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## Do DNA sequence variants in *ABCA1* contribute to HDL cholesterol levels in the general population?

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**HDL has a key role in reverse cholesterol transport, mobilizing cholesterol from the peripheral tissues to liver. In this process, the ABC transporter A1 (ABCA1) protein controls the efflux of intracellular cholesterol to apoAI, the major apolipoprotein of HDL. Since *ABCA1* mutations were discovered to cause Tangier disease, a rare recessive HDL deficiency, it has been speculated that sequence variants in *ABCA1* might also contribute to variations in plasma HDL cholesterol levels in the general population. A new study provides genetic evidence supporting this hypothesis (see the related article beginning on page 1343).**

A decreased level of plasma HDL cholesterol (HDL-C) is a major risk factor for coronary atherosclerosis. The cardioprotective effect of HDL has been attributed to, among other factors, its key role in reverse cholesterol transport (RCT), mobilizing cholesterol

from the peripheral tissues to liver. Approximately 50% of plasma HDL-C variability is determined by genetic factors (1, 2). Variants in several genes, including *ABC transporter A1 (ABCA1)*, *apolipoprotein AI (APOA1)*, and *lecithin cholesterol acyltransferase (LCAT)*, are implicated in rare mendelian forms of HDL deficiency (refs. 3–5; reviewed in refs. 6, 7). Several chromosomal regions have been identified in genome-wide scans for HDL-C, and these regions are likely to harbor genes for common forms of HDL-C deficiency (8–13). In addition, candidate genes, the variants of which have been shown to affect plasma HDL-C levels, include, for example,

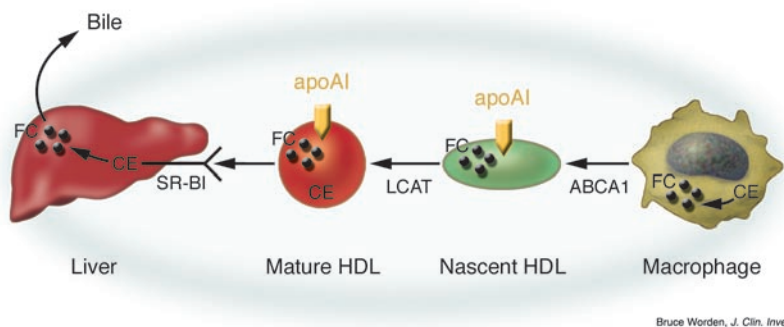
*hepatic lipase* and the *apolipoprotein AI/CIII/AIV/AV* gene cluster (14). However, DNA sequence variants contributing to variation in plasma levels of HDL-C in the general population are largely unknown, especially regarding the prevalence of variants with major effects on HDL-C level.

Generally, complex traits are suggested to be caused by common sequence variants that each may have a small to moderate phenotypic effect (15–17). On the other hand, accumulating data show that most mendelian disorders are caused by a set of different mutations that often reside in coding regions (reviewed in ref. 18). These rare variants tend to have strong phenotypic effects. The extent to which rare versus common variants confer the susceptibility to complex traits is currently not known. Studies such as that featured in this issue of the *JCI* by Frikke-Schmidt and colleagues (19), investigating whether rare and/or common variants contribute to a quantitative trait, are of major importance not only in elucidating the sequence variants

**Nonstandard abbreviations used:** ABCA1, ABC transporter A1; APOA1, apolipoprotein AI; HDL-C, HDL cholesterol; LCAT, lecithin cholesterol acyltransferase; RCT, reverse cholesterol transport; SNP, single-nucleotide polymorphism.

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**Figure 1**  
A schematic overview of the role of the ABCA1 protein in HDL metabolism and RCT. In RCT, excess free cholesterol (FC) is removed from peripheral tissues and returned to the liver for excretion in the bile. The ABCA1 protein is crucial for the initial steps of this process, since it controls the efflux of intracellular cholesterol to lipid-poor apoA1, which is the major apolipoprotein of HDL. The other key molecules in RCT include LCAT and scavenger receptor class-B, type I (SR-BI). CE, cholesteryl ester.

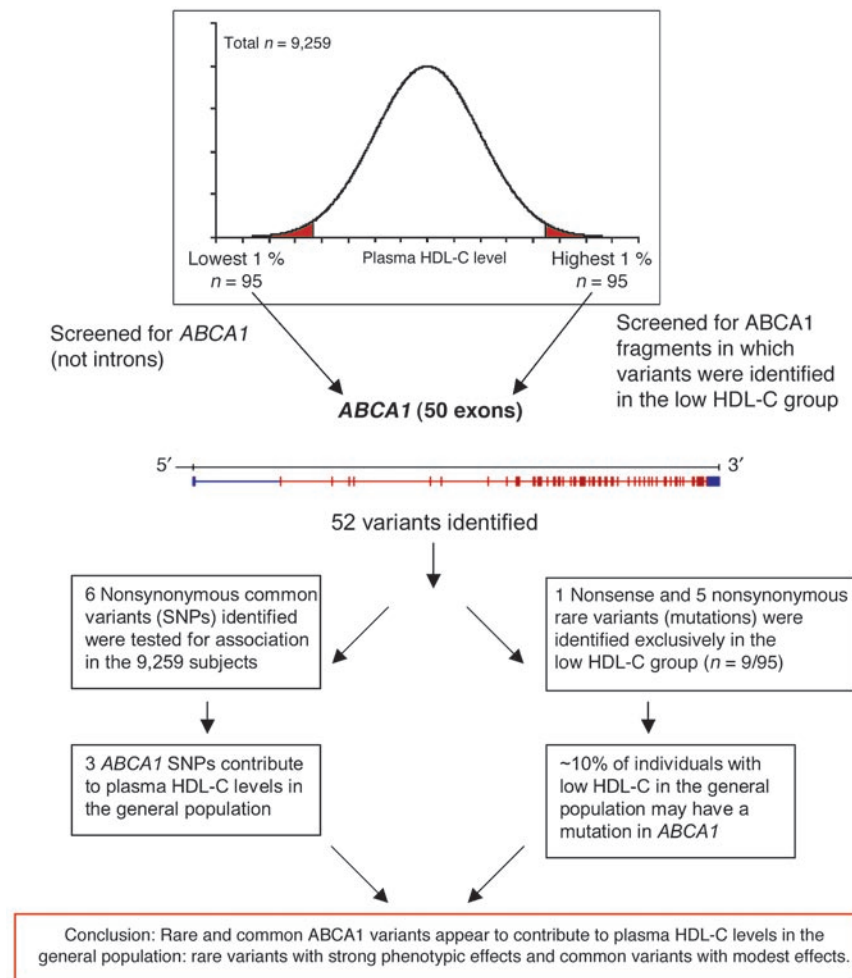
predisposing to low HDL-C levels but also because the results of such studies will help guide future identification of genes underlying complex traits. Particularly, the selection of strategies for genome-wide association studies with hundreds of thousands of common variants, so-called single-nucleotide polymorphisms (SNPs), will depend on the results obtained in these types of studies in large population samples. If the model suggesting that common alleles at several loci interact to cause disease (i.e., the common variation/common disease [CV/CD] model) is correct, the proposed haplotype-based association studies (20) that detect mostly common variants can be successfully used to identify genes for complex traits. If the contribution of rare alleles also turns out to be significant for complex traits, sequence-based approaches (reviewed in ref. 18), focusing on identification and testing for association of variants in coding and regulative regions, will also be needed in order to detect rare alleles.

**Contribution of ABCA1 variants to plasma HDL-C levels in the general population**

The ABCA1 gene is a crucial player in the initiation of RCT and in the lipidation of apoA1, because the ABCA1 protein controls

the efflux of intracellular cholesterol to lipid-poor apoA1, the major apolipoprotein of HDL (3–5) (Figure 1). Furthermore, mutations in ABCA1 are known to cause Tangier disease, a rare recessive HDL defi-

ciency (3–5). In this issue of the JCI, Frikke-Schmidt and colleagues present their data on the contribution of ABCA1 variants to plasma HDL-C levels in the general population (19). The sample consisted of 9,259 individuals from an ethnically homogeneous general-population survey, the Copenhagen City Heart Study (21). The authors explored 3 questions: (a) are heterozygotes for mutations in ABCA1 over-represented in subjects with low HDL-C; (b) do frequencies of SNPs in ABCA1 differ between subjects with low and high HDL-C levels; and (c) do SNPs in ABCA1 have an effect on HDL-C levels in the general population? To answer these questions, they first screened the core promoter and all 50 exons, including exon-intron boundaries, of ABCA1 in individuals with the lowest 1% (n = 95) of HDL-C levels, adjusted for sex and age, from the Copenhagen City Heart Study (Figure 2). Second, ABCA1 variants that were identified were screened in individuals with the highest 1% (n = 95) of HDL-C levels. All common variants



**Figure 2**  
The strategy used by Frikke-Schmidt and colleagues (19) to investigate whether DNA sequence variants in the ABCA1 gene contribute to plasma HDL-C levels in the general population. Any variant that causes a premature stop-codon in synthesis of protein is called a nonsense variant.

**Table 1**

Summarized overview of the SNPs associated with increased or decreased HDL-C levels in the general population

Nonsynonymous SNP	Allele frequency: general population/low-HDL-C group/high-HDL-C group	Effect
V771M	0.03/0.005/0.04	Increased HDL-C
V825I	0.06/0.05/0.08	Increased HDL-C
R1587K	0.24/0.23/0.16	Decreased HDL-C

The information in this table is from ref. 19.

identified in the coding region of the *ABCA1* gene that resulted in amino acid substitutions (i.e., nonsynonymous SNPs) were genotyped in the 9,259 individuals (Figure 2). However, because the authors screened only 28 of the 51 *ABCA1* fragments – those containing mutations and SNPs in the low-HDL-C group – important variants associated with high HDL-C levels may have been missed. The authors may also have missed important variants residing in introns or regulatory regions. Variations in regulatory sequences leading to subtle quantitative differences in phenotype may be particularly relevant to common chronic metabolic diseases, in which a gradual accumulation of damage over many years typically occurs before reaching a critical threshold. All in all, this is, however, a clever strategy (19), because by screening subjects with extremely low and high levels of HDL-C the authors can increase the possibility of detecting functionally significant variants.

#### Rare *ABCA1* variants

In the 95 subjects with low HDL-C levels, 52 *ABCA1* sequence variants were identified, of which 19 were previously unidentified (19). Using a definition of an allele frequency greater than 1% for an SNP (common variant) and less than or equal to 1% for a mutation (rare variant), the authors identified 17 SNPs and 13 mutations in their analyses of the core promoter and exons. Interestingly, 100% of the SNPs, but only 23% of the mutations, identified in the low-HDL-C group were also observed in the high-HDL-C group, which suggests that many of these SNPs may have a neutral phenotypic effect. Further, 83% of the mutations resulting in an amino acid substitution were detected only in the low-HDL-C group, which implies a strong phenotypic effect. The amino acids affected by these mutations were also located in highly

conserved regions of the *ABCA1* protein and are completely conserved between several species. There were 3 individuals heterozygous for the known Tangier mutation, N1800H, among the 95 low-HDL-C subjects, resulting in an allele frequency of 1.58%, which is actually approaching the frequency of an SNP. Therefore, to further evaluate the population effect of this variant, N1800H, it would be interesting to see how many heterozygotes there are among somewhat less severe HDL-C cases, using, for example, the 10% and 25% levels of HDL-C in this extensive population sample. Based on the locations of the 3 new nonsynonymous mutations identified – S364C, P1065S, and G1216V – the authors suggest that these mutations cause an inherited form of HDL-C deficiency, familial hypoalphalipoproteinemia. This could be verified by testing of their effect on cholesterol efflux, because, as in Tangier disease, in this dominantly inherited form of familial hypoalphalipoproteinemia, a reduction in cellular cholesterol efflux is typically observed (22). In addition, to further confirm that the nonsynonymous *ABCA1* mutations identified contribute to HDL-C level, their cosegregation with low HDL-C levels could be investigated in the probands' families.

Because 9 of the 95 subjects with low HDL-C levels had either a nonsynonymous mutation in *ABCA1* or a mutation resulting in a truncated *ABCA1* protein, as many as 10% of individuals with very low HDL-C levels in the general population could be heterozygous for mutations in *ABCA1*, which suggests that rare *ABCA1* variants contribute to HDL-C levels. This finding is further supported by the data of a recent study screening the *ABCA1*, *APOA1*, and *LCAT* genes in white and black subjects from the population-based Dallas Heart Study and in white Canadians with low or high plasma HDL-C levels (23). The exten-

sive sample of 9,259 individuals in the present study (19) enabled Frikke-Schmidt et al. to detect this effect in individuals with the lowest 1% of HDL-C levels, showing once again the importance of using large, carefully collected population samples such as the Copenhagen City Heart Study.

#### Common *ABCA1* variants

Regarding the common variants, all nonsynonymous SNPs identified in this study (19) were genotyped in the 9,259 individuals using a 3-way analysis strategy: (a) the overall effect of each SNP was investigated (regardless of variation at the other 5 sites); (b) the isolated site effect was investigated (six-SNP genotypes differing only at the relevant SNP); and (c) the results were verified using phenotype data from the previous examination of the Copenhagen City Heart Study taken 10 years earlier (21). Using this well-planned analysis strategy, the authors were able to conclude that none of these 6 SNPs, all described previously, had effects on total cholesterol, triglycerides, or apoB levels, and that 3 SNPs affected HDL-C levels, especially in women; the relatively rare SNPs, V771M and V825I, were associated with increases in HDL-C, and the common SNP, R1587K, was associated with decreased HDL-C levels (Table 1). Importantly, these results were seen at 2 examinations 10 years apart. Although the reasoning for the observed sex-specific differences remains unclear, in general, sex-specific differences seem to be a typical phenomenon in complex traits. An extensive study sample is required to investigate multiple-SNP genotypes that differ only at the particular SNP of interest. This strategy can, however, be useful to distinguish a direct association with an SNP from an association with one in tight linkage disequilibrium with the causal variant (i.e., alleles of 2 separate variants appear together on a chromosome more often than expected by chance alone). It is especially effective when, as in this new study (19), it is combined with analysis of phenotype data that originate from 2 examinations a number of years apart. However, the effects of these 3 SNPs, V771M, V825I, and R1587K, on HDL-C levels were relatively modest (19). Furthermore, although frequencies of 2 of these 3 SNPs (V771M and R1587K) and their haplotypes differed between the population extremes of HDL-C levels, all 3 were present in both low- and high-HDL-C groups (Table 1) (19). A variant with a strong phenotypic effect is more likely to be observed in only 1 extreme.



Recently, the only nonsynonymous *ABCA1* SNPs that showed some evidence for association ( $P < 0.05$ ) with HDL-C levels in 2,569 subjects from the Dallas Heart Study, further divided into 4 groups by race and sex, were I883M and V825I, both observed in males (23). These different results may be partly explained by the smaller study sample and by the criterion used for triglyceride level ( $<200$  mg/dl) (23).

To generalize, the results reported by Frikke-Schmidt and colleagues (19) regarding rare variants (represented by *ABCA1* mutations) suggest that extreme levels of a quantitative trait in the general population can be due to rare variants with major phenotypic effects. On the other hand, their results regarding common variants (represented by nonsynonymous *ABCA1* SNPs) are in accordance with the theory that complex traits are caused by common variants, each likely exhibiting a small to moderate phenotypic effect. This elegant study helps us recognize that several different approaches are needed in future studies of complex traits to address the complexity of the human genome.

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