first-generation assays and may overestimate LDL-C in patients with dysbetalipoproteinemia (18, 19). Some of our dysbetalipoproteinemic patients were initially evaluated in laboratories that perform direct LDL-C measurements (various methods), and in our experience (unpublished observation), their measured LDL-C is often much lower than the LDL-C calculated by the Friedewald formula. The Friedewald formula is known to overestimate LDL-C in dysbetalipoproteinemia because of abnormal VLDL composition.

It has been suggested that measuring TC adds little to cardiovascular risk assessment when HDL-C and LDL-C are measured directly (20). This approach is valid for most patients but will underestimate atherosclerotic risk in conditions in which abnormal nonmeasured lipoproteins such as remnants accumulate. ApoB concentrations are an important component of cardiovascular risk assessment (21, 22). Increased apoB is associated with higher numbers of circulating atherogenic lipoproteins and higher cardiovascular risk. In dysbetalipoproteinemia, the situation is somewhat reversed, and apoB concentrations below the 75th North American population percentile (23) are associated with very high cardiovascular risk. In dysbetalipoproteinemia, high TC and TG concentrations indicate high risk, once again highlighting that apoB and lipid measurements are not identical but complementary indices for risk assessment (24).

In conclusion, the apoB/TC ratio is a simple but effective screening test for dysbetalipoproteinemia in patients with mixed hyperlipidemia when lipid values are suggestive of dysbetalipoproteinemia. It best identifies patients that are unlikely to be dysbetalipoproteinemic, for whom additional testing likely will be nondiagnostic. The ratio should not be used in other lipid phenotypes. No additional laboratory work is required if apoB is included as part of the routine assessment of dyslipidemia.

References

Effect of Two Common Polymorphisms in the ATP Binding Cassette Transporter A1 Gene on HDL-Cholesterol Concentration, Petter S. Woll, Naomi Q. Hanson, Valerie L. Arends, and Michael Y. Tsai. (Department of Laboratory Medicine and Pathology, University of Minnesota Medical School, Minneapolis, MN 55455; * address correspondence to this author at: 420 Delaware Street SE, Mayo Mail Code 609, Minneapolis, MN 55455-0392; fax 612-625-5622, e-mail tsai001@tc.umn.edu)

HDL-cholesterol (HDL-C) has long been recognized as having an atheroprotective role (1). Epidemiologic studies have shown that decreased HDL-C concentrations are the most common lipid abnormality in patients with premature coronary artery disease (CAD). Thus, there has been increased recognition of the inverse relationship between HDL-C concentrations and risk for CAD (2, 3). This heightened awareness is underscored by changes in the recommendations of the Adult Treatment Panel (ATP) of the National Cholesterol Education Program. For example, ATP II, published in 1993, first introduced the
concept of a low HDL-C [<0.91 mmol/L (<35 mg/dL)] as a risk factor for CAD (4). More recently, ATP III further revised the cutoff for HDL-C as a risk factor to <1.04 mmol/L (<40 mg/dL) (5).

HDL-C is the primary lipoprotein particle responsible for reverse cholesterol transport (RCT) (6). RCT involves the transport of cholesterol from nonhepatic cells to the liver and its subsequent elimination from the body as bile acid and free cholesterol. A major advance in the understanding of RCT was heralded by the discovery of the gene encoding for ATP binding cassette transporter A1 (ABCA1). ABCA1, a member of the ABC transporter family, facilitates the active transport of cholesterol and phospholipids from the intracellular compartments of peripheral cells to the lipid-poor nascent HDL particle (7), thus representing the first step of the RCT pathway.

Deleterious mutations, when present in both alleles in the ABCA1 gene, were identified as the molecular basis for patients with Tangier disease, a disorder characterized by an almost complete absence of plasma HDL-C and an increased risk for CAD (8–10). Studies from our own laboratory, however, demonstrated that these detrimental mutations of the ABCA1 gene are probably not highly represented in the general population, even in a group of individuals with low HDL (11). On the other hand, many common ABCA1 polymorphisms have been reported, and at least some have been shown to influence plasma HDL-C concentrations and/or CAD progression and severity (12, 13).

Clee et al. (12) investigated the effect of several polymorphisms on plasma HDL-C concentrations and the associated risk of CAD. Of nine nonsynonymous ABCA1 polymorphisms, only the 1051G/A polymorphism (R219K) was significantly correlated with HDL-C concentrations and CAD risk. Moreover, carriers of the 1051A allele were found to have higher HDL-C concentrations and reduced severity of CAD. The association between the ABCA1 1051G/A polymorphism and HDL-C concentrations was confirmed by Kakko et al. (13), but only in the female study population. Lutucuta et al. (14) studied the −477C/T polymorphism located in the promoter region of ABCA1 and found that it was associated with a trend toward higher plasma HDL-C concentrations. Other groups have also investigated the effect of ABCA1 polymorphisms on HDL-C concentrations and found no significant association (15) or an association only in a particular ethnic group (16).

In the current investigation, we studied the prevalence of two frequently occurring variants of the ABCA1 gene, the 1051G/A and the −477C/T polymorphisms, and the effect of these two polymorphisms on plasma HDL-C concentrations. We studied 838 patients with premature CAD, as documented by angiographically confirmed atherosclerosis and/or one or more episodes of myocardial infarction or coronary artery bypass surgery before age 55, recruited over a time period of 4 years from the Minneapolis Heart Institute. We also studied a cohort of the Minnesota Heart Study that included 257 apparently healthy individuals with no family history of CAD. The CAD group had a mean (SE) age of 49.5 (0.2) years and mean HDL-C of 0.91 (0.01) mmol/L. The group without CAD had a mean age of 48.3 (0.5) years and mean HDL-C of 1.24 (0.02) mmol/L. DNA was obtained from all individuals for polymorphism studies. This study was approved by the Institutional Review Board: Human Subjects Committee of the University of Minnesota, and all participants gave informed consent.

HDL-C was measured in serum by precipitation of the non-HDL-C with magnetic M, 50,000 dextran sulfate and magnesium chloride, followed by colorimetric reflectance spectrophotometry on a Vitros analyzer (Johnson & Johnson Clinical Diagnostic, Inc.). Genomic DNA was extracted from peripheral leukocytes isolated from acid-citrate-dextrose-anticoagulated blood with use of commercially available DNA isolation reagents (Puren-gene; Gentra Systems).

To detect the 1051G/A polymorphism, we used a 433-bp fragment selectively amplified by PCR (primers: sense, 5′-CTTCAAAAAGCTTCAAGGACC-3′; antisense, 5′-GGCCCAAAAGTCCTGAAAGACAC-3′). The amplified fragment was digested with 4 U of StyI according to the manufacturer’s instructions (New England Biolabs) and electrophoresed on 2% ultra pureTM agarose-1000 (Life Technologies) gel containing ethidium bromide. DNA from a patient homoyzogous for the G allele appeared as bands 189, 131, and 113 bp in length relative to the size marker. Presence of the A allele abolishes a 78 bp cut site, and DNA from a patient homoyzogous for the A allele appears as bands of 320 and 113 bp. The −477C/T polymorphism was detected by selective PCR amplification of a 351-bp fragment (primers: sense, 5′-CTCGGCTCCTCAGGGACT-3′; antisense, 5′-CCGAGACTCTCTAGTCCAC-3′), followed by digestion of the amplified product with 3.5 U of AcI according to manufacturer’s instructions (New England Biolabs). DNA from a patient homoyzogous for the C allele appears as bands of 148, 130, and 73 bp. Presence of the T allele abolishes an AcI cut site, and DNA from a patient homoyzogous for the T allele appears as bands of 278 and 73 bp.

Statistical analysis was performed by SPSS 10.0 for Windows. We used multivariate ANOVA to identify statistical differences at 95% confidence. The effect of individual genotypes and P values were determined by post hoc analysis using least-significant differences. Mean HDL-C concentrations were adjusted for sex and age. We used the Pearson χ2 test to compare the genotype prevalence in the two populations. Significance was defined as P < 0.05.

The prevalences of the ABCA1 1051G/A and −477C/T genotypes in 838 individuals with documented CAD and 257 control individuals are shown in Table 1. The prevalence of individuals homoyzogous for the 1051A allele was significantly higher (P < 0.05) in the control group compared with the CAD group. We observed no significant difference in genotype frequency between the two populations for the −477C/T polymorphism.

The mean HDL-C concentrations in the CAD patients
Table 1. Prevalence of the ABCA1 1051G/A and −477C/T genotypes in 838 CAD patients and 257 controls and effect on HDL-C concentration.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>CAD patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>HDL-C(^a) mmol/L</td>
</tr>
<tr>
<td>1051GG</td>
<td>457 (53.7)</td>
<td>0.90 (0.01)(^b)</td>
</tr>
<tr>
<td>1051GA</td>
<td>327 (39.0)</td>
<td>0.91 (0.01)(^b)</td>
</tr>
<tr>
<td>1051AA</td>
<td>61 (7.3)(^c)</td>
<td>0.99 (0.03)(^b)</td>
</tr>
<tr>
<td>−477CC</td>
<td>237 (28.3)</td>
<td>0.90 (0.02)</td>
</tr>
<tr>
<td>−477CT</td>
<td>426 (50.8)</td>
<td>0.90 (0.01)</td>
</tr>
<tr>
<td>−477TT</td>
<td>175 (20.9)</td>
<td>0.94 (0.020)</td>
</tr>
</tbody>
</table>

\(^a\) Values are the mean (SE) and are adjusted for age and sex.
\(^b\) \(P < 0.05\) for 1051AA vs 1051GG and 1051GA in CAD group.
\(^c\) \(P < 0.05\) for CAD patients vs controls.

and controls classified according to ABCA1 1051G/A and −477C/T genotypes are also shown in Table 1. Individuals with the 1051AA genotype had a significantly higher (\(P < 0.05\)) mean HDL-C concentration than did individuals with the 1051GG and 1051GA genotypes. This same trend was observed in the controls, but the difference did not reach statistical significance, in part because of the small number of individuals tested and, thus, the lack of statistical power of the control group. With respect to the −477C/T polymorphism, there were no significant differences in mean serum HDL-C concentrations in either group.

The results of the current study are the first reported for the 1051G/A polymorphism in a US population. The results confirm those of Clee et al. (12) and Kakko et al. (13), studies involving a French-Canadian and a Finnish cohort, respectively, that the 1051A allele is associated with a slightly higher HDL-C concentration. Additionally, our finding of a higher frequency of the 1051AA genotype in apparently healthy individuals vs individuals with documented CAD confirms the finding of Clee et al. (12) that the 1051A allele confers protection against CAD. Individuals with the 1051AA genotype had higher HDL-C concentrations than did individuals with the 1051GG and -GA genotypes, suggesting that the 1051AA genotype may confer protection against CAD, at least in part through its association with higher HDL-C concentrations. By confirming the findings of Clee et al. (12), our study strengthens the hypothesis that increased ABCA1 activity reduces the development of atherosclerosis by increasing the net efflux of cholesterol from peripheral cells.

With regard to the −477C/T polymorphism, we did not find a consistent association between the genotype and HDL-C or a significant difference in the allele frequency between CAD and control individuals. This suggests that the ABCA1 −477C/T polymorphism has little to no effect on plasma HDL concentrations. An earlier study (14), involving 429 members of a US cohort with coronary lesions, also showed that the −477C/T genotype did not affect serum HDL-C concentrations, but individuals with the T/T genotype had an increased number of lesions. Thus, additional studies are needed to determine whether this polymorphism is of significance in predisposing individuals to atherosclerosis.

In conclusion, we show that the 1051A allele of the ABCA1 gene is more prevalent in controls than in patients with CAD and that the apparent protective effect of the 1051A allele may be attributable in part to its association with moderately increased HDL-C concentrations. Thus, ABCA1 polymorphisms play a role in the polygenic regulation of HDL-C, as do variants in other genes, such as those encoding apolipoprotein A-I and cholesteryl ester transfer protein. Because ABCA1 protein is the first step in RCT and is responsible for facilitating the transport of cholesterol across the membrane of extrahepatic cells, more studies are needed to determine whether increased ABCA1 protein plays an atheroprotective role beyond its association with serum HDL-C concentrations.

References


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