Annu. Rev. Nutr. 2006. 26:105–29
doi: 10.1146/annurev.nutr.26.061505.111214

First published online as a Review in Advance on May 8, 2006

VARIATIONS ON A GENE: Rare and Common Variants in *ABCA1* and Their Impact on HDL Cholesterol Levels and Atherosclerosis

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Key Words HDL, atherosclerosis, genetics, lipoproteins, lipids

■ **Abstract** Cholesterol and its metabolites play a variety of essential roles in living systems. Virtually all animal cells require cholesterol, which they acquire through synthesis or uptake, but only the liver can degrade cholesterol. The *ABCA1* gene product regulates the rate-controlling step in the removal of cellular cholesterol: the efflux of cellular cholesterol and phospholipids to an apolipoprotein acceptor. Mutations in *ABCA1*, as seen in Tangier disease, result in accumulation of cellular cholesterol, reduced plasma high-density lipoprotein cholesterol, and increased risk for coronary artery disease. To date, more than 100 coding variants have been identified in *ABCA1*, and these variants result in a broad spectrum of biochemical and clinical phenotypes. Here we review genetic variation in *ABCA1* and its critical role in cholesterol metabolism and atherosclerosis in the general population.

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INTRODUCTION

Atherosclerosis and its manifestation in coronary artery disease (CAD) are the leading causes of death worldwide (118). CAD is a multifactorial disease influenced by smoking, obesity, diabetes mellitus, and alterations of plasma lipid levels (77). The central role of elevated low-density lipoprotein (LDL) cholesterol as an important risk factor for and cause of CAD is well established. Dietary saturated and trans fats are critical drivers of plasma hyperlipidemia (101), and indeed have a significantly greater hyperlipidemic effect than does dietary cholesterol, partially through their ability to activate lipogenic genes in the liver (75). The development of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins), which reduce LDL cholesterol by inhibiting hepatic cholesterol synthesis, have been a critical development in the treatment and prevention of CAD (47). However, despite significant reductions in mortality with statin treatment, recent studies continue to document a high incidence of residual disease burden even in aggressively treated populations (20), highlighting the need for new therapeutic approaches to prevent CAD.

A low level of high-density lipoprotein (HDL) cholesterol is the most common lipoprotein abnormality among men with CAD (45, 46). HDL is thought to prevent atherosclerosis by selectively returning cholesterol from peripheral tissues to the liver for excretion, in a process termed reverse cholesterol transport (40, 49). Other properties of HDL that may contribute to its atheroprotective role include modulation of endothelial function through nitric oxide production (117), suppression of monocyte adhesion molecules, and inhibition of LDL oxidation (103). Data from epidemiological studies suggest that for every 1 mg/dl increase in HDL cholesterol, CAD risk is reduced by 2%–3% (21, 48). The Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial further demonstrated that gemfibrozil treatment, which increased HDL cholesterol modestly (by 6%), was associated with a 22% reduction in coronary events (92). Dietary modification (99) and high-intensity exercise (70) can achieve modest increases in HDL cholesterol, but there are currently no approved therapies that substantially raise HDL levels. HDL cholesterol therefore represents an attractive but largely underexploited avenue for therapeutic intervention in CAD (76).

IDENTIFICATION OF GENES THAT REGULATE HDL CHOLESTEROL

About half of the variation in HDL cholesterol levels is under genetic control (13, 88). As with most common phenotypes, the search for the genetic components of HDL levels has been difficult. Both whole-genome linkage analysis and candidate gene association studies have been undertaken to identify the genetic factors that influence HDL levels (8, 54). However, in general these approaches have been unable to identify major loci involved in HDL regulation in humans. Linkage

analysis is generally underpowered to identify all but the most powerful loci, and candidate gene studies necessarily examine only a small number of potential targets out of the entire genome. With the completion of the phase I HapMap (7), whole-genome association studies may soon become a reality. To date, however, one extremely fruitful approach for identifying the genetic factors involved in HDL metabolism has been the study of rare, Mendelian disorders of HDL metabolism.

Tangier disease (TD) (Online Mendelian Inheritance in Man no. 2054000) is one such rare disease, affecting approximately 100 patients worldwide. TD was first identified by Donald Fredrickson on Tangier Island in the Chesapeake Bay area of the United States (42). While performing routine tonsil exams, Fredrickson discovered two siblings with massively enlarged orange tonsils and a virtual absence of alpha migrating (HDL) lipoprotein, thus identifying the two salient features of TD: reduced HDL cholesterol and cholesterol accumulation in cells of the reticuloendothelial system. With remarkable foresight, Frederickson noted that "patients with rare genetically determined diseases offer . . . an occasional view of normal processes obtainable in no other way," and that TD may "provide us with just such a view into some now-clouded aspects of fat transport and metabolism" (42).

In 1999, the molecular cause of TD was identified as mutations in both alleles of the *ABCA1* gene (12, 14, 74, 93). Soon after, mutations in *ABCA1* were also described in familial HDL deficiency, a more common and relatively milder disorder than TD, which results from heterozygous loss of *ABCA1* function (79). To date, more than 100 common and rare variants have been described in the *ABCA1* gene, with a wide range of biochemical and clinical phenotypes (98). Here we review genetic variation in *ABCA1* and how it influences cholesterol transport, HDL metabolism, and risk for atherosclerosis.

ABC GENE SUPERFAMILY

The transport of specific molecules against gradients across cellular membranes is a fundamental feature of biological systems. The ABC transporter superfamily encodes proteins that transport a wide variety of substances including sterols, metabolic products, and drugs across both intra- and extracellular membranes. The ABC gene superfamily is highly diverse and is well conserved between species, hinting at the evolutionarily ancient history and critical importance of this gene family (53). ABC transporters are the largest known membrane transporter family, consisting of 49 members in humans (33). The mammalian ABC gene superfamily is divided into seven subfamilies, A through G, based on similarity in gene structure, order of domains, and sequence homology in the nucleotide-binding folds and transmembrane regions. Sixteen of the 49 human ABC genes are known to be associated with genetic disease (shown in Table 1), including cystic fibrosis, Stagardt disease, sistosterolemia, Dubin-Johnson syndrome, and others, underscoring the fundamental importance of this class of transporters in physiological processes.

TABLE 1 ABC transporters implicated in human disease

Gene	Associated disease	Reference
ABCA1	Tangier disease	(14)
ABCA3	Surfactant deficiency	(97)
ABCA4 (ABCR)	Stargardt disease	(5)
ABCA12	Harlequin ichthyosis	(66)
ABCB1 (MDR1)	Multidrug resistance	(95)
ABCB2 (TAP1)	Defective antigen presentation	(23)
ABCB3 (TAP2)	Immune deficiency	(32)
ABCB4 (MDR3)	Progressive familial intrahepatic cholestasis-3	(34)
ABCB7	X-linked sideroblastosis and anemia	(4)
ABCB11	Progressive familial intrahepatic cholestasis-2	(104)
ABCC2	Dubin-Johnson syndrome	(86)
ABCC6	Pseudoxanthoma elasticum	(10)
ABCC7 (CFTR)	Cystic fibrosis	(67)
ABCC8 (SUR1)	Persistent hyperinsulinemic hypoglycemia of infancy	(82)
ABCD1	Adrenoleukodystrophy	(80)
ABCG5/ABCG8	Sitosterolemia	(9)

MOLECULAR EVOLUTION OF *ABCA1*

ABCA1 is highly conserved between species, showing over 90% identity with mouse ABCA1 at the protein level. The recent availability of genome sequences from many different organisms has made it possible to infer the ancestral state of DNA sequences and thereby determine lineage-specific changes for specific genes. Such an approach was recently employed in a large set of sequences from humans, mice, and chimps (25). To identify genes that have undergone adaptive evolution, this group used a statistical test, the model 2 *P* value, which assesses the likelihood that specific sites in the human lineage display a ratio of nonsynonymous (amino acid sequence is altered) to synonymous (no change in amino acid sequence) substitutions of greater than 1, indicative of positive selection. Interestingly, the model 2 *P* value for *ABCA1* was $P_2 = 0.00211$, putting it in the top 0.6% of the nearly 8000 genes assessed in terms of adaptive evolution since the divergence of the last common ancestor between humans and chimps (25). By comparison, the model 2 *P* value for *FOXP2*, a transcription factor involved in language for which there exists considerable evidence of positive selection (37, 71), is $P_2 = 0.0027$. Therefore, *ABCA1* is among the genes with the strongest evidence for having undergone adaptive evolution in the human lineage.

We examined the rates of synonymous and nonsynonymous substitutions that have occurred in mouse, human, and chimp *ABCA1* sequences. Figure 1 shows that

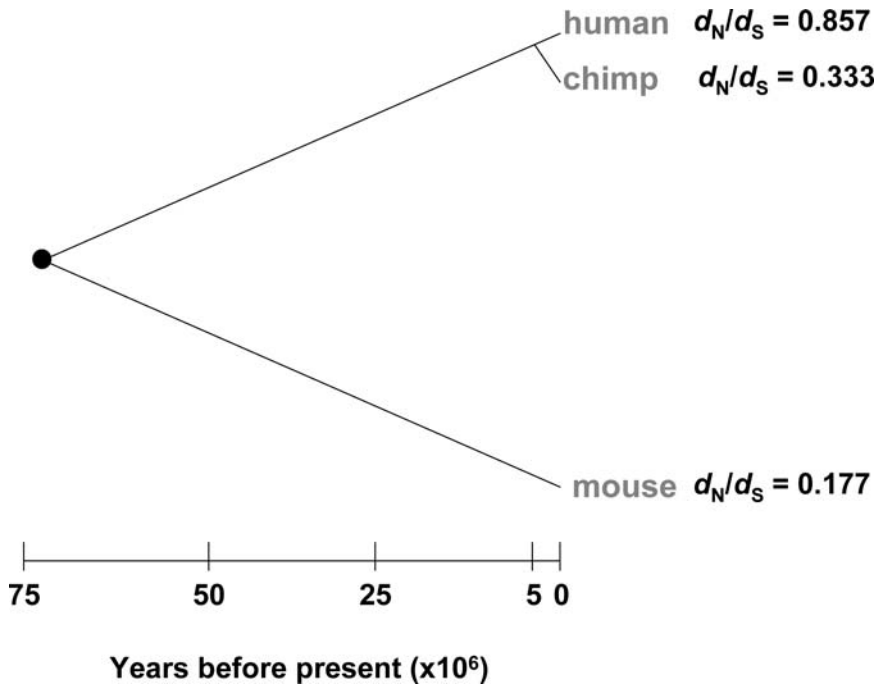

Figure 1 Lineage-specific evolution of *ABCA1*. The ratio of nonsynonymous to synonymous substitutions (d_N/d_S) in each lineage is shown. The lineage leading to modern humans shows a significant acceleration in (d_N/d_S), with six nonsynonymous and seven synonymous substitutions having occurred since the divergence of the last common ancestor between humans and chimps.

the ratio of nonsynonymous to synonymous substitutions (d_N/d_S) is significantly elevated in the human ($d_N/d_S = 0.86$) in comparison with mouse ($d_N/d_S = 0.18$) and chimp ($d_N/d_S = 0.33$) lineages ($P \leq 0.05$, Fishers exact test). An elevation of d_N/d_S indicates either a relaxation of selective pressure or positive selection for amino acid substitution. This analysis therefore provides further support for the concept that human *ABCA1* has undergone adaptive evolution and suggests that the *ABCA1* gene product may have played a critical functional role during the evolution of modern humans, leading to the observed pattern of positive selection for amino acid changes in this protein.

MUTATIONS IN *ABCA1*

To date, 73 mutations have been described in the *ABCA1* gene (2, 3, 6, 12, 14, 16, 26, 39, 43, 50, 51, 55–58, 60, 62, 69, 73, 74, 79, 83, 87, 89, 90, 93, 94, 100, 109, 110) (Table 2). These include 44 missense mutations, 18 nonsense mutations, and

TABLE 2 Mutations in ABCA1

Missense				Nonsense				Insertion/deletion			
Nucleotide[a]	Amino acid	Exon	Ref.	Nucleotide[a]	Amino acid	Exon	Ref.	Nucleotide[a]	Amino acid	Exon	Ref.
C566T	P85L	4	(56)	1210_1217del	277X	9	(16)	IVS2 + 1G > C		—	(6)
—	H160F	6	(87)	C1157T	R282X	9	(6)	IVS25 + 1G > C		—	(14)
C1001T	R230C	7	(110)	—	R557X	13	(87)	110 nt ins/14 nt del exon 12		13	(93)
G1076A	A255T	8	(83)	C2033A	Y573X	12	(69)	IVS16_IVS31del		17–31	(51)
—	E284K	9	(87)	C2194G	Y627X	14	*	5517ins 138 nt		38	(74)
C1406G	S364C	10	(43)	G2219del	635X	14	(12, 93)	IVS39→del		39	(12)
T1509C	V399A	11	(12)	C3038T	R909X	19	(79)	6152ins 14nt		42	(74)
—	Y482C	12	(87)	C2265del	913X	19	(73)	IVS12_IVS14del		—	(51)
G2265T	R587W	14	(74)	3680insG	1145X	23	*	GG5277,8C	1628 frameshift	36	
G2082T	W590L	14	(58)	3738_3739del	1145X	23	(90)	6073_6078del	E1893_D1894del	42	(79)
G2082C	W590S	14	(12)	4242_4245del	1284X	27	(60)	2472_2474del	L693del	15	
A2103G	Q597R	14	(74)	4943insA	1552X	34	(110)				
G2641C	K776N	16	(27)	C5864T	R1851X	41	*				
—	W840R	17	(89)	IVS46delT	2072X	47	(55)				
C3099T	T929I	19	(109)	C6743T	R2144X	49	(79)				
A3117G	N935S	19	(12, 51)	C6825del	2145X	49	(26)				
A3116C	N935H	19	(51)	CTC6952–4TT	2203X	49	(50)				
C3123T	A937V	19	(12)	6968ins 4nt	2215X	49	(16)				
C3450A	A1046D	22	(110)								
C3506T	P1065S	22	(43)								

—	R1068C	22	(89)
G3516A	R1068H	22	(100)
T3585C	M1091T	23	(79)
G3608T	D1099Y	23	(57)
G3960T	G1216V	25	(43)
G4178A	D1289N	27	(16, 60)
C4448T	L1379F	28	(2)
T4742C	C1477R	31	(14)
C4830T	S1506L	32	(73)
A5144G	N1611D	36	(83)
—	R1615P	35	(94)
C5351T	R1680W	37	(62)
T5424A	V1704D	36	(2)
A5711C	N1800H	40	(16)
G5865A	R1851Q	41	(55)
C6002T	R1897W	42	(39)
—	R1901S	42	(87)
G6087A	R1925Q	42	(3)
T6339C	F2009S	45	(57)
C6554T	R2081W	47	(60)
C6762T	P2150L	49	(26)
T6801C	F2163S	49	(89)
—	Q2196H	49	(87)
G7034A	V2244I	50	(89)

[a]Nucleotide position is with respect to NM_005502.

*Previously unpublished data.

11 insertions and deletions. The distribution of mutations in *ABCA1* is nonrandom (Figure 2). The vast majority of mutations occur in the large extracellular loops and the areas surrounding the nucleotide binding folds. Twenty-five mutations occur in the two large extracellular loops, and 28 occur in the intracellular regions, for a total of 53/73 = 73% of mutations occurring in 55% of the protein. In contrast, only 3/73 = 4% of mutations occur in transmembrane regions, which make up 12% of the protein ($P = 0.03$, Fisher's exact test).

Several mutations have been described in the C-terminal region of ABCA1, while there are no common, coding single nucleotide polymorphisms (cSNPs) reported in this region, suggesting that it is a functionally critical domain. The C-terminus of ABCA1, like that of the related CFTR protein (105), has been reported to contain a PDZ domain (16, 19) that could mediate protein-protein interactions. In addition, a VFVNFA motif was recently identified in the C-terminus of ABCA1, and deletion of this domain resulted in diminished apoA-I binding and lipid efflux (41). The C-terminus of ABCA1 therefore appears to be a crucial functional domain, potentially by recruiting other proteins in the efflux pathway. The specific protein interactions necessary for efflux to occur remain to be determined.

Although certain regions of the ABCA1 protein do appear to be more susceptible to deleterious mutations, there are no "common," recurrent mutations in *ABCA1* associated with Tangier disease. A small number of mutations have been reported in more than one unrelated individual, such as N1800H (16, 28, 43), and the K776N variant that was recently reported to occur in a Danish population at a frequency of 0.4% and to be associated with a two- to threefold increased risk for ischemic heart disease (44). The K776N variant was initially described as a SNP (27); however, the Danish study reporting that it occurs at a frequency less than 1% and is associated with a strong phenotypic effect would suggest that this is a relatively "common" *ABCA1* mutation, at least in the Danish population, influencing atherosclerosis susceptibility. However, by far the majority of mutations in *ABCA1* are private mutations occurring only in individual families. This indicates that mutations in *ABCA1* associated with Tangier disease have occurred relatively recently, and are efficiently purged from the population. Interestingly, mice lacking *ABCA1* have reduced fertility and placental defects (24). Although it is not clear if loss of ABCA1 activity is associated with reduced fertility in humans, this offers one hypothetical mechanism by which newly arising mutations are removed from the population, thus explaining the observed high frequency of new mutations in *ABCA1* despite the small number of individuals with TD.

An additional 24 rare variants in *ABCA1* have been described in a population-based cohort with low levels of HDL cholesterol (28). It is likely that many of these variants are pathogenic mutations, but it is not yet clear to what extent they impair ABCA1 function (17) or if they segregate with the low-HDL phenotype. We therefore have not included them in the list of validated *ABCA1* mutations in Table 2.

DIFFERENTIATION BETWEEN NEUTRAL AND FUNCTIONAL VARIANTS IN *ABCA1*

Recently our laboratory employed a bioinformatics approach to predict the functional consequence of genetic variation in *ABCA1*. By comparing the conservation in evolutionarily related proteins at the specific sites at which the variants occur, it is possible to predict the functional impact of any given amino acid variant. Using this approach, we showed that *ABCA1* mutations tend to occur at much more highly conserved positions in related proteins in comparison with *ABCA1* SNPs (Figure 3),

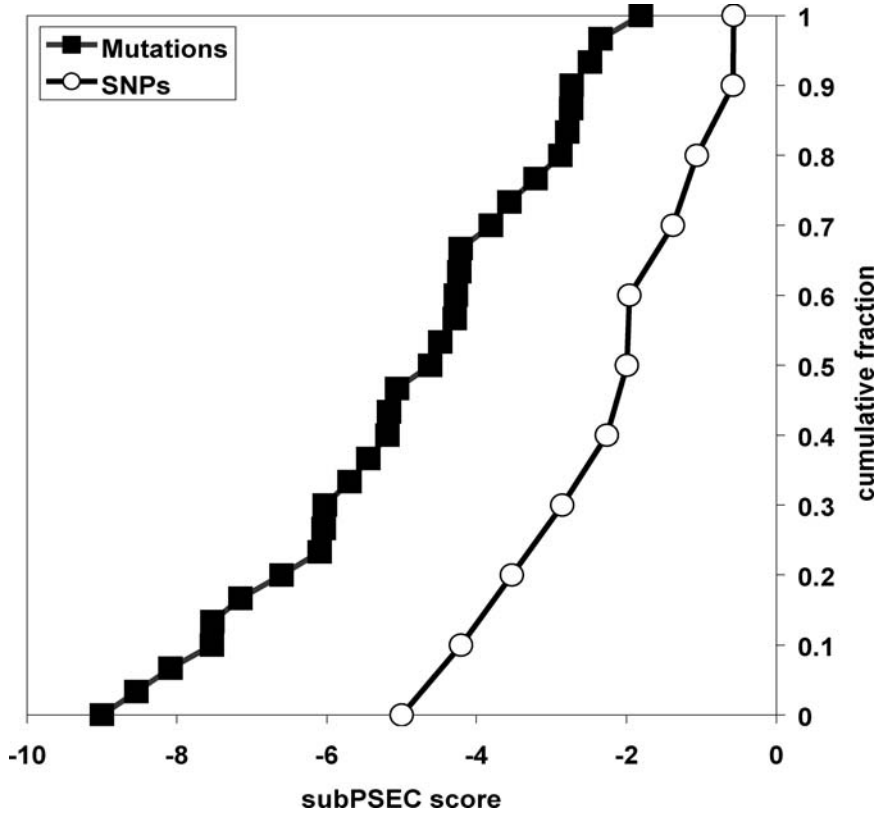

Figure 3 Prediction of functional effect of SNPs and mutations in *ABCA1*. The functional impact of single nucleotide polymorphisms (SNPs) and mutations in *ABCA1* was predicted based on site-specific conservation in evolutionarily related proteins, estimated by the substitution position specific evolutionary conservation (subPSEC) score. *ABCA1* mutations have significantly lower subPSEC scores compared with *ABCA1* SNPs ($P < 0.0001$, Mann Whitney U test), indicating that *ABCA1* mutations occur at much more highly conserved residues in related proteins and are predicted to have a significantly greater functional impact. Adapted from (17).

and that the majority of *ABCA1* mutations are predicted to significantly impair the function of the ABCA1 protein. These data also suggest that it is possible to use this approach to predict accurately whether a newly identified variant will impair ABCA1 function.

Applying this approach to the 24 rare *ABCA1* variants identified in the general population described above (28), we showed that 14 (58%) of these variants are predicted to impair the function of the ABCA1 protein. Therefore, this group of alleles is predicted to be enriched in functionally deleterious variants but to also consist of a significant number of benign variants.

PHENOTYPIC IMPACT OF MUTATIONS IN *ABCA1*

Prior to the identification of *ABCA1* as the molecular defect in TD, patients were identified based on their clinical phenotype, that is, extremely low HDL cholesterol in homozygotes, with the offspring and parents of homozygotes being obligate heterozygotes. There was therefore potential for heterozygotes with more mild phenotypes to be missed and, conversely, for severely affected heterozygotes to be misclassified as suffering from TD. The possibility to determine an individual's *ABCA1* genotype has now allowed for more unambiguous genetic diagnosis.

Such a genetic analysis reveals that mutations in *ABCA1* are associated with a broad range of biochemical defects. Table 3 shows mean plasma HDL cholesterol levels and mean percentage HDL of age- and sex-matched controls for *ABCA1* heterozygotes. Although HDL levels are an insensitive marker and reflect a variety of other environmental and genetic factors besides *ABCA1* genotype, they do allow for some assessment of the severity of each *ABCA1* mutation. A remarkably broad range of phenotypes associated with heterozygous *ABCA1* mutations is evident, ranging from 30% to 100% of control HDL cholesterol. The majority of *ABCA1* mutations are associated with HDL levels approximately 50% of control. This indicates that most of these mutations are complete loss-of-function alleles; removal of one copy of *ABCA1* results in half of normal efflux activity, underlying the observed phenotype of an approximately 50% reduction in HDL cholesterol.

However, a subset of mutations are associated with greater than 50% of control HDL levels, specifically T929I, A947V, R1680W, and W590S. Therefore, these mutations are likely not to be complete loss-of-function mutations, but may retain some residual activity. In this case, overall efflux is reduced, but not by 50%, thus resulting in HDL levels greater than 50% of controls. Consistent with this concept, cholesterol efflux from fibroblasts of an individual carrying the T929I mutation has been reported to be approximately 75% of control levels (26).

Conversely, a small number of mutations are associated with less than 50% of control HDL cholesterol, specifically M1091T, G1216V, and the truncation mutations R2144X, R282X, and R909X. Since a complete loss of function allele would be expected to result in a 50% reduction in HDL levels, a greater than 50% reduction in HDL is most likely explained by a dominant negative allele, in which

TABLE 3 Patient phenotypes associated with heterozygous ABCA1 mutations

Mutation	HDL (mmol/L)	HDL (% of control)	Number of patients
M1091T	0.48 ± 0.5	30 ± 30	4
G1216V	0.50	40	1
R2144X	0.56 ± 0.2	41 ± 18	12
R282X	0.52	41	1
R909X	0.59 ± 0.3	42 ± 19	5
K776N	0.55 ± 0.1	47 ± 5	2
R587W	0.61 ± 0.1	47 ± 8	7
S364C	0.60	48	1
P1065S	0.80	51	1
c-ter deletion	0.75	53	1
N1800H	—	56.5	33
P85L	0.72 ± 0.4	57 ± 33	5
Del693L	0.79 ± 0.2	57 ± 15	8
D1289N	0.80 ± 0.1	59 ± 12	4
R2081W	0.80 ± 0.1	59 ± 12	4
2203X	0.80 ± 0.2	59 ± 20	4
DelED1893,4	0.77 ± 0.2	59 ± 18	8
2145X	0.82 ± 0.1	59 ± 9	4
A1046D	0.70 ± 0.1	60 ± 8	2
Q597R	0.82 ± 0.1	60 ± 5	5
C1477R	0.82 ± 0.2	61 ± 15	9
IVS25 + 1G > C	0.78 ± 0.1	62 ± 12	4
D1099Y	0.83 ± 0.3	63 ± 21	5
1552X	1.00	64	1
F2009S	0.82 ± 0.2	64 ± 19	6
R587W	0.86 ± 0.1	65 ± 17	2
R1068H	0.90 ± 0.3	67 ± 26	9
N935S	1.00 ± 0.3	74 ± 16	7
T929I	1.01 ± 0.2	76 ± 7	8
1284X	1.11 ± 0.2	83 ± 14	5
A937V	1.15 ± 0.6	85 ± 28	2
R1680W	1.22 ± 0.2	87 ± 17	3
635X	1.24 ± 0.5	90 ± 32	7
W590S	1.32 ± 0.6	103 ± 46	15

the mutant protein actually interferes with the activity of the remaining wild-type protein. Recent biochemical data helps to explain how this could occur. ABCA1 was recently shown to exist in dimeric and tetrameric forms in human fibroblasts (35). Therefore, a dysfunctional ABCA1 protein that is still able to oligomerize may sequester wild-type ABCA1 proteins, thus reducing the activity of the wild-type protein. The ability of *ABCA1* truncation mutations to suppress the activity of wild-type ABCA1 has been previously shown in transfected cells (115).

Other factors could also explain the wide range of phenotypic effects associated with *ABCA1* mutations seen in Table 3. In addition to environmental factors and the influence of other genes, the genetic background with respect to *ABCA1* on which the mutation occurs could influence the phenotypic expression. Support for this concept comes from a recent study showing that HDL cholesterol levels in carriers of the R1068H mutation are significantly influenced by the *ABCA1* promoter haplotype associated with the mutation (100). It will be of interest to examine in greater detail whether specific *ABCA1* haplotypes play an important role in influencing the manifestation of mutations in this gene and therefore explain part of the variation among individuals with identical *ABCA1* mutations.

Two recent reports suggest that *ABCA1* mutations may be associated with additional clinical manifestations, besides the classic phenotypes of low HDL cholesterol, tissue cholesterol accumulation, and atherosclerosis. Albrecht et al. (3) reported a case of Scott syndrome, a rare bleeding disorder characterized by defective phosphatidylserine (PS) exposure on the surface of platelets, that was associated with a novel missense mutation in *ABCA1*, R1925Q (3). ABCA1 is known to be expressed on platelets, and prolonged bleeding time has been previously reported in association with TD (36, 84). In addition, ABCA1 has previously been shown to mediate calcium-stimulated PS exposure in red blood cells (52). The 1925Q mutation was shown to cause mislocalization of ABCA1 in transfected cells, suggesting that this is a functionally significant variant (3). Paradoxically, however, this patient had normal levels of HDL cholesterol (1.3 mmol/L). Clearly this observation needs to be extended to other cases, but the case described by Albrecht et al. (3) does raise the intriguing possibility that defective ABCA1-mediated efflux in platelets could result in the aberrant PS externalization associated with Scott syndrome.

Saleheen et al. (94) reported a case of a 33-year-old male with type 2 diabetes and very low HDL cholesterol (0.26 mmol/L)(94). This patient was found to be heterozygous for a novel R1651P missense mutation in *ABCA1*. Because Saleheen et al. did not demonstrate segregation of the diabetic phenotype with the mutation in the kindred, it cannot be excluded that this is a chance finding. However, certain *ABCA1* haplotypes have been found to be overrepresented in a type 2 diabetic compared with normo-glycemic population (30), consistent with a relationship between loss of ABCA1 activity and diabetes. Although the mechanism underlying a relationship between reduced ABCA1 activity and type 2 diabetes is unclear, previous studies have described specific down-regulation of ABCA1 in response to unsaturated free fatty acids (111, 112) and advanced glycosylation end products (85), suggesting that the imbalances associated with the metabolic syndrome—such

as elevated fatty acids and hyperglycemia—may themselves reduce ABCA1 activity. Detailed studies of glucose homeostasis in TD patients would be useful to resolve these issues.

ABCA1 is expressed in a wide variety of tissues (114), but it is likely that only hepatic and intestinal ABCA1 are significantly involved in HDL biogenesis (107, 113). Using tissue-specific gene targeting, we have recently shown that the liver (107) and the intestine (18) are the major sites of HDL biogenesis, contributing to approximately 80% and 30% of the plasma HDL pool, respectively. The studies of Albrecht et al. (3) and Saleheen et al. (94) suggest that detailed study of phenotypes associated with *ABCA1* mutations could point to previously unexpected functions of ABCA1 in other tissues.

THE ROLE OF RARE *ABCA1* VARIANTS IN THE GENERAL POPULATION

Two recent studies addressing the role of rare *ABCA1* variants in the general population provide substantial information about the role of *ABCA1* in modulating HDL levels on a population scale. Cohen et al. (28) sequenced the entire coding region of *ABCA1* (as well as the genes encoding apolipoprotein A-I and LCAT) in 256 individuals representing the top and bottom 5% of HDL cholesterol levels from the Dallas Heart Study population (28). Remarkably, 20 of the 128 individuals with the lowest 5% of HDL cholesterol levels were found to harbor a rare amino acid variant in ABCA1 that was not found in the high-HDL cholesterol group. This finding was replicated in a second population, indicating that in two separate populations, $\sim$15% of individuals with low HDL cholesterol have rare sequence variants in *ABCA1*. In contrast, none of the common ($>$10% frequency) *ABCA1* variants identified were associated with HDL cholesterol levels across all gender and ethnic groups.

Although it remains unclear what percentage of these rare *ABCA1* alleles are truly functionally significant, this study does provide evidence that rare, but not common, variants in *ABCA1* may contribute significantly to low HDL cholesterol in the general population. This notion is supported by a second study (43) in which the *ABCA1* coding region and promoter were sequenced in individuals with the highest and lowest 1% of HDL cholesterol levels (95 individuals per group) from the Copenhagen City Heart Study. Seven rare variants that alter the amino acid sequence of ABCA1 were identified, and of these, six were observed only in the low-HDL cholesterol group, which suggests that they impart a major phenotypic effect. In total, 10% of individuals with low HDL cholesterol were found to harbor a mutation in *ABCA1*. In contrast to the study by Cohen et al. (28), Frikke-Schmidt et al. (43) did observe significant association between common *ABCA1* alleles and HDL levels in the entire population. This may be due to the fact that they examined a much larger population than did Cohen et al. (9259 individuals versus 2569), and that Cohen et al. restricted their association study to variants greater than 10% frequency, whereas Frikke-Schmidt et al. examined all SNPs with a frequency of

1% or greater. However, the study by Frikke-Schmidt et al. does verify the main finding from Cohen et al. that a significant proportion of individuals with low HDL cholesterol from the general population harbor rare variants in *ABCA1*.

These studies have important implications for future research into the role of *ABCA1* in modulating HDL cholesterol levels in the general population. Notably, they indicate that approximately 10%–15% of individuals with low HDL cholesterol from the general population have rare *ABCA1* variants. The relative importance of mutations in *ABCA1* as a cause of low HDL in the general population is an important question and has been the subject of debate. Initial reports suggested that up to 40% of familial HDL deficiency was caused by defective ABCA1-mediated efflux (79, 81). However, later studies suggested that the frequency of *ABCA1* mutations in familial HDL deficiency is much less, about 4.5% (59). However, these studies both suffered from potential selection biases in that the patients were recruited from medical clinics. In contrast, the studies by Cohen et al. (28) and Frikke-Schmidt et al. (43) examined patients from the general population who were selected solely on the basis of their HDL phenotype; in addition, the *ABCA1* gene was sequenced in all individuals with low HDL. These studies, therefore, provide the strongest evidence to date that *ABCA1* is a critically important locus for modulating HDL cholesterol levels in the general population, and that loss of ABCA1 activity underlies a significant proportion of the low HDL cholesterol seen in the population.

These studies also suggest that future work aiming to investigate the relationship of *ABCA1* variants to HDL levels and atherosclerosis should examine rare variants by sequencing, in addition to association studies using common SNPs. More broadly, these studies bring into question the popular theory that common traits, such as low HDL cholesterol, are caused by common alleles, each with moderate phenotypic effects—the so-called common-disease common-variant hypothesis (29, 72, 91). On the contrary, the results of these two studies suggest that for the low HDL phenotype, rare alleles with strong functional effects modulate the variation of HDL levels in the population to a more significant extent than do common alleles. The utility of large-scale SNP projects, such as the International HapMap Project (7), rests on the assumption that genome-scale association studies of common variants will be useful in dissecting the genetics of common phenotypes. If the results of the studies by Cohen et al. (28) and Frikke-Schmidt et al. (43) prove generalizable to other common traits, however, sequence-based approaches to identify rare alleles will also be necessary to identify the genetic factors important in complex traits.

COMMON VARIANTS IN *ABCA1*

Fifteen coding nonsynonymous SNPs have been described in the *ABCA1* gene (Table 4). Many of these variants have been studied in relationship to their association with HDL cholesterol levels and atherosclerosis (11, 15, 22, 27, 28, 38,

TABLE 4 Nonsynonymous single-nucleotide polymorphisms (SNPs) in *ABCA1*

SNP id	Nucleotide[a]	Amino acid[b]	Observed heterozygosity
rs2230806	G969A	R219K	0.488
rs9282541	C1001T	R230C	0.029
rs9282543	T1509C	V399A	0.020
rs4131108	A1556C	M415L	—
rs13306068	A1949G	I546V	—
rs2066718	G2624A	V771M	0.074
rs2472458	G2804A	D831N	—
rs4149313	A2962G	I883M	—
rs2482437	C3326T	E1005K	—
rs13306072	G3473A	V1054I	—
rs13306073	G3599A	V1096I	—
rs1997618	T4977C	I1555T	—
rs2230808	A5073G	K1587R	0.480
rs1883024	T5256C	L1648P	—
—	C5505G	S1731C	—

[a]Nucleotide position is with respect to NM_005502.

[b]Amino acid position is with respect to NP_005493.

43, 44, 61, 64, 65, 68, 78, 96, 102, 106, 108, 110, 116, 119), and these results have been reviewed elsewhere (98). Most of these studies have reported significant association of *ABCA1* SNPs with lipid levels and atherosclerosis, suggesting that common variation in *ABCA1* does influence HDL cholesterol levels and risk for atherosclerosis in the general population. However, not all findings have been replicated and some findings are inconsistent, making it difficult to determine which specific variants mediate these effects.

The largest study of *ABCA1* variants to date examined the relationship between six *ABCA1* cSNPs and HDL cholesterol in more than 9000 individuals from an ethnically homogeneous population (43). This study had the added advantages that the authors considered the effect of each SNP in isolation of variation at the other sites, and assessed phenotypic data collected at two time points ten years apart (43). The only SNP associated with HDL cholesterol in both men and women at both time points was R1587K, for which the minor K allele was associated with modestly but significantly reduced HDL. The V825I and V771M SNPs were associated with increased HDL cholesterol in one, but not both, genders.

This study provides the most convincing data to date that common *ABCA1* SNPs do affect ABCA1 activity and HDL levels in the general population. However, the magnitude of the effect on HDL cholesterol was generally small. As

mentioned, HDL levels are influenced by many other factors, and therefore may be an unreliable measure of ABCA1 activity. Indeed, several studies have documented an association of *ABCA1* SNPs with clinical end points independent of changes in lipid levels, suggesting that small changes in ABCA1 activity, potentially in the important vessel-wall macrophage pool (1), can affect atherogenesis without appreciably impacting steady-state HDL levels. Unfortunately, the large study by Frikke-Schmidt et al. (43) did not examine association of these *ABCA1* SNPs with markers of atherosclerosis.

HAPLOTYPIC ARCHITECTURE OF *ABCA*1

A major limitation in examining the effects of common *ABCA1* variants in modulating HDL cholesterol levels and risk for atherosclerosis has been an incomplete understanding of linkage disequilibrium patterns at the *ABCA1* locus. Figure 4 displays a graphical representation of the patterns of linkage disequilibrium and major haplotype blocks across the approximately 150 kilobase genomic region containing the *ABCA1* gene, as determined by data from the HapMap project. The *ABCA1* locus displays a complex haplotypic structure, with nine major haplotype blocks. The 5' region of *ABCA1* shows particularly complex patterns of linkage disequilibrium. Notably, only two of the 15 common *ABCA1* nonsynonymous SNPs, I883M and R219K, are represented in the HapMap data set, which is an incomplete data set (the phase I project having set out to identify 1 SNP every 5000 base pairs) (7). However, it is apparent from the haplotypes in which I883M and R219K reside that the minor alleles of each of these are unique to one particular, different haplotype, which may explain in part why these two SNPs have been consistently found to be associated with HDL levels and atherosclerosis risk. This also suggests that such associations are not necessarily due to functional effects of these variants, but could instead reflect the functional effects of linked SNPs. Significant linkage disequilibrium does not appear to exist between *ABCA1* and the nearby *NIPSNAP3A* and *NIPSNAP3B* genes.

Considerations of linkage disequilibrium among SNPs can guide selection of a more efficient set of "tag" SNPs, which provide information about nearby linked SNPs without genotyping the linked SNPs directly (63). Based on data from the HapMap project, we assembled a list of tag SNPs for *ABCA1* using the Tagger program (31), based on a correlation coefficient (r^2) between tag SNPs of 0.8. The list of *ABCA1* tag SNPs is shown in Table 5. These 17 SNPs capture most of the genetic variation in the *ABCA1* gene, while dramatically reducing the number of genotypes required. These SNPs are all highly polymorphic, with observed heterozygosities ranging from 0.278 to 0.556, indicating that they are all likely to be informative. The use of a set of tag SNPs selected from an incomplete data set such as the current HapMap was recently shown to result in only minor losses in power compared with a complete data set (31). Of course, the actual selection of tag SNPs for any given association study will also be influenced by details of the

TABLE 5 *ABCA1* tag single-nucleotide polymorphisms (SNPs)

SNP id	Observed heterozygosity	Position[a]	Variation	Location
rs2740484	0.456	14872385	A > G	intron 44
rs2297406	0.478	14872743	C > T	intron 44
rs2740479	0.378	14884642	C > T	intron 34
rs2254884	0.500	14902954	C > T	intron 34
rs2065412	0.422	14919945	C > T	intron 11
rs2487054	0.456	14925927	A > C	intron 8
rs4743764	0.433	14950309	C > T	intron 5
rs2000069	0.456	14957074	C > T	intron 5
rs2575875	0.389	14983699	A > G	intron 2
rs3758294	0.378	14986020	C > T	intron 2
rs2740487	0.556	14986166	C > T	intron 2
rs2740486	0.528	14987718	A > C	intron 1
rs10820743	0.393	14992864	C > T	intron 1
rs2515616	0.389	15003200	C > T	intron 1
rs2472510	0.310	15004327	A > C	intron 1
rs2515614	0.378	15005523	G > T	intron 1
rs2487052	0.278	15007610	C > T	intron 1

[a]Position is in contig NT_008470.

population under study and the genotyping platform used. In addition, it would seem wise to include known nonsynonymous SNPs in a genotyping panel, because of their higher probability of functionality.

This set of SNPs, however, illustrates the principle of selecting a highly informative and efficient set of tag SNPs. The confluence of data from HapMap, and the ability to predict the functional significance of nonsynonymous variants in *ABCA1* (17), should aid considerably in the prioritization of SNP selection for future association studies of *ABCA1*, as well as the entire genome.

CONCLUSION

The identification of *ABCA1* as the molecular defect in Tangier disease has ushered in a new era in our understanding of HDL metabolism and reverse cholesterol transport. This discovery provides further support for the study of rare diseases as an opportunity to gain "an occasional view of normal processes obtainable in no other way" (42) and to provide insights into potential ways to raise HDL levels and protect against a common disease.

ACKNOWLEDGMENTS

This work was supported by grants from the Canadian Institutes of Health Research and the Heart and Stroke Foundation of BC and the Yukon (to MRH). LRB was supported by a CIHR studentship and is a Michael Smith Foundation for Health Research doctoral trainee. MRH is a University Killam Professor and holds a Canada Research Chair in Human Genetics.

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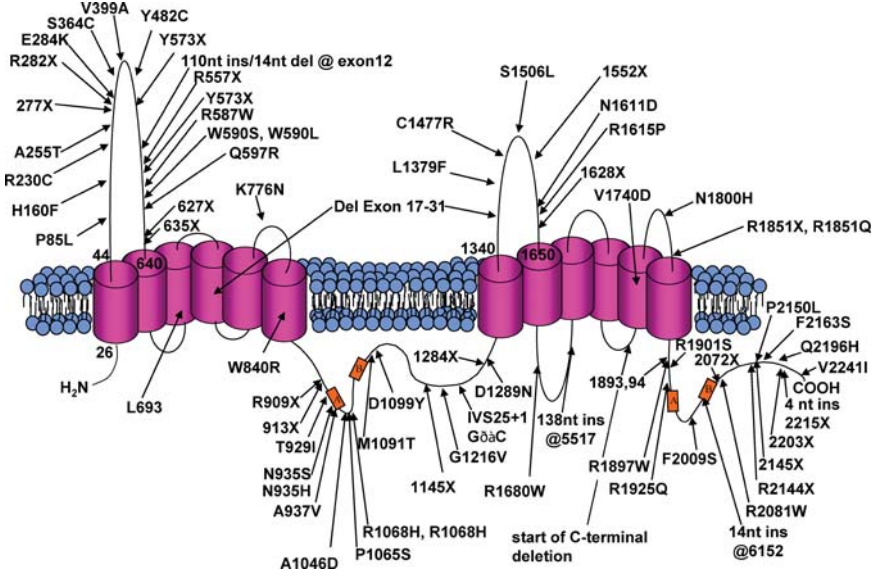

Figure 2 Location of mutations in *ABCA1*. The locations of all reported mutations in *ABCA1* are shown in a diagrammatic representation of the ABCA1 protein.

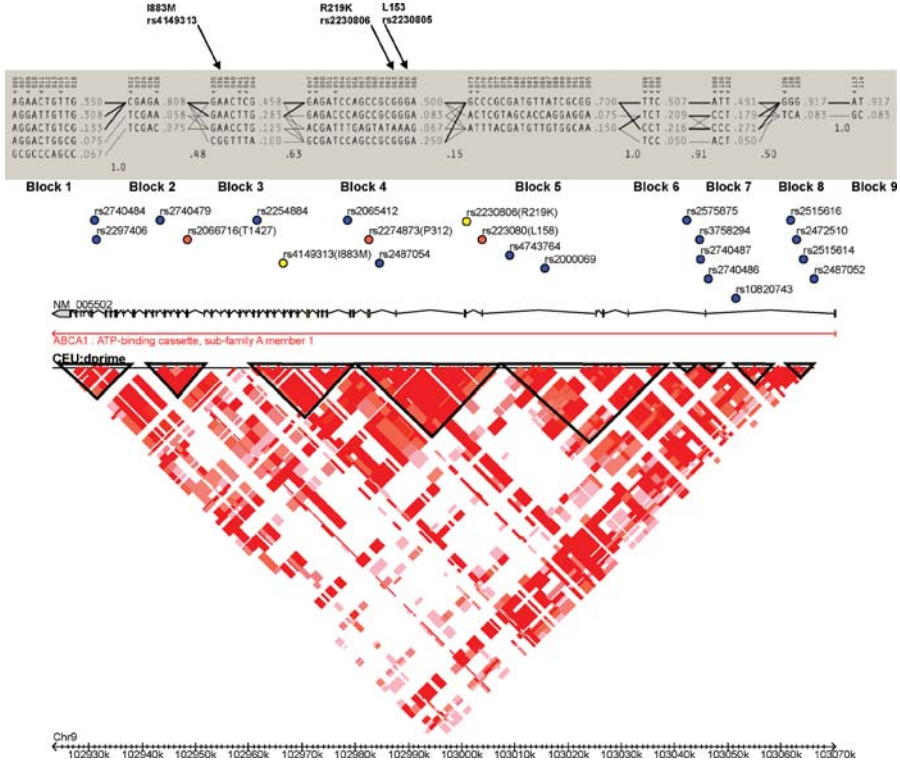

Figure 4 Haplotype structure of *ABCA1* in Americans of Northern and Western European ancestry. The nine major haplotype blocks in the *ABCA1* gene are shown. Below the haplotype blocks, *ABCA1* "tag" SNPs are shown as blue circles, nonsynonymous coding SNPs as yellow circles, and synonymous coding SNPs as red circles. Below, the patterns of linkage disequilibrium, estimated by the r^2 value, are shown. Red blocks indicate regions of significant linkage disequilibrium. Adapted from the HapMap project Web page (http://www.hapmap.org).

Annual Review of Nutrition
Volume 25, 2005

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