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GENETICS (AJ MARIAN, SECTION EDITOR)

# Genetics of Cholesterol Efflux

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**Abstract** Plasma levels of high-density lipoprotein cholesterol (HDL-C) show an inverse association with coronary heart disease (CHD). As a biological trait, HDL-C is strongly genetically determined, with a heritability index ranging from 40% to 60%. HDL represents an appealing therapeutic target due to its beneficial pleiotropic effects in preventing CHD. This review focuses on the genetic basis of cellular cholesterol efflux, the rate-limiting step in HDL biogenesis. There are several monogenic disorders (e.g., Tangier disease, caused by mutations within ABCA1) affecting HDL biogenesis. Importantly, many disorders of cellular cholesterol homeostasis cause a reduced HDL-C. We integrate information from family studies and linkage analyses with that derived from genome-wide association studies (GWAS) and review the recent identification of micro-RNAs (miRNA) involved in cellular cholesterol metabolism. The identification of genomic pathways related to HDL may help pave the way for novel therapeutic approaches to promote cellular cholesterol efflux as a therapeutic modality to prevent atherosclerosis.

**Keywords** HDL · Coronary artery disease · Genetics · Cholesterol efflux · ABCA1 · Apo AI · ABCG1 · SR-BI · Cholesteryl ester hydrolases · NPC1 · NPC2 · LIPA · SMPD1 · StAR D3 · miRNA · Genome wide association studies

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## Introduction

The study of genetic defects of high-density lipoprotein (HDL) has increased our knowledge of the mechanisms of HDL biogenesis and metabolism and has provided surprising insights on the contribution of genetic disorders of HDL in the pathogenesis of coronary heart disease (CHD). Indeed, since the identification of apolipoprotein (apo) AI Milano (Apo AI Arg173Cys, rs28931573), the once firmly held notion that genetically determined low HDL cholesterol (HDL-C) levels are associated with CHD has been questioned. The use of Mendelian randomization principles under which the existence of a causal relationship between HDL-C and CHD would imply that association between a gene variant and HDL-C levels will translate into the CHD risk expected from the effect on HDL-C, has also stirred

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great controversy [1], despite the coherent association of HDL in heart disease. As such, the study of severe cases of HDL deficiency, such as Tangier disease and apo AI deficiency, has suggested that these forms are associated with premature CHD [2]. In this review, we examine significant genes identified from family studies and linkage analyses that pertain to cellular cholesterol efflux, along with candidate genes involved in cellular cholesterol homeostasis, and integrated information derived from genome-wide association studies (GWAS). Recent data on microRNAs (miRNAs) involved in cholesterol metabolism are also reviewed. The identification of novel genes involved in the complex cellular cholesterol efflux pathways may provide novel therapeutic targets to prevent atherosclerosis in man.

## HDL Biogenesis

HDL is associated with protection against cardiovascular disease due to coronary artery disease and other forms of atherosclerosis. Evidence from animal and human studies suggests that HDL, as well as HDL-associated proteins and lipids, possess pleiotropic biological properties that contribute to their antiatherogenic effects. These include anti-inflammatory, antioxidant, and antithrombotic activities, as well as beneficial effects on vascular endothelial function, vascular endothelial cell proliferation, and survival and differentiation of endothelial progenitor cells [3, 4]. The best-recognized property of HDL is reverse cholesterol transport (RCT), the mechanism by which excess cellular cholesterol from peripheral cells, including vessel wall macrophages, is returned to the liver via specific transporters and receptors (ABCA1, ABCG1, ABCG5/8 and SR-BI), for excretion in the bile [3].

Two proteins play a vital role in the process of RCT and in the generation of HDL particles, a process we refer to as HDL biogenesis: apo AI, the major protein within HDL; and ABCA1, a large membrane-associated transporter responsible for cellular cholesterol efflux and apo AI lipidation [5]. This mechanism is remarkably conserved throughout evolution. Cellular cholesterol is toxic to cells and may activate at least two important events: cellular accumulation within the endoplasmic reticulum may trigger the unfolded protein response, leading to alarm, defense, or activation of apoptosis signaling pathways [6], and cholesterol crystals activate the NLRP3 inflammasome by first inducing lysosomal damage; in turn leading to the activation of interleukin-1 and −18 [7, 8]. Thus, the effects of HDL-mediated cholesterol efflux on specific cells such as macrophage point towards a role in innate immunity to protect cells against damage.

In this review, we examined novel and well-known genes and their products involved in cellular cholesterol efflux and provided some insights on the impact of genetic variations within these genes in man. First, key processes involved in cellular cholesterol efflux are summarized (Fig. 1) while presenting an update on candidate genes thus far identified in the cellular cholesterol efflux machinery (Table 1). Then, potential genes identified by GWAS and associated with HDL-C are discussed (Table 2).

## Cholesteryl Ester Hydrolases

Arguably, the first step in removing cellular cholesterol involves the hydrolysis of cholesteryl esters (CE) by enzymes that cleave the fatty acyl chain. Neutral cholesterol ester hydrolase (nCEH1, also called KIAA1363) is the prototypical cellular enzyme involved in this process [9]. In macrophages, where most of the cholesterol is stored in lipid droplets as CE, hormone-sensitive lipase (LIPE) is considered to play a major role in the generation of free cholesterol destined for export in mouse, but not in humans. Interestingly, lysosomal cholesteryl ester hydrolases (CEHs) also hydrolyze CE obtained from circulating lipoproteins. At least one other nCEH has recently been identified [10], and the CE hydrolysis system is redundant [11]. This biological redundancy likely may explain why, so far, none of the genes involved in CE hydrolysis, the first step of RCT, have been identified in GWAS (Table 2), or why targeted gene deletion of LIPE does not reduce CE hydrolysis, supporting the concept of multiple redundant systems for cellular CE hydrolysis [12, 13].

## ABCA1

The interaction between apo AI and ABCA1 is essential and rate-limiting for the initial step in RCT. Data suggest that ABCA1 acts as a phospholipid translocase and contributes to the formation of a non-raft membrane domain that facilitates the lipidation of apo AI and the formation of nascent HDL particles [14•].

The ABCA1 gene was identified over a decade ago as the molecular basis for Tangier disease, a rare disorder of HDL deficiency in which cellular cholesterol efflux is severely reduced [15]. Many mutations in the ABCA1 gene have been identified in Tangier disease and the heterozygous form, familial HDL deficiency [16]. The prevalence of ABCA1 mutations in subjects with HDL deficiency is estimated at approximately 10% to 20% [17, 18]. Insights into the molecular physiology of ABCA1-mediated cellular cholesterol efflux have shown that ABCA1 is predominantly regulated by oxysterols via the

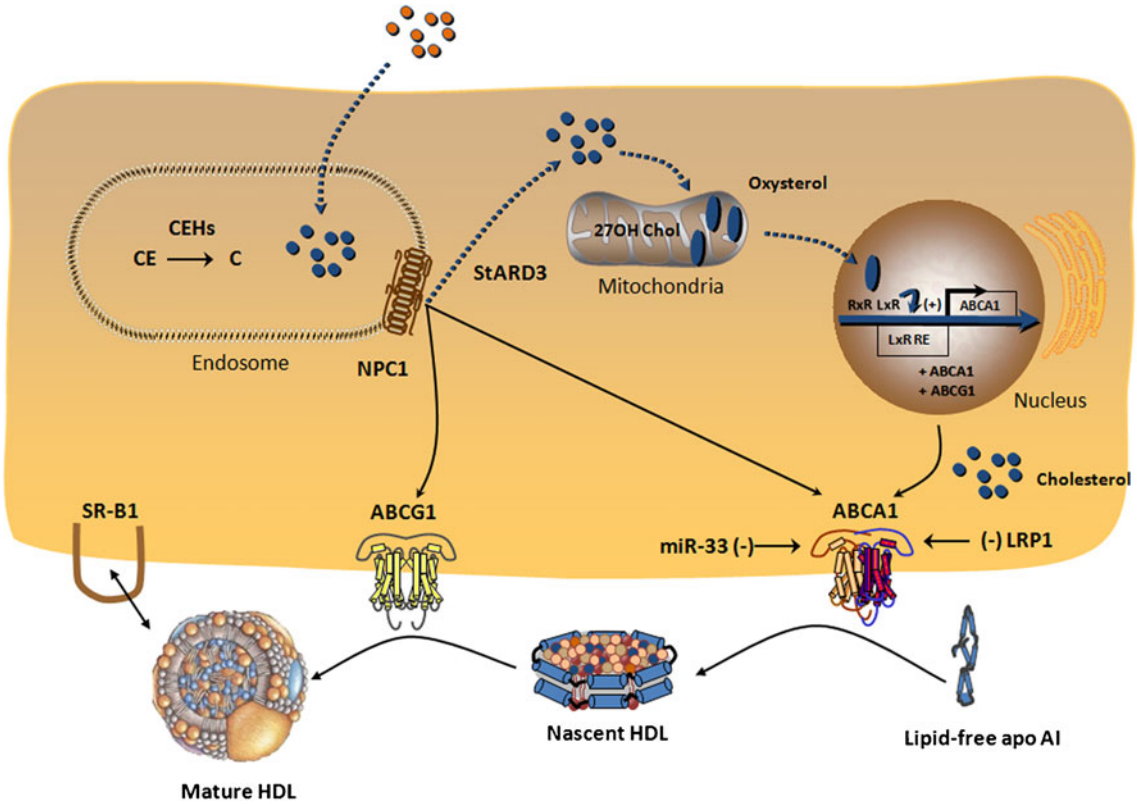


**Fig. 1** Major genes involved in genetic disorders of cellular cholesterol efflux in man. To date, genetic disorders can be associated with defects of cholesterol transport in the cytosol in Niemann-Pick disease type C (NPC), defects in cellular cholesterol efflux to apolipoprotein (apo) AI caused by defective ATP-binding cassette (ABC) transporter A1 (Tangier disease), and mutations within apo AI and mutations within SR-BI. ABCG1—ATP-binding cassette transporter G1; C—free cholesterol; CE—cholesteryl esters; CEHs—cholesteryl esterases (LIPE—hormone sensitive lipase; NCEH1—neutral cholesteryl ester hydrolase-1 also called KIAA1363 and other CE hydrolases); HDL—high-density lipoproteins; LRP1—low density lipoprotein receptor-related protein-1; LXR—liver X receptor; miR—micro RNA; RXR—retinoid X receptor; SR-B1—scavenger receptor B1; StARD3—steroidogenic acute regulator protein

liver specific receptor (LxR) pathway [19] and cyclic adenosine monophosphate (cAMP) in various cell lines [20]. Genetic control of ABCA1 appears to be predominantly through the oxysterols/LxR pathway (Fig. 1). Attempts to modulate the LxR pathway to increase ABCA1 and HDL have been fraught with the multiple roles of LxR on fatty acid

**Table 1** Genes and their proteins associated with cellular cholesterol efflux

| Gene Symbol | Protein | GWAS[a] (Table 2) | Disease in humans |
|---|---|---|---|
| ABCA1 | ATP binding cassette A1 | Yes | Tangier disease |
| ABCG1 | ATP binding cassette G1 | No | – |
| Apo AI | Apolipoprotein AI | Yes | Apo AI deficiency |
| LIPE | Hormone sensitive lipase | No | – |
| LRP1 | LDL receptor related protein-1 | Yes | – |
| miR-33 | Micro RNA −33 | No | – |
| NCEH1 | Neutral cholesteryl ester hydrolase | No | – |
| NPC1 | Niemann Pick Type C −1 | No | Niemann-Pick disease type C |
| SMPD1 | Sphingomyelinase phosphodiesterase-1 | No | Niemann-Pick disease types A & B |
| SR-BI | Scavenger Receptor BI | Yes | Elevated HDL-C |
| STARD3 | Steroidogenic acute regulatory D3 | Yes | |

[a] Several of these genes are also found in GWAS associated with HDL-C (see Table 2)

*GWAS* genome-wide association studies; *HDL-C* high-density lipoprotein cholesterol

**Table 2** Results of GWAS showing 47 loci associated with HDL-C levels as a primary trait (with exception of the 9 last genes, where HDL-C was a secondary trait in the analysis)

| Locus | Gene | Chromosome | Functions and/or biological processes associated with gene of interest |
|---|---|---|---|
| PABPC4 | PolyAdenylate-Binding Protein Cytoplasmic 4 | 1 | Platelet activation |
| ZNF648 | Zinc Finger Protein 648 | 1 | Transcription factor |
| GALNT2 | UDP-N-acetyl-alpha-D-galactosamine: polypeptide Nacetylgalactosaminyltransferase 2 (GalNAc-T2) | 1 | Involved in O-linked oligosaccharide biosynthesis |
| IRS1 | Insulin Receptor Substrate 1 | 2 | Protein phosphorylated by insulin receptor tyrosine kinase, mutations in genes associated with type 2 diabetes and insulin resistance |
| COBLL1 | COBL-Like protein 1 | 2 | – |
| SLC39A8 | Solute Carrier family 39 (Zinc transporter), MEMBER 8 | 4 | Subfamily of proteins with structural characteristics of zinc transporters |
| ARL15 | ADP-Ribosylation Factor-Like 15 | 5 | – |
| C6orf106 | Chromosome 6 Open Reading Frame 106 | 6 | – |
| CITED2 | CBP/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain 2 | 6 | Inhibits transactivation of HIF1A-induced genes, mutations in genes are cause of cardiac septal defects |
| LPA | Lipoprotein (a) | 6 | Serine proteinase that inhibits tissue-type plasminogen activator I, elevated levels are linked to atherosclerosis |
| KLF14 | Kruppel-like factor 14 | 7 | Kruppel-like family of transcription factors |
| PPP1R3B | Protein PhosPhatase 1, Regulatory subunit 3B | 8 | Regulation of glycogen synthase phosphatase activity; may be involved in type 2 diabetes |
| TRPS1 | TrichoRhinoPhalangeal Syndrome I | 8 | Transcription factor that represses GATA-regulated genes, mutations in this genes are a cause of trichorhino-phalangeal syndrome types I-III |
| TTC39B | TetraTriCopeptide repeat domain 39B | 9 | Knockdown in mouse liver increases plasma HDL-C levels |
| ABCA1 | ATP binding Cassette A1[a] | 9 | Cholesterol efflux |
| AMPD3 | Adenosine Monophosphate Deaminase 3 | 11 | Deamination of AMP to IMP in red cells; purine nucleotide cycle |
| LRP4 | Low density lipoprotein Receptor-related Protein 4 | 11 | Regulator of Wnt signaling |
| PDE3A | Phosphodiesterase 3A | 12 | Mediates platelet aggregation and plays a role in cardiovascular function by regulating vascular smooth muscle contraction and relaxation |
| MVK | Mevalonate kinase[a] | 12 | Isoprenoid and sterol synthesis |
| SBNO1 1 | Strawberry notch homolog 1 | 12 | – |
| ZNF664 | Zinc finger protein 664 | 12 | Transcription factor |
| SCARB1 | Scavenger receptor class B, member 1[a] | 12 | Cholesterol uptake and efflux |
| LIPC | Hepatic lipase[a] | 15 | Triglyceride hydrolase; ligand/bridging factor for receptor-mediated lipoprotein uptake |
| LACTB | Lactamase, beta | 15 | Subunit of the mitochondrial ribosome (39S) |
| CETP | Cholesteryl Ester Transfer Protein | 16 | Transfer of cholesteryl ester from HDL to other lipoproteins |
| LCAT | Lecithin-cholesterol acyltransferase | 16 | Extracellular cholesterol esterifying enzyme; mutations in gene cause fish-eye disease and LCAT deficiency |
| CMIP | c-Maf-inducing protein | 16 | T-cell signaling pathway |
| STARD3 | StAR-related lipid transfer (START) domain containing 3[a] | 17 | Cellular cholesterol transport |
| ABCA8 | ATP Binding Cassette A8 | 17 | Member of the ABC1 subfamily (transport various molecules across extra- and intracellular membranes); function of protein has not yet been determined |

**Table 2** (continued)

| Locus | Gene | Chromosome | Functions and/or biological processes associated with gene of interest |
|---|---|---|---|
| PGS1 | Phosphatidylglycerophosphate synthase 1 | 17 | – |
| LIPG | Endothelial lipase | 18 | Phospholipase |
| MC4R | Melanocortin 4 receptor | 18 | Interacts with adrenocorticotropic and MSH hormones |
| ANGPTL4 | Angiopoietin-like 4 | 19 | Regulator of glucose homeostasis, lipid metabolism, and insulin sensitivity |
| LOC55908 | Hepatocellular carcinoma-associated gene TD26 | 19 | – |
| LILRA3 | Leukocyte Ig-like receptor, subfamily A, member 3 | 19 | Soluble immunoreceptor expressed on monocytes and B cells |
| HNF4A | Hepatocyte nuclear factor 4, alpha | 20 | Nuclear transcription factor, regulates expression of several hepatic genes |
| PLTP | Phospholipid transfer protein | 20 | Transfers phospholipids from triglyceride-rich lipoproteins to HDL |
| UBE2L3 | Ubiquitin-conjugating enzyme E2L 3 | 22 | Ubiquitination of several protein |
| APOB | Apolipoprotein B | 2 | Major apolipoprotein of chylomicrons, VLDL and LDL |
| MLXIPL | MLX interacting protein-like | 7 | HLH leucine zipper transcription factor of the Myc/Max/Mad superfamily |
| LPL | Lipoprotein Lipase | 8 | Triglyceride hydrolase; ligand/bridging factor |
| TRIB1 | Tribbles homolog 1 | 8 | – |
| FADS1-2-3 | Fatty acid desaturase | 11 | Regulate unsaturation of fatty acids |
| APOA1 | Apolipoprotein AI[a] | 11 | Major protein of HDL; involved in cholesterol efflux, cofactor for LCAT |
| UBASH3B | Ubiquitin associated and SH3 domain containing B | 11 | Inhibit endocytosis of EGFR and PDGF |
| LRP1 | Low density lipoprotein receptor-related protein 1 | 12 | Multiligand cell surface receptor; involved in intracellular signaling, lipid homeostasis, clearance of apoptotic cells |
| APOE | Apolipoprotein E | 19 | Chylomicron remnants and VLDL remnants clearance by lthe liver |

[a] Gene is related to known efflux defects in humans

*HDL-C* high-density lipoprotein cholesterol

(*Data from* Teslovich et al. [27] the National Center for Biotechnology Information [86])

synthesis and regulation of sterol regulatory element-binding proteins (SREBP)-1c.

The regulation of ABCA1 differs between cell types [21]. In macrophages, a novel pathway has been identified. Chen et al. [22] examined the effect of low-density lipoprotein (LDL) loading in macrophages and found increased levels of phosphorylated specificity protein 1 (Sp1) and protein kinase C-ζ (PKCζ), with increased amounts of Sp1 bound to the ABCA1 promoter. Inhibition of PKCζ or mutations within the Sp1 binding site on ABCA1 attenuated the LDL-induced expression of ABCA1 and the increase in cellular cholesterol efflux [22].

In a recent study of the lipoprotein receptor related protein-1 (LRP1), a cell-surface receptor of the LDL-receptor superfamily, Basford et al. [23] examined the effects of liver-specific LRP1 knockout ($LRP1^{-/-}$) in mice, and found lower levels of HDL-C. Primary hepatocytes from $LRP1^{-/-}$ mice displayed reduced HDL secretion and decreased cell-surface localization of ABCA1 without a change in total cellular ABCA1 content. Their results are consistent with a decreased translocation of ABCA1 to the plasma membrane in $LRP1^{-/-}$ mice hepatocytes (Fig. 1) [23].

## Apo AI

Apo AI is the major apolipoprotein within HDL, accounting for approximately 70% of the protein mass. The interaction between Apo AI and ABCA1 is rate-limiting in the first step of the RCT and thus in promoting efflux of cholesterol from

cells (Fig. 1). It has recently been documented that ABCA1 acts by flipping phospholipids to the outer leaflet of the plasma bilayer, contributing to the formation of a 'high-capacity binding site', facilitating binding of Apo AI to this domain, and subsequent formation of nascent HDL particles [14•].

Mutations within Apo AI have been previously identified, with at least 47 variants affecting Apo AI structure [24, 25], some leading to a marked reduction in Apo AI and HDL-C levels [24], and concomitant CHD, whereas others have low HDL but no incidence of heart disease [26]. Apo AI was also previously identified in GWAS (Table 2) [27••], with a significant association for triglyceride (TG), total cholesterol (TC), HDL (as a secondary lipid trait), and LDL lipid levels. Nevertheless, despite the fact that apo AI genetic variations are well characterized, the role of Apo AI structure on ABCA1-mediacted efflux has not been completely elucidated.

Considerable controversy in the past few years has emerged regarding the lipid-free and lipid-bound structure of Apo AI [28–30] offering different insights into the structure-function of Apo AI domains critical for nascent HDL particle assembly. As such, Huang et al. [31] have recently addressed Apo AI structure in spherical particles by applying chemical cross-linking and mass spectrometry to HDL particles, describing the first models of authentic human plasma HDL in which Apo AI assumes a cage-like structural framework closely resembling that in synthetic HDL. Specifically, Apo AI adopts intermolecular interactions in plasma HDL similar to interactions previously described in the double-belt and trefoil models derived in reconstituted systems, suggesting that HDL particle sizes are modulated through twisting motion of the resident Apo AI molecules [31].

Other studies have focused at better understanding the properties of the tertiary structure domains of Apo AI and their influence on Apo AI functionality in the RCT pathway [32•]. By generating two chimeric N-terminal domain-swap variants of mouse and human Apo AI, and expressing these hybrids in Apo AI-null mice, Alexander et al. [32•] evaluated their abilities to promote macrophage RCT in vivo, in comparison to wild-type (WT) human and mouse Apo AI. More cholesterol was observed to be significantly removed from the macrophages of the mouse-H Apo AI-expressing mice as compared to the other groups, as shown by a marked increase in fecal excretion via the RCT pathway. Furthermore, catalytic efficiencies ($V_{max}/K_m$) of the Apo AI variants were determined and observed to be twofold higher in the mouse-H Apo AI than the WT human Apo AI and 3.5-fold higher than WT mouse Apo AI, suggesting a more efficient ABCA1-mediated cholesterol efflux. These findings, along with a detected increase in the rate of cholesterol uptake into hepatocytes, demonstrated that substitution of the N-terminal domain of the human Apo AI with the mouse Apo AI counterpart created a "gain of function" Apo AI variant showing enhanced nascent HDL particle formation, a more efficient macrophage RCT, and potentially antiatherogenic apo AI. Additional insights into the effects of Apo AI structure on cholesterol efflux were described by the same group through examination of Apo AI C-terminal α-helix hydrophobicity influence on nascent HDL particle formation [33]. By engineering human Apo AI variants, Lyssenko et al. [33] observed that the decrease in Apo AI hydrophobicity of the non-polar face of the C-terminal amphipathic α-helix significantly reduced the catalytic efficacy of vesicle solubilisation and cholesterol efflux, forming large HDL particles, with reversing effects when hydrophobicity is restored [33].

In addition to structural modifications, several studies have documented that oxidative modification of Apo AI could also be a contributing factor in altering RCT levels [34, 35]. As such, Heinecke and Oram [36] have demonstrated that chlorination, but not nitration, of Apo AI through the myeloperoxidase pathway dramatically impaired the ability of Apo AI to interact directly with ABCA1 and to activate the Janus-kinase-2 signaling pathway, preventing Apo AI from promoting cellular cholesterol efflux in macrophages [36].

## ABCG1

The ATP binding cassette transporter G1 (ABCG1) is expressed in a variety of tissues. Although it is predominantly located in the cytosol, ABCG1 promotes the lipidation of nascent HDL particles rather than lipid-free (or lipid-poor) apo AI, playing a significant role in the efflux transport of excess cholesterol to HDL in macrophages, thereby reducing atherosclerosis (Fig. 1). Gao et al. [37] recently identified the first amino acid residue that is critical for ABCG1-mediated cholesterol efflux. Indeed, the cysteine residue located at position 514 is highly conserved and a mutation at this position, although having no effect on the protein stability or trafficking, significantly decreased the efflux of cholesterol onto lipidated apo AI.

Apart from structural changes, ABCG1 expression is mainly regulated by the cellular cholesterol content: it has been shown to be increased following treatment with acetylated LDL (cholesterol-loading condition) and downregulated after treatment with HDL-3 [38]. Nuclear receptors such as the LxR and the peroxisome proliferator-activated receptors (PPARs) have been suggested to regulate the expression of ABCG1. More recently, a significant effort has been put on the study of the various regulators of ABCG1 expression through the LxR pathway, and their effect on the resultant cholesterol efflux. First, Rayner et al. [39••] showed that ABCG1 is targeted by miRNAs (see miRNA section below),

more specifically miR-33a. Hence, in mouse macrophages, miR-33 decreases ABCG1 expression, reducing cholesterol efflux to nascent HDL. Despite the presence of a miR-33a binding site in the human ABCG1 gene, the targeting of ABCG1 by miR-33a only appears to be marginal [39••, 40]. The LxR pathway is also involved in the regulation of ABCG1 expression by group X secretory phospholipase $A_2$ (GX $sPLA_2$). In a recent study by Shridas et al. [41], ABCG1 expression and associated cholesterol efflux were found to be reduced after GX $sPLA_2$ overexpression or exogenous addition in mouse macrophages, suggesting a potential role of GX $sPLA_2$ in atherosclerotic lipid accumulation.

ABCG1-mediated cholesterol translocation also plays an important role in protection against endothelial dysfunction [42, 43], interaction of monocytes with endothelial cells [44], and regulation of insulin secretion [45, 46]. Increase in ABCG1 expression and concomitant cholesterol efflux capacity, in the presence of erythropoietin [47] or after weight loss [48] for instance, were respectively associated with a reduction in lipid accumulation in foam cells and an improvement in the atherogenic profile in human. As for insulin, it was shown to decrease HDL-mediated cholesterol efflux from macrophages from the suppression of nCEH and ABCG1 expressions [49] and to be involved in the insulin secretion process from pancreatic β cells [45]. These new insights on the regulation of ABCG1 present this transporter as having a novel potential target in the inhibition of the process of atherosclerosis in various human metabolic states, including obesity and diabetes.

## Disorders of Cholesterol Trafficking: NPC1, NPC2, LIPA and SMPD1 Genes

Niemann-Pick disease type C (NPC) is a complex lysosomal storage disorder caused by mutations in either the *NPC1* or *NPC2* genes, characterized by the accumulation of unesterified cholesterol in the lysosomal compartment. Choi et al. [50] have reported that the cholesterol trafficking defect in cells from subjects with NPC disease results in a reduced activity of *ABCA1*. Low plasma HDL-C in *NPC1* disease patients occurs independent of their plasma triglyceride levels, suggesting that impaired *ABCA1* regulation as a consequence of reduced efflux of cholesterol out of the late endosome is the cause of the low HDL-C seen in NPC [50, 51]. Reduced plasma HDL-C was the most consistent lipoprotein abnormality found in male and female *NPC1* patients across age groups independent of changes in plasma triglycerides, representing a potential biomarker of *NPC1* disease severity [52]. *NPC1* cells also develop a secondary defect in acid sphingomyelinase (SMase) activity despite a normal acid SMase gene, (*SMPD1*) [53]. Recent advances have provided potential therapeutic approaches for the treatment of NPC disease. In one study, SMase activity defect in fibroblasts from *NPC1* patients was corrected by *SMPD1* transfection or acid SMase enzyme replacement. Both approaches resulted in a dramatic reduction in lysosomal cholesterol [53]. Recently, the use of small histone deacetylase (HDAC) led to a correction of the NPC phenotype [54]. The HDAC inhibitor LBH589 (panobinostat) is currently undergoing clinical trials in man for several types of cancer. In cultured NPC1 fibroblasts, LBH589 restores cholesterol homeostasis, raising the possibility that this class of agents might be useful in a clinical setting [54].

Another form of ABCA1 impairment is seen in mutations in the lysosomal acid lipase A (*LIPA)* gene that result in less than 5% of normal lysosomal acid lipase (LAL) activity causing cholesteryl ester storage disease (CESD) [55]. *LIPA* has been identified on chromosome 10q23 as a novel CHD susceptibility locus. Elevated *LIPA* expression itself was related to lower HDL-C levels that are attributed to reduced cholesterol efflux to apo AI. Furthermore, treatment of fibroblasts from normal patients with chloroquine to inhibit LAL activity was observed to reduce *ABCA1* expression and activity, similar to that of CESD cells [55]. LXR agonist treatment of CESD cells corrected *ABCA1* expression, but failed to correct LDL CE hydrolysis and cholesterol efflux to apo AI. Moreover, LDL-induced production of 27-hydroxycholesterol (27-HC) was decreased in CESD compared to normal fibroblasts. It was further determined that treatment of CESD cells with conditioned medium containing LAL from normal fibroblasts or with recombinant human LAL, rescued *ABCA1* expression, apo AI-mediated cholesterol efflux, HDL particle formation, and production of 27-HC [55].

Sphingomyelin (SM) plays an important role in the structural integrity of cellular membranes. Characterized by its high gel to liquid-crystalline phase transition temperature, SM often segregates with cholesterol within distinct subcellular compartment. As previously described, one of the genes involved in the modulation of SM levels is sphingomyelin phosphodiesterase 1, also known as acid sphingomyelinase (*SMPD1, MIM 607608*). It codes for the lysosomal and secretory SMase, a 70 kDa glycoprotein that hydrolyzes SM to ceramide and phosphorylcholine. Mutations in the *SMPD1* gene cause the autosomal recessive disorder of Niemann-Pick type I disease, which includes type A (MIM 257200) and B (MIM 607616) (NPA and NPB). Deficiency of lysosomal SMase results in a lysosomal accumulation of SM and a secondary accumulation of cholesterol. We have previously reported that mutations in *SMPD1* causing Niemann-Pick disease types A and B are associated with low HDL-C levels but normal cholesterol efflux under the experimental conditions used [56, 57].

Together, these findings provide further evidence that the rate of release of cholesterol from late endosomes/lysosomes is a critical regulator of cellular cholesterol

trafficking (Fig. 1) and this, in turn, may be critical in cellular cholesterol efflux pathways via the ABCA1 transporter.

## SR-BI

The scavenger receptor BI (SR-BI) mediates the selective uptake of cholesteryl esters from HDL into hepatocytes and steroidogenic tissues [58, 59] (Fig. 1). The atheroprotective effects of SR-BI are therefore primarily attributable to its role in cholesterol efflux from lipid-laden macrophages to HDL [60–62] and in the delivery of HDL cholesteryl esters to the liver [63]. Interestingly though, SR-BI$^{-/-}$ mice exhibit higher levels of HDL-C and increased atherosclerosis, a process attributed to a critical block in the RCT to the liver for biliary excretion [64]. In humans, several studies have described genetic *SCARBI* variants associated with HDL-C levels [65, 66], most notably in the recent GWAS by Teslovich et al. [27] (Table 2), where SR-BI was identified among the 95 loci significantly associated with lipid levels in more than 100,000 individuals of European ancestry (rs838880 $P=3\times10^{-14}$), confirming its critical role in lipid metabolism. Furthermore, Vergeer et al. [67] recently brought forth evidence that impaired SR-BI function can affect human physiology. Identifying a kindred with a functional missense mutation in SR-BI, P297S, they observed that carriers had significantly elevated HDL-C levels, although no considerable differences in lipid profile parameters such as atherosclerosis or carotid intima-media thickness. Importantly, primary murine hepatocytes expressing mutant SR-BI exhibited a marked reduction of 56% in cholesterol uptake from HDL of that of wild-type SR-BI, whereas P297S variants carriers showed reduced efflux capacity from monocyte-macrophages comparatively to non-carriers [67].

## StARD3 (MLN64)

The steroidogenic acute regulatory proteins comprise at least 15 proteins involved in cellular cholesterol transport and homeostasis [68]. StARD3 (also called endosomal Metastatic Lymph Node protein 64**)** is involved in the key transfer of cholesterol from the late endosomal compartment, a step that occurs after the NPC1 protein. Recent data from Charman et al. [69] shows that StARD3 transports cholesterol from the endosome to the mitochondria and may provide an essential regulatory step between cholesterol, mitochondria-derived oxysterols, and regulation of the cellular cholesterol efflux machinery (Fig. 1). To date, no human disease linked to StARD3 has been identified. Moreover, targeted mutations of the StAR domain in mice do not lead to a defect in steroidogenesis or to a distinct phenotype [70]. However, in a recent study, Borthwick et al [71] reported that the overexpression of StARD3 in macrophages induces an increase in ABCA1 mRNA and protein, and enhanced cellular cholesterol efflux to Apo AI. Interestingly, overexpression of STARD3 in macrophages also prevents the accumulation of cholesteryl esters in response to acetylated LDL [71]. In GWAS (Table 2), the StARD3 locus is associated with lower HDL-C levels, suggesting an important role for this protein in cellular cholesterol homeostasis and possibly, efflux.

## ABCA8

The *ABCA8* gene, located on chromosome 17 (17q24) and coding for another membrane-associated protein member of the ABC superfamily, has also been identified in GWAS (Table 2). The function of this protein in cellular cholesterol efflux is however still unclear. The protein has been isolated in human brain microvessels [72] where it could play a role in intracellular lipid trafficking rather than trans-plasma membrane transport [73]. The gene could be regulated by extracellular signal-regulated kinases (ERK1 and 2) [74].

## Novel Genes

The study of extensive kindred with HDL deficiency have identified several chromosomal regions harboring genes related to HDL metabolism [75–77, 78•]. We have recently reported a strong associated between the WWOX gene locus and HDL cholesterol levels [79] ($P=6.9\times10^{-7}$). It remains to be determined if these genes are related to cholesterol homeostasis of cholesterol efflux pathways.

## miRNAs

Recently, micro RNAs (miRNAs) as key regulators of lipoprotein metabolism have been uncovered [80, 81]. miRNAs are short (average 22 nucleotides) strands of RNA involved in the repression of gene transcription. Single miRNAs can regulate the transcription of multiple genes by virtue of target sequences usually located within the 3′UTR of target genes. One miRNA, miR-33, located within the SREBP2 gene, has been shown to repress expression of ABCA1 and to lower HDL-C levels (Fig. 1). Using oligonucleotides directed against miR-33, Rayner et al. [39••, 82] showed that inhibition of miR-33 in the LDL-R$^{-/-}$ mouse is associated with an increase in hepatic ABCA1 and ABCG1 expression, increase in HDL-C, increase in reverse cholesterol transport to the liver and feces, and reduced atherosclerosis. This provides compelling support for a therapeutic application in man. More recently, other miRNAs (miR-106b,

miR758) [81, 83] have been shown to control a variety of aspects of lipid metabolism, from HDL biogenesis to cholesterol efflux. These findings have thus highlighted the significant role that miRNAs play in HDL metabolism, opening new avenues for the treatment of CHD.

## Genome-Wide Association Studies

The search for novel genes associated with a measurable trait has been greatly facilitated by GWAS. Using single-nucleotide polymorphisms (SNPs), common genetic variants in different individuals are examined to identify association with a specific, measurable biological trait. GWAS can identify SNPs (and other variations) in DNA, but do not necessarily imply causality between identified genes and the biological trait under study.

GWAS compare the frequency of common DNA variants between people with the trait or the disease (cases) and similar people without (controls). DNA is extracted from various cells in individual participants. High-throughput gene chips can now access over 2,000,000 SNPs, spanning the entire genome. The prevalence of genetic variants at each SNP is then examined between cases and controls and bioinformatics is then used to determine the statistical strength and significance of the observation. It should be emphasized that most of the SNP variations associated with a trait or disease do not always lay in the region of DNA that codes for proteins. They are usually in the large non-coding regions (introns or intergenic regions); such SNPs are probably not causal but may be in linkage disequilibrium with yet unknown genetic variants that have functional significance.

In contrast to careful analyses of individual SNPs, GWAS are hypothesis-free and, theoretically at least, bias-free. It should be noted that the sheer number of statistical tests performed presents an unprecedented potential for false-positive results. Despite these caveats, GWAS in the field of HDL genetics has reassuringly identified many of the same genes (or genomic regions) that harbor genes causing Mendelian disorders in man [78•] (Table 2). Thus, GWAS not only have the potential of finding common variants explaining a relatively small percentage of variation of a biological trait, but also to identify novel genes associated with disease in man.

The investigation of severe traits of HDL will be facilitated by two other techniques that allow even faster progress in the field of human genetics. “Deep” resequencing is a relatively novel technology that allows the sequencing of genomic DNA over a large chromosomal region (usually in millions of base pairs). Reference genome sequences for humans are available [84]. Identifying sequence variations and understanding their biological significance is therefore becoming a major focus of genetics. The second technique is exome-wide sequencing, where every exon (approximately 180,000) is sequenced by several high-throughput technologies. Using reference genome sequences and powerful bioinformatics packages, one can identify genetic variants causing disease in humans [85•].

## Conclusion

Evidence shows that cholesterol efflux and RCT play a major role in preventing atherosclerosis in humans. Multiple studies have also reported genetic data to explain variations in HDL-C levels associated with cellular cholesterol homeostasis. These studies are based on different approaches, including heritability of HDL-C levels in families, candidate genes involved in HDL metabolism, linkage analyses from genome-wide scans, and GWAS, among others. Furthermore, because of their importance in lipoprotein metabolism and vascular endothelial function, the identification of novel genes involved in cholesterol efflux pathways, is warranted. As such, novel genomic pathways related to HDL metabolism may provide novel therapeutic avenues to prevent CHD.

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- • Of importance
- •• Of major importance

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