Association of Cholesteryl Ester Transfer Protein–TaqIB Polymorphism With Variations in Lipoprotein Subclasses and Coronary Heart Disease Risk

The Framingham Study

Jose M. Ordovas, L. Adrienne Cupples, Dolores Corella, James D. Otvos, Doreen Osgood, Antonia Martinez, Carlos Lahoz, Oscar Coltell, Peter W.F. Wilson, Ernst J. Schaefer

Abstract—Cholesteryl ester transfer protein (CETP) facilitates the exchange of triglycerides and cholesteryl esters between lipoprotein particles, a key step in reverse cholesterol transport in humans. Variations at the CETP locus have been shown to be determinants of the levels and activity of CETP and high density lipoprotein (HDL) plasma concentration. The associations of the common CETP polymorphism, TaqIB in intron 1, with lipoprotein levels and particle size distribution, CETP activity, and coronary heart disease (CHD) risk were examined in a population-based sample of 1411 men and 1505 women from the Framingham Offspring Study. The B2 allele frequency was 0.444 in men and 0.433 in women, and its presence was significantly \( P < 0.05 \) associated with decreased CETP activity. B1B1 men had lower HDL cholesterol (HDL-C) levels (1.07 mmol/L) compared with B1B2 (1.14 mmol/L) and B2B2 (1.18 mmol/L) men \( (P < 0.001) \). Likewise, B1B1 women had lower HDL-C levels (1.40 mmol/L) compared with B1B2 (1.46 mmol/L) and B2B2 (1.53 mmol/L) women \( (P < 0.001) \). In men, the B2 allele was associated with increased particle size for HDL and low density lipoprotein. In women, a similar effect was demonstrated only for HDL particle size. The odds ratio for prevalent CHD associated with the B2 allele was 0.696 \( (P = 0.035) \) in men. After adjusting for age, body mass index, systolic blood pressure, diabetes, smoking, alcohol consumption, \( \beta \)-blocker use, total cholesterol, and HDL-C, this odds ratio was 0.735 \( (P = 0.187) \), suggesting that the protective effect of the B2 allele was due in part to its association with HDL-C levels. No significant protective effects were observed in women. These data demonstrate that variation at the CETP gene locus is a significant determinant of HDL-C levels, CETP activity, and lipoprotein size in this population. Moreover, these effects appear to translate into a lower CHD risk among those men with the B2 allele. (Arterioscler Thromb Vasc Biol. 2000;20:1323-1329.)

Key Words: cholesteryl ester transfer protein \( \square \) coronary heart disease \( \square \) lipoproteins \( \square \) gene polymorphisms

Cholesteryl ester transfer protein (CETP) facilitates the exchange of triglycerides and cholesteryl esters between lipoprotein particles. In humans, CETP mRNA encodes a polypeptide of \( M, 53,000 \), which is \( n \)-glycosylated at 4 sites, giving rise to the mature form of CETP of \( M, 74,000 \). CETP is expressed primarily in liver, spleen, and adipose tissue, and lower levels have been detected in the small intestine, adrenal gland, heart, kidney, and skeletal muscle.\(^{1,2}\) The CETP gene encompasses 16 exons, and it has been localized on chromosome 16q21 adjacent to the lecithin-cholesterol acyltransferase gene. Several mutations at the CETP locus have been identified, resulting in the absence of detectable CETP mass and/or activity.\(^{3}\) These mutations are common in Japanese populations,\(^{4-7}\) although some have been recently reported in white subjects.\(^{8,9}\) CETP deficiency is associated with hyperalphalipoproteinemia, which is primarily due to an increase of cholesteryl ester–enriched large-sized HDL. Conversely, the triglyceride-rich lipoproteins and LDL are smaller, reflecting the role of CETP in neutral lipid exchange.\(^{3}\)

Several common restriction fragment length polymorphisms (RFLPs) have been reported in the CETP gene locus.\(^{10-12}\) The most studied RFLP to date has been \( TaqI \), which has been shown to be a silent base change affecting the 277th nucleotide in the first intron of the gene.\(^{10}\) The B2 allele (absence of the \( TaqI \) restriction site) at this polymorphic site has been associated in normolipemic subjects with increased HDL cholesterol (HDL-C) levels and decreased CETP activity and levels,\(^{13-16}\) thus resembling a mild form of CETP deficiency. It has been suggested that this association may be population specific\(^{17,18}\) and highly influenced by environment-
tal factors, such as alcohol consumption and tobacco smoking.\textsuperscript{15,19,20} Moreover, Kuivenhoven et al\textsuperscript{21} have shown an interaction between the \textit{TaqIB} genotype and the progression of coronary heart disease (CHD) after therapy. These observations could be of significant relevance, because low plasma HDL levels are associated with an increase in the risk of coronary artery disease.\textsuperscript{22,23} Moreover, clinical evidence suggests that an increase of 1% in the plasma HDL-C levels is associated with a reduction in cardiovascular morbidity and mortality of 2% to 3%.\textsuperscript{24} Therefore, CETP could have a relevant role in atherogenesis through its effects on HDL metabolism.

The aim of the present study was to determine the frequency, phenotypic expression, and potential modulation of CHD risk in the general population by \textit{TaqIB} RFLP in the first intron of the CETP gene. Specifically, we studied the interindividual variability in lipid levels, lipoprotein subclass profiles, and cardiovascular risk associated with this CETP polymorphism among Framingham Offspring Study participants.

**Methods**

**Subjects**

The details of the design and methods of the Framingham Offspring Study have been presented elsewhere.\textsuperscript{25} Starting in 1971, a total of 5124 subjects were enrolled.\textsuperscript{26} Blood samples for DNA were collected between 1987 and 1991. Lipid phenotypes, DNA, and information on CHD risk factors were available for 1411 men and 1505 women who attended the fourth and fifth examination visits of the Framingham Offspring Study conducted between 1987 and 1995 and for whom lipid values were available in the absence of lipid-altering medication. Nearly all subjects were white. Data on smoking, blood pressure, height, weight, and diabetes were obtained for these subjects as previously described.\textsuperscript{26} CHD included the presence of myocardial infarction, angina pectoris, coronary insufficiency, and coronary death. All suspected CHD events were reviewed by a panel of 3 physicians to ascertain the presence of CHD. Subjects taking a lipid-lowering medication (\(n = 100\)) were included for the analyses of CHD prevalence at examination 5 but excluded in all other analyses.

**Plasma Lipid, Lipoprotein, Apolipoprotein, and CETP Measurements**

Twelve-hour fasting venous blood samples were collected in tubes containing 0.1% EDTA. Plasma was separated from blood cells by centrifugation and immediately used for the measurement of lipids. Plasma total cholesterol, HDL-C, and triglyceride levels were measured as previously described.\textsuperscript{28} HDL-C was measured after precipitation of apoB-containing lipoproteins with dextran sulfate.\textsuperscript{29} LDL cholesterol (LDL-C) concentrations were estimated with the equation of Friedewald et al.\textsuperscript{30} Coefficients of variation for total cholesterol, HDL-C, and triglyceride measurements were each <5%.\textsuperscript{31} Plasma levels of apoA-I and apoB were measured by noncompetitive ELISA with the use of affinity-purified polyclonal antibodies.\textsuperscript{32,33}

Plasma lipoprotein concentrations and subclass distributions were determined by proton nuclear magnetic resonance (NMR) spectroscopy as previously described.\textsuperscript{34,35} Each profile displays the concentration of 6 VLDL, 1 HDL, 3 LDL, and 5 HDL subclasses and the weighted-average particle sizes of VLDL, LDL, and HDL. The 10 lipoprotein subclass categories used were the following: large VLDL and remnants (80 to 220 nm), intermediate VLDL (35 to 80 nm), small VLDL (27 to 35 nm), large LDL (21.3 to 27.0 nm), intermediate LDL (19.8 to 21.2), small LDL (18.3 to 19.7 nm), large HDL (8.8 to 13.0 nm), intermediate HDL (7.8 to 8.8 nm), and small HDL (7.3 to 7.7 nm). Levels of VLDL subclasses are expressed in millimolar units of triglyceride, and those of LDL and HDL subclasses are expressed in millimolar units of cholesterol. LDL and HDL subclass distributions determined by gradient gel electrophoresis and NMR have been shown to be closely correlated.\textsuperscript{36} However, it should be noted that given the characteristics of this methodology, there could be some overlap between the IDL fraction and the small HDL subclass. Nevertheless, this should not have a major effect on the associations examined, given the low concentrations of HDL found in fasting plasma levels of normal subjects.

CETP activity was determined by using a CETP Activity Kit by Roar Biomedical, Inc. This kit includes donor (synthetic phospholipid and cholesteryl ester) and acceptor (VLDL) particles. The fluorescent neutral lipid is present in a self-quenched state when contained within the core of the donor. The CETP-mediated transfer is determined by the increase in fluorescence intensity as the fluorescent neutral lipid is removed from the self-quenched donor to the acceptor. Briefly, for each sample assayed, 10 \(\mu\)L of plasma was diluted (1:10) in 90 \(\mu\)L of sample buffer (10 mmol/L Tris, 150 mmol/L NaCl, and 2 mmol/L EDTA, pH 7.4). In a fluorescence-compatible microtiter plate (Dynex Laboratories), 20 \(\mu\)L of the plasma dilution was combined with 4 \(\mu\)L of donor and 4 \(\mu\)L of acceptor in a total volume of 200 \(\mu\)L and incubated for 3 hours at 37°C. The assay was read in a fluorescence spectrometer at excitation wavelength of 465 nm and emission wavelength of 535 nm. A standard curve was used, according to the manufacturer’s guidelines, to derive the relation between fluorescence intensity and mass transfer. Plasma controls were run in each plate to account for plate-to-plate variation. For standardization, the unquenched fluorescence intensity of the fluorescent cholesteryl ester contained within the donor particle core was determined by dispersing 5 \(\mu\)L of donor (fluorescent cholesteryl ester concentration 146 \(\mu\)g/mL, as reported by the manufacturer) in 2 mL of 100% isopropanol. Serial dilutions of the dispersion were made to generate a standard curve of fluorescence intensity (excitation 465 nm/emission 535 nm) versus mass of fluorescent cholesteryl ester. The fluorescence intensity transferred in the assay of plasma samples was applied to the standard curve to determine mass transfer. The intra-assay and interassay coefficients of variation were <5%.

**DNA Analysis**

Genomic DNA was isolated from peripheral blood leukocytes by standard methods.\textsuperscript{36} CETP genotype was performed as described by Fumeron et al.\textsuperscript{19} A fragment of 535 bp in intron 1 of the CETP gene was amplified by polymerase chain reaction (PCR) in a DNA Thermal Cycler (PTC-100, MJ Research, Inc) with the use of oligonucleotide primers (forward 5’-CAGTACGGCAGAGAGGAGAGTGC-3’, reverse 5’-CTGAGCCCATGCACTTAC-3’). Each amplification was performed by using 100 ng of genomic DNA in a volume of 50 \(\mu\)L containing 40 pmol of each oligonucleotide, 0.2 mmol/L dNTPs, 1.5 mmol/L MgCl\textsubscript{2}, 10 mmol/L Tris, pH 8.4, and 0.25 U of Taq polymerase. DNA templates were visualized by UV illumination. The resulting fragments were 174 and 361 bp for the B1 allele and 535 bp for the uncut B2 allele. Restriction enzyme digestion of the amplified DNA was carried out as previously described.\textsuperscript{37}

**Statistical Analyses**

To compare men and women who participated in the present study, we used \(\chi^2\) tests for categorical measures and 2-sample \(t\) tests for continuous measures. We estimated the allele frequency of the B2 allele and apoE alleles with a chromosome-counting method and used a \(\chi^2\) test to compare the frequency in men and women. To evaluate the relation between the CETP genotypes and lipid levels, we used ANCOVA techniques, which accounted for the familial relations among the members of the study (mostly siblings and
cousins). We used 2 approaches to accomplish these analyses. First, we used a repeated-measures approach, which assumed an exchangeable correlation structure among all members of a pedigree (PROC MIXED, SAS). Second, because this approach does not accurately represent the true correlation structure within these pedigrees, we used a measured-genotype approach, as implemented in SOLAR, a variance component analysis computer package for quantitative traits measured in pedigrees of arbitrary size. The latter approach fully accounts for the different types of relations within a pedigree in performing an ANOVA on the defined genotypes. In these analyses, we used several different models to adjust for potential confounders. First, we obtained essentially crude results, which accounted only for the family structure; second, we adjusted for age, body mass index (BMI), smoking, alcohol consumption, β-blockers, and (in women) menopausal status and hormonal replacement therapy. In our final analysis, we added apoE genotypes to the model with E2/E2 and E2/E3 in one group, E3/E4 and E4/E4 in another group, and E3/E3 as the reference group. Subjects with E2/E4 genotypes, of which there were very few, were excluded.

A sensitivity analysis was carried out to estimate the validity and precision of the regression coefficients for the CETP genotypic variables when additional independent terms were included into the model. Because similar results were obtained for both sexes, data from men and women were analyzed together to improve statistical power. Regression coefficients and 95% CIs for B1B2 and B2B2 compared with B1B1 genotypes were calculated by fitting several linear regression models with dummy variables for categorical and interaction terms as follows: model 1, CETP genotype (B1B1, B1B2, and B2B2); model 2, model 1 + sex; model 3, model 2 + BMI; model 4, model 3 + tobacco smoking (nonsmoker and smoker); model 5, model 4 + alcohol consumption (consumption and no consumption); and model 6, model 5 + apoE genotypes (E2, E3, and E4). In all cases, the first category was taken as a reference. Regression diagnostics were used to check the assumptions and to assess the accuracy of computations.

Finally, using a χ² analysis, we estimated the odds of prevalent CHD at examination 5 for those with the B1B2 or B2B2 genotypes relative to those with the B1B1 genotype. CHD includes myocardial infarction, angina pectoris, and coronary insufficiency. To adjust this estimated odds ratio for covariates, we used logistic regression. We also applied generalized estimating equations with a logit link to account for the correlation among the observations and obtained essentially the same results. Hence, we report the results assuming independent observations.

Results

Subject Characteristics
To investigate the frequency and phenotypic association of the TaqIB-CETP polymorphism at the population level, we analyzed a total of 2876 subjects (1411 males and 1505 females) who participated in the Framingham Offspring Study and for whom lipid values were available in the absence of lipid-altering medication. Table 1 provides a summary of the demographic, genotypic, and biochemical characteristics of the participants according to sex. The mean age of men and women at examination was 51.6 and 51.2 years, respectively. Although a similar proportion of men and women were smokers (23.4% and 22.8%, respectively), male subjects smoked more cigarettes per day (5.8±12.5) than did the female subjects (4.7±10.3, P<0.001), and over half of the female participants (54.2%) were postmenopausal. There was no significant difference in the frequency of the B2 allele between men and women, and the distribution of alleles was consistent with Hardy-Weinberg equilibrium. Alcohol consumption, BMI, plasma LDL-C, total apoB, triglyceride, and glucose levels were significantly higher in men than in women, and total HDL-C, HDL₂-C, and HDL₃-C concentrations were significantly higher in female participants. The apoE genotypic distribution was similar in men and women (P=0.398).

Association of TaqIB Polymorphism With Variations in Plasma Levels of Lipids, Lipoproteins, Apolipoproteins, and CETP Activity
Table 2 shows that in men and women, the 3 genotype groups were equivalent with respect to age and BMI. Male homozygotes for the B1 allele had lower HDL-C levels (1.07±0.27 mmol/L) than did B1B2 (1.14±0.28 mmol/L) and B2B2 (1.18±0.34 mmol/L) male subjects (P<0.001). Likewise, female homozygotes for the B1 allele had lower HDL-C levels (1.40±0.38 mmol/L) than did B1B2 (1.46±0.39 mmol/L) and B2B2 (1.53±0.40 mmol/L) female subjects (P<0.001). Similar associations were noted for apoA-I values. The higher HDL-C levels associated with the B2 allele were due to increases in HDL₂-C and HDL₃-C subfractions. A significant association was noted between the TaqIB genotype and CETP activity. Male and female carriers of the B2 allele had significantly lower CETP activity than did those homozygotes for the B1 allele. In both sexes, there were no statistically significant differences among the geno-
type groups in the plasma levels of total cholesterol, LDL-C, and apoB. These results were confirmed by the variance component approach and revealed that TaqIB accounts for $\approx 1\%$ of the variability in HDL-C.

To test the consistency of the association between the CETP-TaqIB genotype and HDL-C levels, a sensitivity linear regression analysis was carried out as described in Methods. The Figure shows regression coefficients and 95% CIs for B1B2 and B2B2 compared with B1B1 genotypes when each indicated variable was included into the linear regression models (models 1 to 6). First, the only variables included were dummies for the TaqIB genotype (model 1). This genetic factor accounted for $1\%$ of the variability of HDL-C ($P<0.001$). The initial regression coefficients for B1B2 and B2B2 after controlling for the effect of sex (model 2) were 0.06 (95% CI 0.03 to 0.09) mmol/L ($P<0.001$) and 0.14 (95% CI 0.09 to 0.18) mmol/L ($P<0.001$), respectively. When other variables were progressively added to the core

### Table 2: Plasma Levels of Lipids, Lipoproteins, and Apolipoproteins of Framingham Offspring Study Subjects According to TaqIB-CETP Genotypes

<table>
<thead>
<tr>
<th>Men</th>
<th>B1B1</th>
<th>B1B2</th>
<th>B2B2</th>
<th>$P^*$</th>
<th>$P^†$</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>428</td>
<td>713</td>
<td>270</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>51.2±10.3</td>
<td>52±10.0</td>
<td>51.3±10.1</td>
<td>0.313</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.9±4.0</td>
<td>27.50±3.80</td>
<td>27.6±3.8</td>
<td>0.169</td>
<td></td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>5.28±0.93</td>
<td>5.25±0.96</td>
<td>5.22±0.96</td>
<td>0.639</td>
<td>0.889</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>3.49±0.83</td>
<td>3.47±0.88</td>
<td>3.41±0.85</td>
<td>0.288</td>
<td>0.363</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.07±0.27</td>
<td>1.14±0.28‡</td>
<td>1.18±0.34§</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL₃-C, mmol/L</td>
<td>0.12±0.09</td>
<td>0.14±0.10</td>
<td>0.15±0.11§</td>
<td>&lt;0.001</td>
<td>0.033</td>
</tr>
<tr>
<td>HDL₄-C, mmol/L</td>
<td>0.95±0.21</td>
<td>1.00±0.22‡</td>
<td>1.03±0.26§</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.63±1.16</td>
<td>1.52±1.14</td>
<td>1.45±0.95</td>
<td>0.059</td>
<td>0.098</td>
</tr>
<tr>
<td>ApoA-I, g/L</td>
<td>1.32±0.25</td>
<td>1.35±0.23</td>
<td>1.37±0.24§</td>
<td>0.017</td>
<td>0.025</td>
</tr>
<tr>
<td>ApoB, g/L</td>
<td>1.03±0.25</td>
<td>1.02±0.24</td>
<td>1.00±0.25</td>
<td>0.135</td>
<td>0.662</td>
</tr>
<tr>
<td>HDL-C/ApoA-I</td>
<td>0.81±0.14</td>
<td>0.84±0.13</td>
<td>0.86±0.13</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TC/HDL ratio</td>
<td>5.3±1.5</td>
<td>4.9±1.5‡</td>
<td>4.8±1.6§</td>
<td>&lt;0.001</td>
<td>0.011</td>
</tr>
<tr>
<td>CETP, nmol·L⁻¹·h⁻¹</td>
<td>160±10.0</td>
<td>156±10.0</td>
<td>139±9.0</td>
<td>0.026</td>
<td>0.045</td>
</tr>
<tr>
<td>VLDL size, nm</td>
<td>49.12±10.24</td>
<td>48.52±9.23</td>
<td>47.34±8.58</td>
<td>0.054</td>
<td>0.649</td>
</tr>
<tr>
<td>LDL size, nm</td>
<td>20.56±0.60</td>
<td>20.69±0.58‡</td>
<td>20.80±0.53§</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL size, nm</td>
<td>8.83±0.37</td>
<td>8.92±0.40‡</td>
<td>8.98±0.45§</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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</tbody>
</table>

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>n</td>
<td>477</td>
<td>754</td>
<td>274</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>51.2±9.7</td>
<td>50.8±9.41</td>
<td>51.3±10.1</td>
<td>0.413</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.6±5.4</td>
<td>25.8±5.12</td>
<td>26.5±5.5</td>
<td>0.081</td>
<td></td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>5.28±0.98</td>
<td>5.30±1.03</td>
<td>5.33±1.03</td>
<td>0.901</td>
<td>0.794</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>3.34±0.93</td>
<td>3.28±0.91</td>
<td>3.23±0.98</td>
<td>0.297</td>
<td>0.383</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.40±0.38</td>
<td>1.46±0.39‡</td>
<td>1.53±0.40§</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL₃-C, mmol/L</td>
<td>0.24±0.15</td>
<td>0.26±0.14</td>
<td>0.28±0.17§</td>
<td>0.008</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL₄-C, mmol/L</td>
<td>1.16±0.28</td>
<td>1.20±0.29</td>
<td>1.25±0.29§</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.21±0.86</td>
<td>1.24±1.38</td>
<td>1.23±0.84</td>
<td>0.834</td>
<td>0.646</td>
</tr>
<tr>
<td>ApoA-I, g/L</td>
<td>1.52±0.28</td>
<td>1.55±0.32</td>
<td>1.57±0.32</td>
<td>0.040</td>
<td>0.097</td>
</tr>
<tr>
<td>ApoB, g/L</td>
<td>0.95±0.24</td>
<td>0.94±0.27</td>
<td>0.95±0.28</td>
<td>0.775</td>
<td>0.648</td>
</tr>
<tr>
<td>HDL-C/ApoA-I</td>
<td>0.92±0.15</td>
<td>0.94±0.16</td>
<td>0.97±0.15</td>
<td>0.003</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TC/HDL ratio</td>
<td>4.0±1.5</td>
<td>3.9±1.50</td>
<td>3.7±1.30§</td>
<td>0.006</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CETP, nmol·L⁻¹·h⁻¹</td>
<td>178±11.0</td>
<td>159±10.0‡</td>
<td>148±11.00§</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VLDL size, nm</td>
<td>43.99±8.59</td>
<td>44.11±8.40</td>
<td>45.81±8.89§</td>
<td>0.019</td>
<td>0.129</td>
</tr>
<tr>
<td>LDL size, nm</td>
<td>21.05±0.52</td>
<td>21.07±0.46</td>
<td>21.09±0.41</td>
<td>0.547</td>
<td>0.194</td>
</tr>
<tr>
<td>HDL size, nm</td>
<td>9.35±0.45</td>
<td>9.40±0.43‡</td>
<td>9.44±0.46§</td>
<td>0.027</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are mean±SD.

* After adjustment for the familial relations.

† After adjustment for the familial relations, age, BMI, smoking, alcohol intake, use of β-blockers, menopausal status and estrogen therapy (in women), and ApoE.

Shown at significant differences between the B1B1 and B1B2 (‡), B1B1 and B2B2 (§), and B1B2 and B2B2 (†) groups after adjustment for the familial relationships, age, BMI, smoking, alcohol intake, use of β-blockers, menopausal status and estrogen therapy (in women), and ApoE genotype.
CETP-TaqIB Genotype and Risk of CHD

To examine the associations of the TaqIB polymorphism with CHD risk, we also included subjects on lipid-lowering medications; CHD was present in 163 men and 62 women. When we examined CHD prevalence in men at examination 5 versus the absence (B1B1) or presence of the B2 allele (B1B2 or B2B2) by χ² analysis, we demonstrated a significantly (P = 0.035) lower frequency of carriers of the B2 allele (58.7% versus 70.6%) among those subjects with positive CHD. Likewise, the odds ratio for CHD associated with the presence of the B2 allele was 0.696 (95% CI 0.50 to 0.98, P = 0.035). After adjusting for age, BMI, systolic blood pressure, diabetes, smoking, and alcohol consumption, this odds ratio remained at 0.700 (95% CI 0.46 to 1.05), but the statistical significance dropped to P = 0.090. After additional adjustment for the previous factors plus β-blocker use, cholesterol-lowering drugs, total cholesterol, and HDL-C, the odds ratio was 0.735 (95% CI 0.46 to 1.162, P = 0.188). These odds ratios were similar after excluding those subjects on lipid-lowering medications (data not shown). In women, we did not find any significant association between the presence of the B2 allele and CHD risk by χ² analysis (75.8% versus 67.9%, P = NS) or by logistic analysis (data not shown). However, there were too few CHD cases to draw definitive conclusions about the association between TaqIB polymorphism and CHD risk in women.

Discussion

In the present study, we found an association of CETP-TaqIB polymorphism with HDL-C levels and apoA-I in men and with HDL-C in women. No statistically significant differences in other lipid levels were observed across TaqIB genotypes after controlling for familial relations, age, BMI, smoking, alcohol intake, use of β-blockers, apoE genotype, and (in women) menopausal status and estrogen therapy. Moreover, we found that the B2 allele was significantly associated with an ~30% lower risk of CHD, which was no longer statistically significant after adjustment for multiple CHD risk factors, including HDL-C levels, indicating that a proportion of this effect may be due to the increased HDL-C levels associated with the presence of the B2 allele. This association with HDL-C has been previously reported in several other studies.13,16,18–21,40,41 Some of them also found significant associations with LDL-C or triglyceride levels.13,21,42 In agreement with some previous studies,16,20,21,43 we have found a significant association between the CETP-
TaqI B polymorphism and CETP activity, with the B1 allele being associated with increased activity compared with the B2 allele. However, a lack of significant association between CETP activity and TaqI B polymorphism has also been reported by other investigators.14 The mechanism by which TaqIB polymorphism may affect CETP activity or HDL-C levels is not known. It is unlikely that this polymorphism located in an intron represents a functional mutation. Given the reported associations of the B2 allele with increased CETP mass and/or activity, the most plausible explanation is that this polymorphism is in linkage disequilibrium with a still unknown functional mutation in the regulatory region of the CETP gene.

The role of CETP in atherogenesis is still under debate.43,44 CETP may play a proatherogenic role, in view of the fact that it mediates a redistribution of plasma cholesterol from lipoproteins, which is associated with a protection against atherosclerosis, into the proatherogenic apoB-containing lipoproteins. This concept is also supported by the fact that animal species that are resistant to diet-induced atherosclerosis have little CETP activity. However, CETP mediates 1 of the steps in reverse cholesterol transport, an antiatherogenic process. The results of the present study support the concept that increased HDL-C levels resulting from lower CETP activity appear to be associated with a lower risk of CHD in male subjects.

Human and transgenic mouse experiments have shown that environmental factors play an important role in the modulation of CETP gene expression.45 Various studies in human populations have analyzed the possible interaction between some environmental factors and the CETP-TaqIB polymorphism on plasma HDL-C levels. In this regard, Kondo et al13 showed that the association of the CETP gene with HDL-C levels was present only in nonsmokers. In another study carried out in Finland,20 male smokers with the B2 allele tended to have HDL-C levels 10% lower than those levels of male smokers with the B1 allele, but this effect was not observed in women; thus, the authors also concluded that allele effects differed according to sex. Fumeron et al19 did not find an interaction with tobacco smoking, but they found an important interaction with alcohol consumption in the Etude Cas-Temoins de l’Infarctus du Myocarde (ECTIM) study. In the present study, when genetic-environmental variables were tested, no statistically significant interactions of the TaqIB genotypes with alcohol consumption or tobacco smoking were found. This was also true in terms of apoE-CETP interactions. These observations allow us to conclude that the effect of this polymorphism on plasma HDL-C in this cohort seems statistically independent and uniform across several levels of these environmental factors, as well as across the different apoE alleles. The study of these gene-environment and gene-environmental interactions can provide an important basis for refining the predictive value of traditional epidemiological risk factors and for targeting intervention and prevention activities for high-risk individuals.

In addition to the reported associations between classical risk factors and TaqIB polymorphism, our results show that this genetic variant is significantly associated with differences in the distribution of lipoprotein subclasses as determined by NMR. The B2 allele was significantly associated in men and women with higher levels of the large, more antiatherogenic HDL subfraction.46–48 This effect is consistent with the fact that the B2 allele is associated with lower CETP activity and results in an increase of cholesteryl ester–enriched large-sized HDL, such as those found in CETP-deficient subjects. The findings from the NMR data are also supported by the increase in the HDL-C/apoA-I ratio that we found associated with the presence of the TaqIB2 allele. In addition to this effect, which was observed in men and women, we found significant effects for LDL subfraction distribution in men only. The B2 allele was associated with a less atherogenic LDL particle size distribution, consisting of decreased levels of the more atherogenic small LDL subfraction and increased levels of the less atherogenic large LDL.46–49,50 These effects were not significant in women. Therefore, the protective effects associated with the B2 allele in men may not only be due to quantitative changes in lipid profiles but may also be due to qualitative changes in particle composition, which are manifested as differences in size distribution. It is interesting to highlight that the B2 allele was not protective in women despite its association with higher HDL-C levels and increased HDL size. The most plausible explanation for this lack of effect could be the small number of CHD events in this group of women. However, one could speculate that the sex differences observed for the association between the B2 allele and CHD risk could be due to the fact that in men the B2 allele was associated with less atherogenic HDL and LDL subclass distributions, whereas in women no effect was observed in the LDL subfraction. These observations point out the relevant role of LDL subclass distribution as a predictor of CHD risk.

In summary, the present study, which was carried out in a large population-based white cohort, supports the hypothesis that the CETP gene locus, as examined with TaqIB polymorphism, plays a significant role in determining HDL-C variability in men and women and apoA-I levels and LDL size in men and accounts for ≈1% of the variability in HDL-C. These associations translate into a less atherogenic lipid profile in both sexes and a lower CHD risk in men. Further studies need to be carried out to demonstrate whether the lower CHD risk associated with this polymorphism is also found in women.

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Association of Cholesteryl Ester Transfer Protein–TaqIB Polymorphism With Variations in Lipoprotein Subclasses and Coronary Heart Disease Risk: The Framingham Study
Jose M. Ordovas, L. Adrienne Cupples, Dolores Corella, James D. Otvos, Doreen Osgood, Antonia Martinez, Carlos Lahoz, Oscar ColteI, Peter W. F. Wilson and Ernst J. Schaefer

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