I needed to remove cellular cholesterol and generate HDL particles. Taken together, these studies implicate the disruption of ABC transport pathways as playing a role in the abnormal HDL and increased CVD associated with diabetes, the metabolic syndrome, and other inflammatory disorders. Additional studies with animal models are needed to confirm that these processes are occurring in vivo.

ABC Cholesterol Transporters As Therapeutic Targets

The ability of ABCA1, ABCG1, ABCG5, and ABCG8 to coordinate the depletion of excess tissue cholesterol and its elimination from the body has made these transporters important new therapeutic targets for treating CVD. The assumption is that increased ABCA1 and ABCG1 activities in arterial macrophages would prevent foam-cell atherosclerotic lesion formation, that increased liver ABCA1 activity would elevate plasma HDL levels and thus enhance the diverse cardioprotective functions of this lipoprotein, and that increased ABCG5 and ABCG8 in the liver and intestines would decrease dietary sterol absorption and increase hepatobiliary cholesterol secretion. The possibility of producing these beneficial changes in cholesterol homeostasis has launched multiple programs in the pharmaceutical industry designed to produce agents that enhance these cholesterol transporters.

Most of these programs to date have focused on developing agonists to increase transcription of these transporters. This approach is made feasible by the fact that each ABC is induced robustly by the same nuclear receptor ligands, thus providing an opportunity to simultaneously increase the expression levels all 4 of them. The first generation drugs shown to be effective for this endpoint have been LXR agonists. An additional advantage of these agonists is that they induce other macrophage proteins that help coordinate cholesterol export, including apolipoproteins that can act as cholesterol acceptors and enzymes and transfer proteins that can remodel extracellular HDL particles to regenerate lipid-free apolipoproteins. Two nonsteroidal synthetic LXR agonists (TO901317 and GW3965) have been shown to increase plasma HDL levels and reduce atherosclerosis in mouse models.

The problem with these LXR agonists is that they also increase hepatic fatty acid synthesis and esterification and thus generate fatty livers and hypertriglyceridemia when administered to animals. LXR agonists induce fatty acid synthase and a transcriptional factor named sterol regulatory binding element protein-1c (SREBP-1c) that activates genes for several enzymes in the fatty acid biosynthetic pathway. Moreover, SREBP-1c induces stearoyl-CoA desaturase, which converts saturated fatty acids to unsaturated fatty acids that destabilize ABCA1 protein.

Several studies have provided proof-in-principle that LXR agonists can be developed that are more selective for the cholesterol transport pathway than for lipogenesis. It has been reported that GW3965 can be administered to mice at a concentration that elevates plasma HDL levels without causing hypertriglyceridemia, although this agonist activates SREBP-1c at high concentrations in vitro. A synthetic oxysterol ligand for the LXR receptor was shown to stimulate ABCA1 synthesis with only a minor increase in hepatic SREBP-1c. A plant sterol derivative was shown to be a potent LXR agonist that selectively activates intestinal ABC transporters with only limited induction of lipogenic enzymes. A major challenge is to produce marketable LXR agonists that selectively induce cholesterol transporters.

The possibility of targeting posttranscriptional regulation of these ABCs has received less attention. It is clear from studies of ABCA1 that this is a highly unstable protein that may be regulated in vivo by metabolic factors associated with common disorders, such as diabetes and inflammation. Under these conditions, stimulating transcription may have only a limited ability to enhance the overall activity of this cholesterol export pathway. It is also possible that transcription of ABCA1 and ABCG1 in highly cholesterol-loaded cells may already be maximally stimulated. Additional studies with animal models are needed to assess the relative importance of transcriptional and posttranscriptional regulation of these ABCs under various atherogenic conditions.
Conclusions

Studies of human disorders, mouse models, and cultured cells have shown that four ABC cholesterol transporters have a major impact on whole-body cholesterol homeostasis and CVD. ABCA1 and ABCG1 work independently and synergistically to remove excess cholesterol from cells, particularly macrophages. Hepatic ABCA1 is largely responsible for generating cardioprotective HDL particles. ABCG5 and ABCG8 form heterodimers that limit sterol absorption by the intestine and promote hepatobiliary secretion of circulating cholesterol. Transcription of these 4 transporters is regulated by the same LXR/RXR nuclear receptor system, which is induced robustly by intracellular sterols and nonsterol agonists.

These beneficial effects on cholesterol homeostasis have made these transporters important new therapeutic targets for preventing and reversing atherosclerotic CVD. First-generation protocols have focused on developing LXR agonists that stimulate transcription of ABCA1 and other genes involved in cholesterol transport. Administering LXR agonists to atherogenic mouse models markedly reduces their atherosclerotic lesions. The potential benefits of these agents have been overshadowed by their broad specificity for ligandic genes. Several studies, however, have offered proof-in-principle that drugs selective for cholesterol transport genes can be developed in the near future.

The most common atherogenic disorders in humans appear to damage ABC transporters or suppress their expression. Metabolites that are elevated in both diabetes and in the metabolic syndrome reduce expression of ABC transporters by several mechanisms that might contribute to the increased CVD common to these disorders. These patients could be relatively resistant to interventions that use transcriptional regulation to elevate their ABC cholesterol transporters. Thus, with many individuals, it may be necessary to direct therapeutic approaches at the underlying mechanisms that impair ABC transporters or at the actual steps in their cellular pathways that are affected.

In summary, we have gained a great deal of knowledge about the biology and pathology of the ABC cholesterol transporters ABCA1, ABCG1, ABCG5, and ABCG8. These transporters, however, have complex cellular and regulatory pathways that are far from being completely understood. Additional studies are needed to identify the molecular players in these pathways, to determine the array of substrates targeted for transport, and to characterize the diverse processes that regulate their expression and activity. These studies will not only provide insight into the role of ABC cholesterol transporters in health and disease, but will uncover novel therapeutic targets for treating these diseases.

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