Efflux and Atherosclerosis
The Clinical and Biochemical Impact of Variations in the ABCA1 Gene

Roshni R. Singaraja,* Liam R. Brunham,* Henk Visscher, John J.P. Kastelein, Michael R. Hayden

Abstract—Approximately 50 mutations and many single nucleotide polymorphisms have been described in the ABCA1 gene, with mutations leading to Tangier disease and familial hypoalphalipoproteinemia. Homozygotes and heterozygotes for mutations in ABCA1 display a wide range of phenotypes. Identification of ABCA1 as the molecular defect in these diseases has allowed for ascertainment based on genetic status and determination of genotype-phenotype correlations and has permitted us to identify mutations conferring a range of severity of cellular, biochemical, and clinical phenotypes. In this study we review how genetic variation at the ABCA1 locus affects its role in the maintenance of lipid homeostasis and the natural progression of atherosclerosis. (Arterioscler Thromb Vasc Biol. 2003;23:1322-1332.)

Key Words: ABCA1 • genetics • efflux • atherosclerosis • HDL

The ABCA1 Gene and Its Biological Role
Atherosclerotic coronary artery disease (CAD) constitutes a major public health burden in developed countries and by 2020 is predicted to be the single greatest cause of death worldwide.1-3 Decreased HDL cholesterol (HDL-C) is the most common lipid abnormality in patients with premature CAD.4,5 ABCA1 encodes the key protein regulating the efflux of lipids from peripheral cells to HDL, following which these lipids are transported back to the liver and excreted as bile in a process termed reverse cholesterol transport.6 At least 50 mutations have been identified in the ABCA1 gene, leading to the allelic disorders Tangier disease (TD) and familial hypoalphalipoproteinemia (FHA), which are associated with a wide range of phenotypic consequences.

*These authors contributed equally to this study.
Correspondence to Dr Michael R. Hayden, Centre for Molecular Medicine and Therapeutics, 950 West 28th Ave, Vancouver, BC, V5Z 4H4, Canada.
E-mail mnh@cmmt.ubc.ca

© 2003 American Heart Association, Inc.

Arterioscler Thromb Vasc Biol. is available at http://www.atvbaha.org

DOI: 10.1161/01.ATV.0000078520.89539.77
and putative biochemical defects. In this study we review how genetic alterations of the ABCA1 gene highlight its role in lipid homeostasis and atherosclerosis. Although TD is exceedingly rare and ABCA1 mutations seem to be an infrequent cause of FHA, the study of mutations in this gene has shed new light on a key pathway in the pathogenesis of atherosclerosis and opened up new approaches for its prevention and treatment.

Superfamily: The ATP-Binding Cassette Transporters

The transport of specific molecules across membranes is critical for survival, and the ATP-binding cassette (ABC) proteins transport a wide variety of substances, including lipids and sterols, metabolic products, and drugs across both intracellular and extracellular membranes. The first ABC transporter was cloned in 1982. ABC transporters are the largest membrane transporter family, consisting of 48 members in humans, 52 in the mouse, 56 in Drosophila, 58 in Caenorhabditis elegans, 31 in yeast, and 129 in Arabidopsis. The genome of Escherichia coli contains 80 ABC transporters, corresponding to 2% of its genome. Despite their large numbers and substrate diversity, all ABC proteins bind and hydrolyze ATP and use the derived energy for transport of the various molecules.

Classification and Topology of ABC Proteins and ABCA1

Knowledge of the normal topology and organization of ABC proteins and ABCA1 is important, because it provides insight into potential functional domains. ABC transporters are defined based on the presence of ATP binding domains, also known as nucleotide binding folds (NBFs), that contain 3 characteristic conserved regions, the Walker A and B domains, which are separated by approximately 90 to 110 amino acids, and a signature (C) motif, located just upstream of the Walker B site. Using flag tags and antibodies and also by deciphering the glycosylation status of ABCA1, the most current topological analysis reveals that ABCA1 consists of 2 large extracellular loops, one between the first...
TABLE 1. Human Diseases Caused by Mutations in ABC Genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCA1</td>
<td>Tangier disease, Familial Hypoalphalipoproteinemia</td>
</tr>
<tr>
<td>ABCA4</td>
<td>Stargardt disease, retinitis pigmentosum 19, cone-rod dystrophy, age-related macular degeneration</td>
</tr>
<tr>
<td>ABCB4</td>
<td>Progressive familial intrahepatic cholestasis type 3 (PFIC)</td>
</tr>
<tr>
<td>ABCB7</td>
<td>X-linked sideroblastic anemia and cerebellar ataxia</td>
</tr>
<tr>
<td>ABCB11</td>
<td>Progressive familial intrahepatic cholestasis type 2</td>
</tr>
<tr>
<td>ABCG6</td>
<td>Pseudoxanthoma elasticum</td>
</tr>
<tr>
<td>ABCG8</td>
<td>Persistent hyperinsulinemic hypoglycemia of infancy</td>
</tr>
<tr>
<td>ABCG5 and ABCG8</td>
<td>Sitosterolemia</td>
</tr>
</tbody>
</table>

and second transmembrane domains and the other following the intracellular NBF1 domain18,19 (Figure 1).

In addition to the NBFs, ABC transporters consist of 1 or 2 sets of membrane-spanning domains, each usually comprised of 6 membranes spanning α-helices, which provide substrate specificity.20

The mammalian ABC genes are divided into 7 subfamilies, ABCA through ABCG, based on similarity in gene structure, order of the domains, and sequence homology in the NBF and TM domains. Figure 2 shows the phylogenetic relationship of selected ABC family members and the percent identity and similarity of these proteins.

Mutations in ABC Genes Cause Many Human Genetic Diseases

Genetic variation in ABC genes have been shown to be the cause of at least 12 genetic diseases (Table 1). The ABCA1 gene is highly conserved between species (Figure 3). Human ABCA1 is 95.2% identical to mouse, 85.3% to chicken, 25.5% to drosophila, 21.6% to C. elegans, and 10.2% to fugu ABCA1. We have performed identity and similarity searches and generated a phylogenetic tree (Figure 3). Although the C. elegans gene CED7 was proposed as the orthologue of ABCA1 based on similarities of function,36 the C. elegans transporter CE2 (Q9TXV8) is the closest member to ABCA1, as supported by similar analysis by Peelman et al.37 Thus, ABCA1 and CED7 are likely to be paralogs and not orthologs.

Variation in ABCA1: Insights Into Protein Function and Its Contribution to Atherosclerosis

Mutations in ABCA1

At least 50 mutations in the ABCA1 gene have been identified31,38–54 (also Hayden et al, unpublished data, 2003). These include 23 missense, 6 nonsense, and 21 insertions or deletions. Forty-nine of the reported mutations occur in exons. One mutation in intron 2 leads to an abnormally spliced transcript lacking exon 2 or exon 4 or both.56 All mutations by definition result in decreased lipid efflux. The extremely high correlation between phospholipid and cholesterol efflux (r = 0.86, P < 0.0001) in more than 15 mutations tested (Singaraja and Hayden, unpublished data, 2003) indicates that ABCA1 influences efflux of both lipid types.

Nonrandom Distribution of Mutations in the ABCA1 Gene

Although mutations do occur throughout the gene, mutations in ABCA1 are not in random distribution. Four mutations cluster between amino acids 230 and 282, 6 between residues 587 and 635, 8 between amino acids 909 and 1099, 5 between residues 1145 and 1289, and 5 between 2144 and 2215 (Figure 4). Conversely, with the exception of a large deletion, only 1 mutation occurs in the transmembrane regions between residues 636 and 908, and no mutations occur in the second set of transmembrane domains. Many of the residues harboring mutations are highly conserved with C. elegans, a nematode that is estimated to have diverged from other metazoans 600 to 1200 million years ago,55,56 thus indicating their functional importance (Table 2).

Functional Effects of Mutations in the Extracellular Loops of ABCA1

Approximately half of the missense mutations in the ABCA1 gene associated with TD and FHA fall within the 2 extracellular loops. Mutations in the first and second extracellular loops might be expected to result in a lack of lipid efflux caused by dysfunctional interaction of ABCA1 with apolipoprotein AI (ApoA-I), because lipid-poor pre-β HDL particles would require either direct interaction or close proximity to ABCA1 for the lipid transfer to occur. Thus, the extracellular loops of ABCA1 might provide a potential binding target for ApoA-I. Impaired transport of ABCA1 to the plasma membrane could prevent interaction with ApoA-I. It is also possible that mutations in the extracellular domains

Figure 3. A, Phylogenetic tree of ABCA1 orthologs from Homo sapiens (NM 005930), Mus musculus (NM 013454), Gallus gallus (AF 362377), C. elegans (AF 101313), Drosophila melanogaster (NM 134601), and Fugu rubripes (Hayden laboratory, unpublished data, 2003) were aligned using ClustalX.1,8 Neighborhood-Joining tree was generated in ClusterX and viewed using phylo-dendron web service at http://iubio.bio.indiana.edu/treeapp/treeprint-form.html as above. B, An identity/similarity matrix consisting of the orthologues of ABCA1 was generated as described above.
will result in a disruption of the tertiary structure of ABCA1, thereby preventing its function at the plasma membrane, where it is normally localized. This concept is additionally supported by studies of ABCR, which show very similar topology to ABCA1.57 The two halves of ABCR each contain a large extracellular loop, a transmembrane domain region, and a nucleotide binding domain that interact together through 1 or more disulfide bonds, putatively involving cysteines located in the extracellular loops in ABCR.57 Interestingly, most of the cysteines are conserved in these regions between ABCA1 and ABCR,57 suggesting that these residues are essential for folding and interactions between the different domains. Of note, ABCA1 contains a cysteine at position 1477 that is mutated to an arginine residue21 and could thereby disrupt proper 3-dimensional folding of the ABCA1 protein necessary for its ability to efflux lipids.

Additional insights into how the mutations R587W, W590S, and Q597R that occur in the extracellular loops affect ABCA1 function have recently been described.58–60 Two studies have reported that ABCA1 containing the point mutation Q597R, which occurs in the first extracellular loop, does not localize to the plasma membrane.59,60 However, other studies have reported that this mutant is expressed at the plasma membrane but at reduced levels relative to wild-type ABCA1.58,44 R587W, another missense mutation in the first extracellular loop, also prevents the trafficking of ABCA1 to the plasma membrane, although results with this mutant have been variable.58–60 Both the R587W and Q597R mutants are resistant to PNGase digestion, indicating that they are not glycosylated, suggesting that ABCA1 harboring these mutations does not traverse the medial and trans Golgi network. However, not all mutations in the extracellular loops prevent the export of ABCA1 to the plasma membrane. ABCA1 harboring the W590S mutation does reach the cell surface, and cross-linking studies reported normal interaction of the W590S mutant with ApoA-I despite defective efflux, suggesting that interaction with ApoA-I may not be sufficient for lipid efflux.58

ABCA1 that does not reach the plasma membrane cannot induce the binding of ApoA-I. This is indeed the case with mutant Q597R,58,60 which shows no ApoA-I binding. However, failure of binding may also occur because of disruption of residues crucial for this function. Indeed, the variants C1477R and S1506L, which are both localized in the second large extracellular loop, are normally translocated to the plasma membrane but show no ApoA-I binding, indicating that specific amino acids in the large extracellular loops are

**Figure 4.** Location of mutations in the ABCA1 protein. At least 50 mutations have been reported thus far in the ABCA1 gene, many of which show a nonrandom distribution and cluster in specific regions of the protein (yellow boxes).

**TABLE 2. Conservation of Amino Acid Residues Mutated in Humans**

<table>
<thead>
<tr>
<th>Mutation</th>
<th>H. sapiens</th>
<th>M. musculus</th>
<th>G. gallus</th>
<th>D. melanogaster</th>
<th>C. elegans</th>
</tr>
</thead>
<tbody>
<tr>
<td>P85L</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>...</td>
<td>P</td>
</tr>
<tr>
<td>R230C</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>P</td>
<td>G</td>
</tr>
<tr>
<td>A255T</td>
<td>A</td>
<td>A</td>
<td>S</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>R587W</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>W590S</td>
<td>W</td>
<td>W</td>
<td>W</td>
<td>R</td>
<td>Q</td>
</tr>
<tr>
<td>Q597R</td>
<td>Q</td>
<td>Q</td>
<td>Q</td>
<td>Q</td>
<td>Q</td>
</tr>
<tr>
<td>L693</td>
<td>L</td>
<td>L</td>
<td>L</td>
<td>L</td>
<td>L</td>
</tr>
<tr>
<td>T929I</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>T</td>
</tr>
<tr>
<td>N9355/H</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>A937V</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>A1046D</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>M1091T</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>D1099Y</td>
<td>D</td>
<td>D</td>
<td>D</td>
<td>D</td>
<td>D</td>
</tr>
<tr>
<td>D1289LN</td>
<td>D</td>
<td>D</td>
<td>D</td>
<td>D</td>
<td>D</td>
</tr>
<tr>
<td>C1477R</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>S1506L</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>N1611D</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>S</td>
</tr>
<tr>
<td>R1680W</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>N1800H</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>A</td>
<td>W</td>
</tr>
<tr>
<td>F2009S</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>I</td>
<td>M</td>
</tr>
<tr>
<td>R2081W</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>P2150L</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>R</td>
<td>N</td>
</tr>
<tr>
<td>ΔE1893</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>D</td>
<td>S</td>
</tr>
<tr>
<td>ΔD1894</td>
<td>D</td>
<td>D</td>
<td>D</td>
<td>D</td>
<td>D</td>
</tr>
</tbody>
</table>

Twenty-three of 24 (95.83%) amino acids affected by mutations are conserved with G. gallus, reflecting the functional importance of these residues.
also necessary for ApoA-I binding. Although thus far only a small subset of the naturally occurring mutations have been biochemically characterized, these in vitro studies of ABCA1 mutations have begun to provide valuable information on structure-function relationships of the protein.

Mutations in the Transmembrane Domain and Impact on ABCA1 Function

Only 1 mutation has been described in the transmembrane domain of ABCA1. Five mutations have been described in the transmembrane domains of the ABCR gene. Small deletions and mutations that introduce charged amino acids into transmembrane regions of ABCR result in greatly reduced amounts of ABCR protein.22,61 Mutations in the transmembrane domain region of ABCA1 might also disrupt the integration of ABCA1 into membranes and therefore prevent it from exiting the endoplasmic reticulum and the golgi or prevent its integration into the plasma membrane. This could result in the rapid turnover of the mutant ABCA1 protein. The only described mutation in ABCA1 that occurs in the transmembrane domain, H9004/L693, results in the deletion of one amino acid. ABCA1 with this mutation does not exit from the endoplasmic reticulum, and therefore it also shows no ApoA-I binding.60

Integrity of the Nucleotide Binding Folds Is Essential for ABCA1 Function

Several mutations have been described in the NBF region of the ABCR gene, and these mutants are defective in ATP binding.22,61 Thus, ABCA1 harboring mutations in the NBFs may not generate the ATP necessary for active transport of substrates. Although ABCA1 harboring these mutations would be expected to show defects in lipid efflux that may be energy-requiring, no defect in localization to the plasma membrane is expected in these mutants. A total of 6 missense mutations have been described in the NBF region between the Walker A and B motifs. None of these mutations have yet been characterized biochemically for their ability to bind ATP, their localization, their ability to bind ApoA-I, or their ability to induce lipid efflux. Interestingly, of the 6 mutations in the NBFs, 5 occur in the first NBF but only 1 occurs in the second NBF. The 2 NBFs in ABCR show significantly different ATP binding and hydrolysis properties, with the NBF1 being active as an ATPase and binding ATP, CTP, GTP, and UTP.62 The NBD1 of the CFTR protein also shows greater affinity for ATP than does NBD2.63,64 Studies of the MRP1 molecule have shown that ATP binding at NBD1 induces conformational changes in the protein and enhances ADP trapping at NBD2.65 These data suggest that the 2 NBD domains in ABCA1 have differential function, with NBD1 being rate-limiting for proper function.

Critical Role of the C-Terminus of ABCA1

The CFTR protein, another homolog of ABCA1, is usually targeted to the apical surface of cells. When the C-terminal portions of CFTR are disrupted, there is a redistribution of the protein to both the apical and basolateral surfaces of cells as well as a reduction in its half-life.66 In addition, the C-terminus of the CFTR protein contains a PDZ binding domain that when mutated causes redistribution of the protein.67 ABCA1 also contains a PDZ binding domain in its C-terminus,43,68 which when mutated could lead to protein mislocalization. The functional significance of the PDZ domain remains to be determined, although binding of PDZ proteins has been shown to occur.68 Mutations in the C-terminus of ABCA1 might similarly impact the normal targeting of ABCA1 to the basolateral surface in polarized cells and influence its stability. The 5 naturally occurring mutations in the C-terminal region of ABCA1 have yet to be functionally characterized.

Nature of Mutations in ABCA1 Contributes to the Biochemical, Cellular, and Clinical Phenotype

It is likely that the phenotypic heterogeneity in TD patients or in those heterozygous for ABCA1 deficiency might at least in part be accounted for by the nature of the mutation and its effect on the protein. Until the genetic basis for TD and FHA were discovered, these patients were diagnosed based on their phenotype. Since the genetic defect underlying both diseases has been discovered, the assignment of disease has been mainly based on genotype, with heterozygotes for ABCA1...
mutations being classified as having FHA and those homozygous for ABCA1 mutations being categorized as having TD. Thus, in the past, there was a potential for underascertainment, with TD patients carrying mutations conferring milder phenotypes being classified as having FHA and those heterozygous for very mild mutations not being recognized at all. The converse is also true, with those carrying severe heterozygous mutations being designated as having TD. Indeed, phenotypic variability of TD is now readily apparent, with some TD patients having very low HDL levels (<1%) and others having >10% compared with age- and sex-matched controls.

Based on the knowledge of the mutations in affected individuals, it is now possible to ascertain functional deficits for a spectrum of phenotypes associated with either heterozygosity or homozygosity for mutations in ABCA1. For TD, individuals with severe clinical phenotypes may show no ABCA1 protein at the plasma membrane or have ABCA1 at the plasma membrane that is completely lacking in function. This could result from 2 null alleles for ABCA1 preventing export of the protein to the plasma membrane or from ABCA1 at the plasma membrane harboring mutations in residues crucial for its function. Indeed, patients harboring the mutations 635X, N935S, N1800H, 1851X, and 2203X and the large C-terminal deletion all have below 1% of HDL-C levels of age- and sex-matched controls from the Lipid Research Clinic population.

Patients homozygous for the mutations A255T and R1680W show HDL-C levels that are greater than 10% of age-and sex-matched population controls. These patients with a less severe clinical phenotype imply that the ABCA1 protein shows some residual activity.

In general, of those patients heterozygous for ABCA1 mutations, those with a mutation resulting in completely dysfunctional protein would be expected to show HDL-C levels that are approximately 50% of those for age- and sex-matched controls. The protein generated from this allele either would not be expected to reach the plasma membrane or would affect a residue essential for its function. Most patients harboring heterozygous mutations do show HDL-C levels that are close to 50% of those of normal age- and sex-matched controls.

Some patients heterozygous for ABCA1 mutations show >50% HDL-C levels compared with controls when environmental effects such as age are controlled for. These patients would be hypothesized to synthesize a mutant ABCA1 protein that shows some residual activity. In this case, mutant ABCA1 would be localized at the plasma membrane and show a mild defect. This is indeed the case in heterozygous patients harboring mutations A255T, W590S, T929I, R1680W, and A937V, who all show HDL-C levels >75% of normal age- and sex-matched controls.

Another possibility is that heterozygotes for mutations in ABCA1 show <50% of normal levels of HDL-C. This could be caused by dominant-negative effects of ABCA1, as previously shown for truncation mutations. Patients harboring the mutation M1091T show HDL-C levels that are 30% of age- and sex-matched controls. This finding suggests that ABCA1 acts as a dimer or as part of a complex in the exertion of its function.

**ABCA1 Heterozygotes Have Increased Atherosclerosis**

Before the cloning of the ABCA1 gene, studies of obligate heterozygotes had reported conflicting findings on whether individuals heterozygous for mutations in ABCA1 are at an increased risk of developing CAD.

This is not surprising considering that patients harboring mutations in ABCA1 show a wide range of phenotypes and thus misclassification of patients was likely. Cloning of the gene and descriptions of the mutations allowed for the direct assessment of atherosclerosis in heterozygotes. In one large study of 13 different mutations in 11 families, both with TD and FHA, phenotypic analysis in a cohort of heterozygous individuals was undertaken. The control cohort consisted of unaffected family members. A greater than 3-fold increase in CAD in adult heterozygotes compared with controls with earlier age of onset (by 10 years) was evident. Intriguingly, the relative cholesterol efflux levels were directly related to CAD, with families with the clearest evidence for premature CAD having individuals with the lowest cholesterol efflux.

However, several caveats were evident in this study. First, the collection of the kindreds may have been biased by clinical sampling, because only families with the most severe phenotypes may have presented at clinics. Second, a very low number of events was seen. Third, using an end point of CAD as an outcome measure might have underestimated the effects. CAD is an insensitive marker for atherosclerosis and does not address the effects of mutations on the natural history of presymptomatic atherogenesis.

To address these issues, a second study elucidating the association between mutations in ABCA1 and surrogate...
markers, namely, increased arterial wall thickness and ABCA1-mediated cholesterol efflux, was performed. The study group consisted of 30 individuals heterozygous for 4 different missense mutations in the ABCA1 gene, C1477R, M1091T, P2150L, and T929I. Importantly, the mean intima-media thickness in carriers was higher than in controls, and carriers for mutations in ABCA1 also showed increased progression of arterial thickening that reached the upper limit of normal (0.8 mm) much earlier (55 years) compared with controls (80 years, \( P < 0.0001 \)). Similar to the previous study, regression analysis of the data from this study indicated that a 50% increase in ABCA1-mediated cholesterol efflux would result in a 30% increase in HDL-C concentrations and that this could translate into a 35% to 50% reduction in the risk of CAD.

Interestingly, mutations in ABCA1 do affect susceptibility to atherosclerosis not only by influencing lipids but by direct effects on the vessel wall. Nitric oxide is derived from the vascular endothelium and is a crucial antiatherogenic agent that maintains vascular homeostasis. Diminished NO availability represents an early step in atherosclerosis. ABCA1 heterozygotes show significantly impaired endothelial function with impaired basal and stimulated nitric oxide activity compared with controls, indicating that ABCA1 affects vessel wall function.

Single Nucleotide Polymorphisms in ABCA1

Ten coding single nucleotide polymorphisms (cSNPs) along with hundreds of noncoding SNPs have been described in the ABCA1 gene (see Table 3). Among the noncoding SNPs are 9 promoter and 5’ UTR variants that have been analyzed for functional significance. Most cSNPs are found distal to known functional domains (Figure 6). In addition, the amino acid residues affected by cSNPs are less conserved compared with those affected by mutations (50% compared with >95%, Table 4).

Association of ABCA1 cSNPs and Regulatory SNPs With HDL and Atherosclerosis

Soon after mutations in ABCA1 were found to be causative of TD and FHA, our laboratory and others investigated whether common variation in ABCA1 could contribute to variation in HDL-C levels and atherosclerosis in the general population. Remarkably, of the 10 cSNPs described, 6 are associated with potential functional effects, including alterations of lipid levels or measures of atherosclerosis (see Table 5). However, it is important to note that few of these findings have been replicated, and some results are inconsistent. Still, it is remarkable that so many cSNPs have been associated with functional effects, suggesting that ABCA1 may be a major atherosclerosis susceptibility locus in the general population.

Remarkably, of the 10 cSNPs described, 6 are associated with potential functional effects, including alterations of lipid levels or measures of atherosclerosis (see Table 5). However, it is important to note that few of these findings have been replicated, and some results are inconsistent. Still, it is remarkable that so many cSNPs have been associated with functional effects, suggesting that ABCA1 may be a major atherosclerosis susceptibility locus in the general population. The R219K, V771M, and I883M variants have been recognized as putative antiatherogenic polymorphisms, associated with increased HDL-C and decreased triglyceride levels (K219 and M883) and increased HDL-C and ApoA-I (M771). The E1172D, R1587K cSNPs have been reported to be associated with decreased HDL-C.

Five promoter SNPs are associated with increased severity of atherosclerosis, including the –191C/–320C/–477T haplotype as well as the G-191C and A-1096G SNPs. In
contrast, the C-17G variant was associated with less atherosclerosis.78 Interestingly, these SNPs are not associated with changes in lipid levels, suggesting that changes in ABCA1 activity can occur without changes in steady-state plasma lipid levels. The V825I, I883M, and E1172D SNPs have also been associated with increased clinical events and severity of atherosclerosis.75,77

The R219K Variant

The R219K SNP has been most studied and highlights many of the difficulties associated with the study of SNPs in general. At least 8 studies have examined the role of this SNP in lipid homeostasis or atherogenesis75,77,79,81–84 (Table 6). Of these, 5 have reported positive association with either increased HDL-C or reduced severity of atherosclerosis. This number of positive replications in independent studies consistently in the same direction indicates that this is truly an important variant with a significant atheroprotective effect.

In 3 studies, the atheroprotective effect of the K219 allele was observed only in certain circumstances, for example in women,83 in individuals with elevated lipoprotein a [Lp(a)] or with the ApoE3/E3 genotype,82 or in smokers.84 These findings indicate the functional effect of K219 may be particularly significant in certain genetic and environmental backgrounds.

The 3 reports that found no association with HDL-C or severity of atherosclerosis may have been confounded by population admixture, because 2 of these studies examined ethnically mixed urban populations in the United States.77,81 The frequencies of ABCA1 cSNPs are highly divergent across populations (Table 7), and interestingly, the minor K219 allele is actually the wild-type allele in a cohort of 327 Japanese school-aged children (Yamakawa-Koboyashi, personal communication, 2002).

Linkage disequilibrium (LD) among ABCA1 cSNPs is an additionally confounding variable, which to date has not been adequately addressed. Clee et al75 reported significant LD between the R219K and the V771M, K776N, I883M, and R1587K cSNPs but found that after carriers of these variants were excluded, R219K remained significantly associated with degree of atherosclerosis and triglyceride levels. Additional

### Table 5. Functional Effects of cSNPs and Regulatory SNPs in the ABCA1 Gene

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>Amino Acid/Position</th>
<th>Lipids</th>
<th>CAD/Atherosclerosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-17G</td>
<td>Promoter</td>
<td>No change</td>
<td>↓</td>
</tr>
<tr>
<td>InsG319</td>
<td>5'UTR</td>
<td>No change</td>
<td>↓</td>
</tr>
<tr>
<td>G1051A</td>
<td>R219K</td>
<td>↓ TG, ↑ HDL, ↑ ApoA-1, ↑ ApoB, ↑ LDL</td>
<td>↓</td>
</tr>
<tr>
<td>A3044G</td>
<td>I883M</td>
<td>↓ TG, ↑ HDL</td>
<td>↑</td>
</tr>
</tbody>
</table>

### Table 6. Associations of the R219K cSNP With Lipid Levels and Atherosclerosis

<table>
<thead>
<tr>
<th>Reference</th>
<th>HDL-C</th>
<th>Atherosclerosis</th>
<th>Triglyceride</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broussseau et al77</td>
<td>NC</td>
<td>...</td>
<td>...</td>
<td>Differences in changes of HDL-C with age observed</td>
</tr>
<tr>
<td>Clee et al70</td>
<td>NC</td>
<td>↓</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>Takagi et al79</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td></td>
</tr>
<tr>
<td>Lutucuta et al81</td>
<td>NC</td>
<td>...</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>Yamakawa-Kobayashi et al (personal communication, 2002)</td>
<td>↑</td>
<td>...</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>Evans et al82</td>
<td>↑</td>
<td>...</td>
<td>↓</td>
<td>Only in ApoE3/E3 (HDL-C) and elevated Lp(a) (triglyceride) individuals</td>
</tr>
<tr>
<td>Kakko et al83</td>
<td>↑</td>
<td>...</td>
<td>NC</td>
<td>Only in women</td>
</tr>
<tr>
<td>Cenarro et al84</td>
<td>NC</td>
<td>↓</td>
<td>NC</td>
<td>FH patients</td>
</tr>
</tbody>
</table>

Five of the 8 studies have reported a positive association of the R219K variant with either lipid levels or atherosclerosis. NC indicates no change; ellipsis, no data are available.
ABCA1 SNPs May Be Associated With Changes in Atherosclerosis Independent of Changes in HDL-C Levels

Of the 12 cSNPs and regulatory SNPs associated with alterations in plasma lipid levels or atherosclerosis, 7 display altered severity of atherosclerosis without detectable changes in lipid levels. This suggests that although ABCA1 may be an important atherosclerosis susceptibility locus, the mechanism by which it exerts this effect is not necessarily by altering steady-state HDL-C levels. The noncoding SNPs G-191C, C-69T, C-17G, and InsG319 and the cSNPs R219K, V771M, and V825I have all been found to be associated with differences in severity of atherosclerosis but not with changes in HDL-C levels in at least 1 study. The implication is that HDL quality and composition, as determined by ABCA1-mediated efflux, may be a determinant of the efficiency of reverse cholesterol transport, without actually affecting the levels of circulating HDL-C. These results are consistent with efflux influencing atherosclerosis without necessarily changing lipid levels. Several studies have recently provided evidence of how this may occur. Bone marrow transplant experiments between ABCA1-null and wild-type mice have demonstrated that deficiency of macrophage ABCA1 is associated with small changes in lipid levels but significant increases in atherosclerosis.\(^{85,86}\) This concept has been recapitulated by the study of ABCA1 BAC transgenic mice, in which a significant protection from atherosclerosis is evident with minimal changes in HDL-C levels.\(^{87}\) Taken together, these studies indicate that ABCA1 can influence atherogenesis independent of steady-state HDL-C levels.

Conclusions

The study of TD, a rare disorder of lipoprotein metabolism with less than 60 reported cases worldwide, led to the identification of the functional impact of the ABCA1 gene and protein. Variation of this protein has now been shown to confer a risk for atherosclerosis in the general population and has provided an answer to a question asked for many years, namely, how lipids are effluxed from cells in the first step of reverse cholesterol transport. The study of this rare disorder in a few has led to the identification of a validated drug target that offers hope for raising HDL and prevention of atherosclerosis in many.

Acknowledgments

This work was supported by grants from the Canadian Institutes of Health Research and the Heart and Stroke Foundation of British Columbia and Yukon and Xenon Genetics, Inc. M.R.H. is a holder of a Canada Research Chair in human genetics. J.J.P.K. is an established investigator of the Netherlands Heart Foundation (2000D039).

References


Efflux and Atherosclerosis: The Clinical and Biochemical Impact of Variations in the ABCA1 Gene
Roshni R. Singaraja, Liam R. Brunham, Henk Visscher, John J.P. Kastelein and Michael R. Hayden

Arterioscler Thromb Vasc Biol. 2003;23:1322-1332; originally published online May 22, 2003;
doi: 10.1161/01.ATV.0000078520.89539.77
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272
Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://atvb.ahajournals.org/content/23/8/1322

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published
in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the
Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for
which permission is being requested is located, click Request Permissions in the middle column of the Web
page under Services. Further information about this process is available in the Permissions and Rights
Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online
at:
http://atvb.ahajournals.org//subscriptions/