Genetics of HDL regulation in humans

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Purpose of review

To review gene regulation of HDL-cholesterol and discuss molecular abnormalities in HDL candidate genes that may lead to human pathologic states.

Recent findings

The inverse association between HDL-cholesterol and vascular disease, especially coronary heart disease, has long been recognized, but understanding gene regulation of HDL in humans gained considerable momentum following the identification of ABCA1 as playing a pivotal role in reverse cholesterol transport. Recent data suggest that potentially important targets for upregulating HDL in humans include upregulators of *ABCA1* and *APOA1* (e.g. peroxisome proliferator activated receptor and liver X receptor agonists) and downregulators of *CETP* (e.g. JTT-705). A host of other nuclear receptors under investigation in animal models may advance to human testing in the near future.

Summary

Disorders affecting HDL metabolism are complex because monogenic disorders causing low HDL do not necessarily correlate with premature vascular disease. To date, pathologic phenotypes have only been deduced among several HDL candidate genes. Understanding the genetic underpinnings associated with variant HDL and reverse cholesterol transport provides an exceptional opportunity to identify novel agents that may optimize this process and reduce vascular event rates beyond currently available LDL lowering therapies.

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Abbreviations

Apo A1	apolipoprotein A1
CETP	cholesterol ester transfer protein
CHD	coronary heart disease
FHA	familial hypoalphalipoproteinemia
LCAT	lecithin:cholesterol acyltransferase
LXR	liver X receptor
PPAR	peroxisome proliferator-activated receptor
SR-BI	scavenger receptor class B type I

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Introduction

Beginning with the clinical description of orange tonsils and associated absence of HDL-cholesterol in a brother and sister residing on Tangier Island more than 40 years ago, there has been considerable interest in understanding the genetics of HDL regulation in humans. During the past two decades, numerous candidates such as apolipoproteins, lipases, antioxidants, transfer proteins and regulators of cholesterol efflux and esterification have been studied and at least 25 candidates are believed to contribute to the anti-atherothrombotic effects of HDL. Genome wide scans have supplemented these efforts with identification of 22 different loci in 14 chromosomal regions contributing to HDL levels in humans (see ref. [1•]). Because genetic influences are the primary contributors to HDL levels, the identification and characterization of variants have provided useful information related to genotype–phenotype relationships and attendant risk of vascular disease. However, the relationship between HDL concentration and cardiovascular disease is not as straightforward as initially hypothesized with several genetic hurdles offsetting this relationship at both ends of the HDL spectrum (e.g. apolipoprotein $A1_{Milano}$ (Apo $A1_{Milano}$) and cholesterol ester transfer protein (CETP) deficiency). Moreover, while the overwhelming majority of low HDL syndromes (20–40 mg/dL) result from polygenic influences, single gene defects are more likely to predominate with HDL <10 mg/dL in the absence of identifiable metabolic irregularities. This review evaluates genetic factors affecting HDL regulation in humans, with a focus on single gene variants that predispose to premature vascular disease and potential targets for therapeutic intervention aimed at maximizing HDLs' cardioprotective properties.

HDL genetic variants associated with low HDL cholesterol and human pathology

While at least 10 candidate genes are involved in the regulation of HDL metabolism, human pathology has only been consistently reported among several candidates in whom structural variation may cause low HDL.

ABCA1

ABC transporters are involved in the mobilization of various substrates across plasma membranes. Defective gene products have been implicated in pathologic conditions ranging from cystic fibrosis and acute bronchopulmonary aspergillosis to pseudoxanthoma

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elasticum (ABCC6), retinitis pigmentosa (ABCA4) and sitosterolemia (ABCG5/ABCG8). Mapped to 9q31.1 and expressed in liver, macrophages and steroidogenic tissues, *ABCA1* mediates cholesterol efflux from macrophages to HDL. Defects in both *ABCA1* alleles as a result of consanguinity or compound heterozygosity may result in Tangier disease, characterized by yellowish–orange discoloration on tonsillar and mucosal surfaces owing to accumulation of carotenoid pigment and retinyl esters in reticuloendothelial cells. Lipid accumulation in Schwann cells may result in peripheral neuropathy [2].

As with other ABC proteins, ABCA1 consists of two transmembrane domains each composed of six hydrophobic membrane spanners. Surrounding each transmembrane domain is the ATP binding cassette which contains the Walker A and B motifs connected by a family specific signature region. Extracellular loops containing glycosylation sites are present within the hydrophobic membrane spanners. At least 30 different structural variants have now been reported in *ABCA1* and encompass most of the regulatory regions (Table 1). Tangier fibroblasts demonstrate reduced cholesterol efflux with the greatest inhibition of efflux correlating with HDL levels [3]. Compared with missense mutations, other ABCA1 variants (e.g. frameshift and in-frame deletions) that encode a truncated protein appear to be less amenable to transcriptional upregulation by oxysterols [4•], which are presently under active investigation as potential targets for enhancing reverse cholesterol transport (see below).

In addition to Tangier disease, a single defective *ABCA1* allele has been reported to cause familial hypoalphalipoproteinemia (FHA), a disorder characterized by moderately low HDL-cholesterol and premature coronary heart disease (CHD). To date, affected FHA subjects with *ABCA1* variants have considerably lower HDL levels than more typically identified in CHD patients (e.g. <20 mg/dl compared to >30 mg/dl). This suggests that FHA caused by *ABCA1* variants likely accounts for only a minority of such cases in the population. In fact, one recent population study of FHA subjects ($n=515$) found no significant association between *ABCA1* polymorphisms and low HDL-cholesterol [5•]. Similarly, fibroblasts from FHA subjects are heterogenous in cholesterol efflux capacity; a range of normal efflux to 50% reduction [6] has been correlated to HDL levels. In association with reduced cholesterol efflux is enhanced carotid intima medial thickness, a non-invasive predictor for future CHD event rates [7]. However, not all ABCA1 variants associated with FHA have been correlated with increased intima–media thickness [8•] and, therefore, it is clinically useful to identify other potentially important covariates (e.g. hypertriglyceridemia, insulin resistance) that may impart increased risk of CHD because failure to adjust for these factors may overstate the true association between isolated low HDL and premature CHD.

In addition to atherosclerotic vascular disease, compound variation in *ABCA1* resulting in HDL deficiency was recently reported in a subject with cerebral amyloid angiopathy [9•]. Increased formation of β-amyloid has also been linked to Alzheimer's disease and one study has suggested that genetic variability of *ABCA1* may impact on the development of Alzheimer's disease [10••].

ABCA1 gene expression is upregulated by liver X receptor (LXR) and retinoid X receptor agonists. In response to cholesterol loading, oxysterols are produced which activate LXR [11] to facilitate cholesterol efflux. Recently a synthetic LXR agonist, acetyl-podocarpic dimer, was shown to be much more effective than the oxysterols, 22-hydroxycholesterol and 27-hydroxycholesterol, in promoting cellular cholesterol efflux [12••]. The extent to which this and other potent LXR agonists (e.g. T0901317) [13] may impact on vascular disease event rates awaits clinical trials.

Peroxisome proliferator-activated receptors (PPARs) also enhance *ABCA1* gene expression by inducing LXR. Fibrates (PPARα) and glitazones (PPARγ) represent two classes of PPAR activators that may raise HDL cholesterol through this pathway [14].

Apolipoprotein A1

As the primary apolipoprotein of HDL, Apo A1 serves as a cofactor for cholesterol esterification and is an important component of reverse cholesterol transport. Apolipoprotein A1 consists of four coding regions and is complexed with apos C3 and A4 on chromosome 11. Complete apo A1 deficiency owing to chromosomal aberrations or deletions results in premature CHD [15]. The impact of genetic variants in apo A1 is highly variable; affected subjects may attain an elderly age without CHD [16•] whereas others may be at increased risk in association with other CHD risk factors [17]. Two notable variants, apo $A1_{Milano}$ and apo $A1_{Paris}$ are associated with reduced vascular risk. Recent data evaluating apo $A1_{Milano}$ have indicated that the additional cysteine residue encoding by the amino acid substitution dimerizes with apo A2 [18], facilitating reverse cholesterol transport despite very low HDL-cholesterol levels. Affected subjects with apo $A1_{Milano}$ also demonstrate reduced carotid intima–media thickness [19] and greater antioxidant capacity [20••]. Transcriptional regulators of *APOA1* include PPARα agonists, steroid hormones and and retinoids [21,22]. A common variant in the apo A1 promoter (–76 G/A) has been shown to be associated with elevated HDL-

Table 1. Pathologic mutations in *ABCA1*

Disease	bp location	NT change	Exon	*Protein change	Location	Reference
TD	IVS2	+5G/C	on original	EX2 or EX4	abnormal protein	[73•]
FHA	648	C/T	4	P85L	extracellular loop # 1	[72•]
TD	1051	G/A	7	R219K	extracellular	[67,68]
FHA	1083	C/T	7	R230C	extracellular	[70]
FHA	1158	G/A	8	A255T	extracellular	[75•]
TD	1210–1217 8bp del		9	277X	truncation	[65]
TD	1239	C/T	9	R282X	truncation	[73•]
TD	1591	T/C	11	V399A	extracellular	[68]
TD	1979 (110bpA*lu* Ins)		12	truncated	truncation	[60]
TD/FHA	2154	C/T	14	R587W	extracellular	[67,69]
TD	2164	G/C	14	W590S	extracellular	[61]
TD	2185	A/G	14	Q597R	extracellular	[59,67]
TD	2219	G/del	14	truncated, 635X	truncated	[60,61]
FHA	2472–2474	3bp del	15	Del L693	TM domain #3 phosphorylation	[59]
	2706	G/A	16	V771M	extracellular	[68]
	2715	A/C	16	T774P	extracellular	[68]
	2723	G/C	16	K776N	extracellular	[68]
	2868	G/A	17	V825I	TM domain #6	[67,68]
TD/FHA	3044	A/G	18	I883M phosphorylat site	cytoplasmic	[68]
FHA	3120	C/T	19	R909X	truncation	[63,71]
TD	3181	C/T	19	T929I	cytoplasmic	[62]
TD	3199	A/G	19	N935S	Walker A	[61]
TD	3205	C/T	19	A937V	Walker A	[61]
TD	3532	C/A	22	A1046D	cytoplasmic, Walker A/B	[70]
FHA	3667	T/C	23	M1091T	cytoplasmic	[63]
	3690	G/T	23	D1099Y	cytoplasmic	[9]
TD	3738	2bp del	23	1145X	truncation	[66]
FHA	3911	G/C	24	E1172D	linker/cytoplasmic	[68]
FHA	4242	4bp del	27	1297X	truncated	[64]
TD	4260	G/A	27	D1289N	linker cytoplasm	[64,65]
TD	4824	T/C	31	C1477R	extracellular	[59]
TD	4912	C/T	32	S1506L	extracellular loop #2	[71]
TD	5025	ins A	34	A1544S→1552X	truncation	[70]
	5059	T/C	34	I1555T	extracellular loop #2	[67]
	5155	G/A	35	R1587K	extracellular loop #2	[68]
FHA	5226	A/G	36	N1611D	extracellular loop #2	[75••]
	5338	T/C	36	L1648P	extracellular loop #2	[67]
TD	5443	C/T	37	R1680W	cytoplasmic	[74•]
TD	3′ deletion (intron 38)			truncated	truncation	[61]
	5587	C/G	38	S1731C	extracellular	[68]
TD	5793	A/C	40	N1800H	extracellular loop, sm	[65]
FHA	5946	C/T	41	R1851X	truncation	[75••]
FHA	6068	del	42	del 1893–1894(E,D)	cytoplasmic	[63]
TD	6152 (14bp Ins) (42–43)			truncated	truncation	[67]
	6316	A/G	44	K1974R	cytoplasmic	[67]
	6421	T/C	45	F2009S	cytoplasmic	[9]
TD	6636	C/T	47	R2081W	cytoplasmic	[64]
FHA	6825	C/T	49	R2144X	cytoplasmic	[63]
TD	6825	del C	49	2145X	truncation	[62]
FHA	6844	C/T	49	P2150L	cytoplasmic	[62]
	6898	C/T	49	P2168L	cytoplasmic	[67]
TD CTC6952–4TT			49	2203X	truncation	[62]
TD	6968 (4bp Ins)		49	2215X, truncated PDZ binding (cyto)		[65]

*Location in accordance with Santamaria-Fojo *et al.* (Proc Natl Acad Sci U S A 2000; 97:7987–7992). TD, Tangier disease; FHA, familial hypoalphalipoproteinemia.

cholesterol and apo A1 levels in some [23] but not other studies [24]. Fat feeding studies have suggested that the presence of this polymorphism was associated with greater LDL increases [25] and in a follow-up postprandial study, carriers of this polymorphism exhibited reduction in apo A4 in large triacylglycerol-rich lipoproteins following the fat load [26]. Although the mechanism between the postprandial changes observed in association with –76 G/A remains to be delineated, the increased postprandial response may reflect less efficient catabolism of triacylglycerol-rich lipoproteins with reduction in apo A4.

Lecithin:cholesterol acyltransferase

Lecithin:cholesterol acyltransferase (LCAT) deficiency is either associated with reduced esterification in plasma (classic LCAT deficiency) or in HDL (fish eye disease). At least 30 different mutations have now been reported [27]. Absence of LCAT leads to accumulation of lecithin and free cholesterol as well as production of an abnormal lipoprotein and results in a clinical pattern of anemia, corneal opacification and renal insufficiency. However, premature CHD is uncommon, even in the presence of cardiovascular risk factors [28,29].

HDL genetic variants associated with high HDL-cholesterol and variable human pathology

Alterations in several HDL regulating genes that result in hyperalphalipoproteinemia (hyperα) have not consistently been associated with cardioprotection. Scavenger receptor BI (SR-BI) deficiency has been included in this discussion even though molecular defects in humans that may impact on atherothrombosis await publication.

Cholesterol ester transfer protein deficiency

CETP is a key plasma protein that influences circulating levels of HDL-cholesterol by facilitating the transfer of esterified cholesterol from HDL to very low density lipoproteins (VLDL), and the transfer of triacylglycerol from VLDL particles to HDL [30]. In conditions where hypertriglyceridemia exists, the efficiency of transfer of triacylglycerol is enhanced leading to triacylglycerol enrichment and cholesterol depletion of HDL. This cholesterol-poor HDL particle is cleared more rapidly from the kidney, and thereby leads to lower circulating HDL-cholesterol levels.

The lipid profile typically seen in subjects with CETP deficiency includes elevated HDL-cholesterol levels with LDL-cholesterol levels generally in the normal range [31]. The HDL_2 subfraction is increased and enriched with cholesterol, consistent with the fact that CETP is unable to facilitate the transfer of esterified cholesterol from HDL_2 to VLDL. Some investigators have suggested that despite the elevations in plasma HDL-cholesterol levels, these particles are dysfunctional and may not be cardioprotective [32], while others suggest that CETP deficiency is associated with a low prevalence of coronary artery disease [33].

The *CETP* gene is located on chromosome 16 and consists of 16 exons and 15 introns and is a member of the lipopolysaccharide binding protein gene family [34]. Most cases of CETP deficiency have been described in Japanese kindreds but cases have also been described in Caucasian and other Asian populations. The first Japanese subject identified as CETP deficient was found to have a G-to-A mutation in the 5'-splice donor site of intron 14 [35]. Other genetic permutations include a missense mutation in exon 15 (442D:G) and several polymorphisms in introns 1 and 8.

The association of CETP deficiency and atherosclerosis had been examined in males of Japanese ancestry in the Honolulu Heart Study [36]. The two *CETP* gene mutations, exon 15 442D:G and intron 14 G:A, were associated with increased HDL-cholesterol and decreased CETP levels. Increased risk for CHD was found in men with the mutations, particularly 442D:G, and HDL-cholesterol levels between 41 and 60 mg/dl, while men with or without mutations and HDL-cholesterol levels >60 mg/dl had a lower prevalence of CHD. However, these data have been challenged and a recent prospective analysis found no significant association between CETP mutations (in heterozygotes) and CHD [37•]. In a clinical trial using the CETP inhibitor, JTT-705, it was shown that the compound effectively raised HDL-cholesterol by 34% and decreased LDL by 7%, without major side effects [38•]. Taken together, these studies suggest that partial inhibition or deficiency of CETP may not be a deleterious effect. Importantly, clinical outcome studies are needed to be sure that raising HDL-cholesterol via CETP inhibition is of clinical benefit.

Hepatic lipase deficiency

Hepatic lipase is a glycoprotein of approximately 65 kDa and is synthesized in hepatocytes, then secreted and bound to hepatocyte and hepatic endothelial surfaces. Hepatic lipase specifically catalyzes the hydrolysis of triglycerides, diglycerides and phospholipids in native lipoproteins. In animal studies Lambert *et al.* [39] have shown that hepatic lipase is involved in the selective uptake of cholesterol ester from HDL.

The human hepatic lipase gene, *LIPC*, is located on chromosome 15q21. It comprises 9 exons and 8 introns, and spans a length of more than 30 kb. It encodes a protein of 449 amino acids with a signal peptide of 23 amino acids [40,41]. Four polymorphisms have been detected in relation to the human hepatic lipase gene. They are: –250 G to A, –514 C to T, –710 T to C, and –763 A to G. Deeb and Peng [42] examined the promoter polymorphisms at positions –250 and –514 to determine whether they have any effect on hepatic lipase activity and whether they are in linkage disequilibrium. They reported that the –514 C→T allele is responsible for decreased hepatic lipase activity.

Vega and colleagues [43] reported the frequency of the –514 T allele to be significantly higher in African-Americans (0.52) than in white Americans (0.17, $P<0.0001$). Nie *et al.* [44] researched two polymorphisms in the coding region of the hepatic lipase gene: an

A→G substitution at +651 (ASP→SER at codon 193), and an A→C substitution at +1075 that (LEU→SER) (LEU→PHE) at codon 334. The +651 G allele was twice as common in African-American men than in white men. The +1075 C allele was nearly 20 times more common in African-American men compared to white men. This study adds further insight to the genetic predisposition of African-Americans to higher HDL-cholesterol and lower hepatic lipase activity. The +651, +1075 and –514 alleles are associated with decreased hepatic lipase activity in African-Americans. Recent data evaluating polymorphisms in endothelial lipase [45••] have also disclosed ethnic differences that may be associated with high HDL. However, the clinical relevance of these molecular alterations remains to be determined [46].

Scavenger receptor class B type I deficiency

The HDL receptor, scavenger receptor class B, type I (SR-BI), was isolated and characterized as a functional receptor by Acton *et al.* [47]. It is a member of the scavenger receptor class B family, whose members also include CD36 and LIMPII [48]. SR-BI participates in the selective uptake of cholesterol ester and is regulated by a number of factors including corticotrophin, estrogens, testosterone, cyclic AMP, gonadotropins, PPARα and PPARγ agonists, and polyunsaturated fatty acids [49,50,51•,52–54]). A number of ligands bind with high affinity to SR-BI, including LDL, HDL, VLDL, modified LDL, and apolipoproteins [55]. In animal models, it has been shown to control levels of plasma HDL-cholesterol and non-HDL-cholesterol levels, and the propensity for atherosclerosis [56]. The *SR-BI* gene has been localized to chromosome 12, it spans 75 kilobase pairs, and contains 13 exons [55]. The gene encodes a receptor protein of approximately 80 kDa, whose weight can vary based on its extent of glycosylation [55]. SR-BI is highly expressed in liver and steroidogenic tissue (adrenal, ovaries, and testes) [55], and has been localized in atherosclerotic plaques (predominantly in macrophages) [56,57].

Acton *et al.* have identified and associated polymorphisms of the *SR-BI* gene locus in a white European population with plasma lipid levels and body mass index [58]. These investigators identified five variants at the human *SR-BI* gene locus, with three variants at exons 1 and 8 and intron 5 having allele frequences >1.0. The exon 1 variant was significantly associated with higher HDL and lower LDL levels in men but not in women. The exon 8 variant was associated with lower LDL levels in women but not in men, and the intron 5 variant showed an association with body mass index in women. The authors concluded that SR-BI might influence LDL and HDL levels.

Conclusion

Abnormalities in several HDL regulatory genes have been associated with human pathology. Specifically, HDL deficiency states may culminate in premature vascular disease (ABCA1 and apo A1) or renal insufficiency (LCAT) whereas variants causing CETP and hepatic lipase deficiency result in elevated HDL but they may also pose elevated risk of vascular disease. Potential pathologic (and/or cardioprotective) consequences of structural alterations in other HDL regulating genes (e.g. apos A2, A4, C1 and C3, phospholipid transfer protein, lipoprotein and endothelial lipase, SR-BI and paraoxynase) remain to be defined. Finally, considerable promise holds for agents that upregulate (e.g. LXR agonists) or downregulate (e.g. CETP inhibitors) gene expression of HDL target genes and enhance reverse cholesterol transport.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

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- •• of outstanding interest

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ABCA1 expression was assessed in human fibroblasts harvested from subjects with and without truncated mutations. Truncated mutations blunted protein expression of the full-length *ABCA1* allele and suppressed upregulation by oxysterols.

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Subjects with low HDL-cholesterol and premature CHD ($n=35$) were screened for ABCA1 mutations and family studies evaluated whether the ABCA1 locus segregated with low HDL. In addition, the prevalence of common ABCA1 polymorphisms was studied in a Finnish sample ($n=515$). No linkage was identified between ABCA1 and HDL-cholesterol and one of the five mutations screened for were found in this high-risk group. None of the ABCA1 polymorphisms was significantly associated with HDL.

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Carriers of R219K in ABCA1 were found to have reduced TC (33%) in cerebrospinal fluid and delayed onset of Alzheimer's disease. In contrast, no association was found in carriers of another variant (R1587K). The authors speculate that variability in ABCA1 may impact on the pathogenesis of Alzheimer's disease.

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This paper describes a 69-year-old woman with apoA-I deficiency resulting from a homozygous base-pair deletion (codon 184) and predicted protein truncation at amino acid 199. Despite markedly reduced HDL (<0.18 mmol/l) and mild elevation in apo B (119 mg/dl) there was no history of symptomatic CHD.

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