Cholesteryl Ester Transfer Protein TaqI B2B2 Genotype Is Associated With Higher HDL Cholesterol Levels and Lower Risk of Coronary Heart Disease End Points in Men With HDL Deficiency

Veterans Affairs HDL Cholesterol Intervention Trial

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Objective—We have previously reported that genetic variation at the cholesteryl ester transfer protein (CETP) TaqIB locus is correlated with plasma lipid levels and coronary heart disease (CHD) risk in the Framingham Offspring Study (FOS). In FOS, the B2 allele was associated with increased levels of high density lipoprotein (HDL) cholesterol (HDL-C), decreased CETP activity, and reduced CHD risk for men having the B2B2 genotype. The present study was undertaken to further define the relationship between this polymorphism and CHD risk at the population level.

Methods and Results—We tested for associations between the CETP TaqIB genotype and plasma lipoprotein levels, response to gemfibrozil therapy, and CHD end points in 852 men participating in the Veterans Affairs HDL-C Intervention Trial (VA-HIT), a study designed to explore the potential benefits of raising HDL levels in men having established CHD with low HDL-C (≤40 mg/dL) as their primary lipid abnormality. In VA-HIT, 13.9% of the men had the B2B2 genotype relative to 19.1% of the men in FOS (227%, P<0.03), whereas more men in VA-HIT had the B1B1 genotype (15%, P<0.05). Similar to our finding in FOS, B2B2 men in VA-HIT had the highest mean level of HDL-C (32.6±4.8 mg/dL), followed by B1B2 men (32.0±5.3 mg/dL), and, last, by B1B1 men (30.9±4.9 mg/dL). Interestingly, B1B1 men, who had the least favorable plasma lipid profile at baseline, had the greatest triglyceride-lowering response to gemfibrozil (−34%, P=0.006). CETP TaqIB genotype was also associated with the risk of CHD end points in VA-HIT, with an adjusted risk ratio of 0.52 for B2B2 men (P=0.08).

Conclusions—Our data demonstrate that in men with CHD and HDL deficiency, the CETP TaqI B2B2 genotype is (1) significantly reduced and (2) associated with higher levels of plasma HDL-C and lower CHD risk. Together with our earlier report, these results support the concept that increased HDL-C levels, resulting from reduced CETP activity, are associated with decreased CHD risk. (Arterioscler Thromb Vasc Biol. 2002;22:lll–lll.)

Key Words: cholesteryl ester transfer protein ■ coronary heart disease ■ high density lipoproteins ■ polymorphism ■ Veterans Affairs HDL-C Intervention Trial

Numerous population studies have shown that a strong inverse relationship exists between plasma HDL cholesterol (HDL-C) levels and coronary heart disease (CHD) risk.1–4 It has long been theorized that the atheroprotective effect of HDL is primarily due to its role in reverse cholesterol transport (RCT), the process by which HDL mediates the transport of excess cholesterol from peripheral cells back to the liver for excretion into the bile.5 RCT is a complex pathway, involving transport proteins, modifying enzymes, and cell surface receptors. One of the enzymes with a key role in RCT is cholesteryl ester transfer protein (CETP), which promotes the exchange of cholesteryl esters from HDL to the apoB-containing lipoproteins, thus, providing an avenue for the uptake of cholesteryl esters by hepatic receptors.6

The CETP gene, located on chromosome 16q21, consists of 16 exons and spans a region of ≈25 kb.7,8 Several rare mutations that result in the absence of detectable CETP mass and/or activity have been reported at the CETP gene locus.
Although the majority of these mutations have been identified in subjects of Japanese ancestry,\textsuperscript{9–12} they have also been found in subjects of German\textsuperscript{13} and North American\textsuperscript{14,15} descent. In humans, CETP deficiency is characterized by the presence of increased concentrations of large, cholesteryl ester–enriched HDL particles in the plasma and, often, reduced concentrations of LDL cholesterol (LDL-C).\textsuperscript{16} The former is the result of delayed catabolism of cholesteryl ester–enriched HDL particles,\textsuperscript{17} whereas the latter is due to accelerated catabolism of triglyceride–enriched LDL particles.\textsuperscript{18}

In addition to rare mutations, several common mutations, or polymorphisms, have been identified in the CETP gene. Polymorphisms identified in the coding sequence of the CETP gene include Ala373→Pro,\textsuperscript{13} Ile405→Val,\textsuperscript{8} and Arg451→Gln.\textsuperscript{13,19} Two of these variants, Ala373→Pro and Arg451→Gln, are associated with increased CETP activity and reduced HDL-C levels,\textsuperscript{20} whereas the Ile405→Val variant is associated with reduced CETP mass and increased HDL-C levels.\textsuperscript{21,22} Among the most widely studied CETP variants is TaqIB, a silent base change affecting the 277th nucleotide in the first intron of the CETP gene.\textsuperscript{23} In normolipidemic subjects, absence of the TaqIB restriction site (B2 allele) is associated with decreased CETP activity and, in turn, increased HDL-C levels,\textsuperscript{24–26} resembling a mild form of CETP deficiency. Finally, a promoter polymorphism, CETP –629 A/C, has been described by Dachet et al.\textsuperscript{27} This polymorphism is tightly linked to the CETP TaqIB variant,\textsuperscript{27} providing a potential explanation for the observed associations between the former variant and plasma CETP activity and lipid levels. Alternatively, it has also been suggested that the effect of the CETP TaqIB variant may be due to another functional polymorphism in the CETP gene yet to be discovered.\textsuperscript{28}

The role of CETP deficiency in atherosclerosis remains controversial. Although the complete absence of CETP has been associated with an increased lifespan in patients with homozygous CETP deficiency,\textsuperscript{9} others have observed increased atherosclerosis susceptibility in heterozygotes for this disease.\textsuperscript{29} The results of epidemiological studies have, likewise, been equivocal, with some studies suggesting that the association of the CETP TaqIB polymorphism with plasma HDL-C concentrations may be population specific\textsuperscript{30,31} and highly influenced by environmental factors, such as smoking\textsuperscript{25} and alcohol consumption.\textsuperscript{26,26} Recently, Kuivenhoven et al\textsuperscript{32} reported that a significant interaction exists between CETP TaqIB genotype and the progression of CHD in men from the Regression Growth Evaluation Statin Study (REGRESS).\textsuperscript{33}

The present study represents an extension of our earlier work with the Framingham Offspring Study (FOS). In FOS males, the B2B2 genotype was associated with decreased CETP activity, increased concentrations of HDL-C, and decreased CHD risk.\textsuperscript{34} To further explore the role of the CETP TaqIB variant in CHD risk, we examined its associations with plasma lipoprotein levels, response to gemfibrozil therapy, and CHD end points in 852 men participating in the Veterans Affairs HDL-C Intervention Trial (VA-HIT), a study designed to examine the potential benefits of raising HDL levels in men having established CHD with low HDL-C (≤40 mg/dL) as their primary lipid abnormality. Our data demonstrate that the CETP TaqIB variant is associated with plasma HDL-C levels and lipoprotein subclass distribution in men with established CHD and HDL deficiency, with carriers of the B2 allele having a lipoprotein profile more favorable than that of noncarriers. Men with the B2B2 genotype also had fewer CHD end points than did noncarriers, suggesting that the relatively favorable lipoprotein profile observed in these men was atheroprotective.

**Methods**

**Subjects**

The rationale, design, and methods for VA-HIT have been described elsewhere in detail.\textsuperscript{35} Briefly, men were recruited at 20 Veterans Affairs medical centers throughout the United States. Eligibility for the trial required a documented history of CHD, an age of <74 years, an absence of coexisting conditions, an HDL-C level of ≤40 mg/dL (1.0 mmol/L), an LDL-C level of ≤140 mg/dL (3.6 mmol/L), and a plasma triglyceride concentration of ≤300 mg/dL (3.4 mmol/L). Information on age, alcohol consumption, smoking status, blood pressure, body mass index, and diabetes was available for all subjects enrolled in VA-HIT. However, informed consent for DNA analysis was obtained from only some of the subjects (n=1014); thus, only these samples could be used in our genotyping analysis. Ninety-three percent of these subjects were white, with no differences noted in the race distribution of subjects within each genotype. Data used in our statistical analyses were obtained at baseline, with the exception of those data used to examine the relationships between genotype and the plasma lipid response to gemfibrozil (1200 mg/d), which were obtained at month 7.

**Measurement of Plasma Lipid, Lipoprotein, and Apolipoprotein Levels**

Blood samples were collected from subjects, after a 12- to 14-hour fast, into tubes containing 0.1% EDTA. Plasma was isolated and frozen for subsequent analysis of plasma lipid, lipoprotein, and apolipoprotein concentrations. Plasma total cholesterol (TC) and triglyceride concentrations were determined by using enzymatic assays.\textsuperscript{36} Plasma HDL-C concentrations were measured after dextran sulfate–magnesium precipitation of apoB-containing lipoproteins,\textsuperscript{37} and HDL subclass separations were performed by differential polyanion precipitation.\textsuperscript{38} LDL-C levels were calculated with the equation of Friedewald et al.\textsuperscript{39} ApoA-I and apoB levels in the plasma were measured with an immunoturbidimetric assay with the use of reagents and calibrators from Incstar Corp.\textsuperscript{40,41}

**Analysis of LDL and HDL Subclass Concentrations and Particle Size by NMR**

The distributions of LDL and HDL subclasses and particle size were determined by proton nuclear magnetic resonance (NMR) spectroscopy, as previously described.\textsuperscript{42,43} The concentrations of 3 LDL (L3, L2, and L1) and 5 HDL (H5, H4, H3, H2, and H1) subclasses, listed from largest to smallest with respect to particle size, are provided by this methodology. L3 is classified as large LDL (21.3 to 27.0 nm); L2, as intermediate LDL (19.8 to 21.2); and L1, as small LDL (18.3 to 19.7 nm). For HDL, H5 and H4 are categorized as large (8.8 to 13.0 nm); H3 and H2, as intermediate (7.8 to 8.8 nm); and H1, as small (7.3 to 7.7 nm). Concentrations of LDL and HDL subclasses are expressed in units of cholesterol (milligrams per deciliter). LDL and HDL subclass distributions determined by NMR have been shown to be closely correlated with those obtained by gradient gel electrophoresis.\textsuperscript{42}
DNA Analysis

Genomic DNA was extracted from whole blood samples by using either QIAamp mini kits (Qiagen) or Generation Capture Column kits (Gentra Systems). CETP genotyping was performed as previously described.32,34 Briefly, a 535-bp fragment in intron 1 of the CETP gene was amplified by polymerase chain reaction, with use of the following oligonucleotide primers: F-5′-CACTAGCCCAGAGAGAGGAGTGCC-3′ and R-5′-CTGAGCCCAGCCGCACACTAAC-3′. DNA templates were denatured at 95°C for 3 minutes, followed by 30 cycles of 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 45 seconds, and last, by 1 cycle at 72°C for 5 minutes. Polymerase chain reaction products (16 µL) were digested with TaqI restriction endonuclease (GIBCO-BRL) at 65°C for 2 hours, and the fragments were separated by electrophoresis in a 1.5% agarose gel. The resulting DNA fragments were 174 and 361 bp for the B1 allele and 535 bp for the undigested B2 allele.

Statistical Analyses

To evaluate the relationships between CETP TaqIB genotype and plasma lipids, lipoproteins, and apolipoproteins, we used ANCOVA techniques, adjusting for the potential confounders of age, smoking status, hypertension, and diabetes. For categorical measures, χ² tests were used, and either 2-sample t tests or Wilcoxon rank sum tests were used for continuous measures. SAS version 8 was used for all analyses. Analyses of time-to-event data were performed, and relative risks were calculated by using Cox proportional hazards models, with CETP TaqIB genotype as the explanatory variable and adjustments made for the aforementioned confounders as well as treatment group.

Results

Subject Characteristics

To further investigate the frequency distribution and phenotypic effects of the CETP TaqIB polymorphism, we analyzed a total of 852 men in VA-HIT who had consented to DNA analysis. The demographic, biochemical, and genotypic characteristics of these subjects are presented in Table 1. This group of men had a mean age of 64 years and a mean body mass index of 29 kg/m², with normal levels of TC, LDL-C, and triglycerides. Because of the selection criteria of VA-HIT, the mean level of HDL-C in these men was low, at 32±6 mg/dL. The substantial reduction in HDL-C concentration resulted in a mean ratio of TC to HDL-C of 5.8, a value that is significantly higher than that defined as favorable.44 DNA analysis revealed that 34.7% of these men were homozygous carriers of the B1 allele, whereas 51.3% were heterozygous carriers. Only 13.9% of men in VA-HIT were homozygous carriers of the B2 allele. This genotype distribution was significantly different relative to that previously reported by us for normolipidemic CHD-free men from FOS.34 Relative to FOS, significantly more men in VA-HIT were homozygous carriers of the B1 allele (15%, P<0.05), whereas significantly fewer were homozygous carriers of the B2 allele (27%, P<0.03). These differences resulted in a significantly higher B1 allele frequency (0.604) and a significantly lower B2 allele frequency (0.396) in men from VA-HIT versus men from FOS (0.556 and 0.444 for B1 and B2, respectively). The fact that the B2 allele was significantly reduced in this population of men having established CHD and HDL deficiency supports the concept that this polymorphism contributes to variation in HDL-C levels and, possibly, CHD risk at the population level.

| TABLE 1. Demographic, Biochemical, and Genotypic Characteristics of VA-HIT Subjects |
|-----------------------------|-----------------------------|
|                             | Value                       |
| Age, y                      | 64±7                        |
| Body mass index, kg/m²      | 29.3±4.6                    |
| TC, mg/dL                   | 179±25                      |
| LDL-C, mg/dL                | 115±22                      |
| HDL-C, mg/dL                | 32±5                        |
| HDL₁-C, mg/dL               | 5±2                         |
| HDL₂-C, mg/dL               | 27±5                        |
| TC:HDL-C                    | 5.8±1.1                     |
| Triglycerides, mg/dL        | 163±67                      |
| ApoA-I, mg/dL               | 107±17                      |
| ApoB, mg/dL                 | 97±20                       |
| Race                        |                             |
| White, %                    | 93                          |
| Non-White,* %               | 7                           |
| CETP TaqIB genotype, %      |                             |
| B1B1                        | 34.7                        |
| B1B2                        | 51.3                        |
| B2B2                        | 13.9                        |
| B1 allele frequency         | 0.604                       |
| B2 allele frequency         | 0.396                       |

*Non-White includes the categories of Black and Other.

Data are mean±SD or percent for 852 men from VA-HIT. To convert values for cholesterol and triglycerides to millimoles per liter, multiply by 0.02586 and 0.01129, respectively.
Association of CETP TaqIB Genotype With Fasting Plasma Lipid and Apolipoprotein Concentrations and Particle Size

Table 2 shows the associations between CETP TaqIB genotype and plasma lipid and apolipoprotein concentrations in men from VA-HIT. Despite being a relatively homogeneous population regarding plasma lipid concentrations, plasma total HDL-C levels differed significantly among the genotypes in VA-HIT. Specifically, a dosage effect of the B2 allele on HDL-C levels was observed such that men with the B1B1 genotype had the lowest mean level of HDL-C (30.9 ± 4.9 mg/dL) in the plasma, followed by men with the B1B2 genotype (32.0 ± 5.3 mg/dL), and last, by men with the B2B2 genotype (32.6 ± 4.8 mg/dL). This allele dosage effect on HDL-C levels was similar to that previously observed by us in the FOS population, in which men with the B1B1 genotype had the lowest mean HDL-C value (41.3 ± 10.4 mg/dL), men with the B1B2 genotype had an intermediate value (44.0 ± 10.8 mg/dL), and men with the B2B2 genotype had the highest value (45.6 ± 13.1 mg/dL).

In VA-HIT, the relatively higher total HDL-C levels noted in carriers of the B2 allele were due to increases in the HDL2 and HDL3 subfractions. The mean level of HDL-C observed for B2 carriers was significantly higher than that for noncarriers (3.4%, \( P = 0.02 \)). Although not statistically significant, a similar trend was noted in plasma HDL-C levels, with B1B2 (5.9%) and B2B2 (7.8%) men having higher mean levels of HDL-C compared with levels in B1B1 men. In turn, the significant differences observed in plasma HDL-C levels among the genotypes led to differences in the ratio of TC to HDL-C. The TC/HDL-C value for the B1B1 group was significantly elevated (\( P = 0.01 \)) relative to values observed for the B1B2 and B2B2 groups. No other associations were detected between CETP TaqIB genotype and plasma lipids or apolipoproteins in this population of men.

Association of CETP TaqIB Genotype With LDL and HDL Subclass Concentrations and Particle Size

To better characterize the relationship between CETP TaqIB genotype and plasma lipoprotein levels, we determined LDL and HDL subclass concentrations, as well as particle size, in 378 men from VA-HIT with the use of automated NMR spectroscopy. The concentrations of 3 LDL (L3, L2, and L1) and 5 HDL (H5, H4, H3, H2, and H1) subclasses, listed from largest to smallest with respect to particle size, are provided by this methodology. As shown in Table 3, no statistically significant differences were observed in LDL subclass distribution or particle size in VA-HIT. However, a trend was noted between the presence of the B2 allele and increased cholesterol (13%) in the large LDL3 fraction in VA-HIT. Conversely, the B2 allele was associated with reduced cholesterol content (−12%) in the small LDL1 fraction. Consistent with the changes in LDL cholesterol distribution, trends in LDL particle size were also observed among the groups in VA-HIT. As seen in Table 3, men with the B2B2 genotype had the largest LDL particles (20.49 nm), whereas those with the B1B1 genotype had the smallest (20.32 nm). The preceding findings were very similar to those in men from FOS, in which men with the B2B2 genotype had increased levels of LDL3 (18%), reduced levels of LDL1 (−7%), and increased LDL particle size (20.80 versus 20.56 nm) relative to homozygous carriers of the B1 allele. Also of note is the fact that even the largest mean LDL particle size observed in VA-HIT (B2 homozygotes, 20.49 nm), is substantially smaller than that of the smallest mean LDL particle size seen in FOS (B1 homozygotes), 20.56 nm.

Analysis of HDL subclasses in VA-HIT revealed that variation at the CETP TaqIB locus was significantly associated with concentrations of the large HDL4 fraction (Table 3). Homozygous (18%) and heterozygous (22%) carriers of the B2 allele, compared with B1 homozygotes, had significantly elevated concentrations (\( P = 0.02 \)) of cholesterol distributed in the H4 subclass. No other differences in HDL subclass distribution were observed between the groups in VA-HIT. Consistent with increased cholesterol content in the large HDL subclass, an increase in mean HDL particle diameter was seen in heterozygous and homozygous carriers of the B2 allele. The mean HDL particle size for B2 carriers was

**TABLE 2.** Association of CETP TaqIB Genotype With Fasting Plasma Lipid and Apolipoprotein Concentrations in Men From VA-HIT

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>64±7</td>
<td>64±7</td>
<td>64±7</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>29.4±4.6</td>
<td>29.0±4.6</td>
<td>29.6±4.7</td>
</tr>
<tr>
<td>TC, mg/dL</td>
<td>179±25</td>
<td>177±24</td>
<td>183±26</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>115±23</td>
<td>114±22</td>
<td>117±21</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>30.9±4.9</td>
<td>32.0±5.3*</td>
<td>32.6±4.8*</td>
</tr>
<tr>
<td>HDLr-C, mg/dL</td>
<td>5.1±2.6</td>
<td>5.4±2.3</td>
<td>5.5±2.1</td>
</tr>
<tr>
<td>HDL2-C, mg/dL</td>
<td>26.2±5.3</td>
<td>27.1±4.8†</td>
<td>27.1±4.1†</td>
</tr>
<tr>
<td>TC/HDL-C</td>
<td>5.9±1.2</td>
<td>5.7±1.1†‡</td>
<td>5.7±1.2‡†</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>167±68</td>
<td>160±67</td>
<td>166±67</td>
</tr>
<tr>
<td>ApoA-I, mg/dL</td>
<td>106±16</td>
<td>107±18</td>
<td>107±16</td>
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<tr>
<td>ApoB, mg/dL</td>
<td>98±19</td>
<td>97±21</td>
<td>99±20</td>
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</table>

Values are mean±SD. To convert values for cholesterol and triglycerides to millimoles per liter, multiply by 0.02586 and 0.01129, respectively.

\*P<0.001, †P<0.01, ‡P<0.02 for difference between genotypes, after adjustment for age, diabetes, hypertension, and smoking.
TABLE 3. Association of CETP TaqIB Genotype With LDL and HDL Subclass Concentrations and Particle Size in Men From VA-HIT

<table>
<thead>
<tr>
<th>CETP TaqIB Genotype</th>
<th>LDL3 (mean±SD)</th>
<th>LDL2 (mean±SD)</th>
<th>LDL1 (mean±SD)</th>
<th>LDL size (mean±SD)</th>
<th>LDL particles (mean±SD)</th>
<th>HDL5 (mean±SD)</th>
<th>HDL4 (mean±SD)</th>
<th>HDL3 (mean±SD)</th>
<th>HDL2 (mean±SD)</th>
<th>HDL1 (mean±SD)</th>
<th>HDL size (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1B1 (n=134)</td>
<td>38.2±29.1 (33)</td>
<td>41.6±32.6 (36)</td>
<td>43.3±28.9 (37)</td>
<td>20.32±0.62</td>
<td>1416±282</td>
<td>1.6±3.1 (5)</td>
<td>6.8±4.2 (22)</td>
<td>4.9±5.3 (16)</td>
<td>15.1±5.1 (49)</td>
<td>0.9±2.6 (3)</td>
<td>8.53±0.24</td>
</tr>
<tr>
<td>B1B2 (n=199)</td>
<td></td>
<td></td>
<td></td>
<td>20.36±0.67</td>
<td>1404±312</td>
<td>1.3±2.7 (4)</td>
<td>8.3±5.0 (26)</td>
<td>5.1±6.5 (16)</td>
<td>14.5±5.7 (46)</td>
<td>0.7±2.4 (2)</td>
<td>8.56±0.24</td>
</tr>
<tr>
<td>B2B2 (n=45)</td>
<td></td>
<td></td>
<td></td>
<td>20.49±0.55</td>
<td>1349±281</td>
<td>1.6±2.8 (5)</td>
<td>8.0±4.4 (24)</td>
<td>5.2±5.1 (16)</td>
<td>14.7±5.1 (45)</td>
<td>1.0±2.9 (3)</td>
<td>8.56±0.21</td>
</tr>
<tr>
<td>P*</td>
<td>0.23</td>
<td>0.45</td>
<td>0.25</td>
<td>0.18</td>
<td>0.27</td>
<td>0.59</td>
<td>0.02</td>
<td>0.65</td>
<td>0.41</td>
<td>0.97</td>
<td>0.39</td>
</tr>
</tbody>
</table>

All data are presented as mean±SD. Numbers in parentheses are percent of total plasma LDL or HDL cholesterol concentration distributed in subfraction. LDL and HDL subclass concentrations are expressed in units of cholesterol (mg/dL). To convert values for cholesterol to millimoles per liter, multiply by 0.02586. LDL and HDL particle size are given in nanometers.

*Adjusted for age, diabetes, hypertension, and smoking status.

8.56±0.23 nm, relative to 8.53±0.24 nm for B1 homozygotes (P=0.20). This was likewise the case for men in FOS, in which those with the B2B2 genotype had the largest mean HDL particle size (8.98±0.45 nm), followed by those with the B1B2 genotype (8.92±0.40 nm), and, last, by those with the B1B1 genotype (8.83±0.37 nm). Again, it is interesting to note that even the largest HDL particle size observed in VA-HIT (8.56 nm) is considerably smaller than the smallest HDL particle size (8.83 nm) seen in CHD-free men in FOS.34

Association of CETP TaqIB Genotype With Plasma Lipid Response to Gemfibrozil

The Figure shows the relationship between CETP TaqIB genotype and the plasma lipid response to gemfibrozil (1200 mg/d) in the treatment group of VA-HIT. The mean percent reduction observed in plasma TC levels was small, regardless of TaqIB genotype, with only a 2.2% reduction in the B1B1 group and a 3.6% reduction in the B2B2 group. Although plasma LDL cholesterol levels were increased to the greatest extent in homozygous carriers of the B1 allele (7.3%), this change was not significantly different (P=0.20) from that in men in either the B1B2 (2.3%) or B2B2 (0.4%) groups. However, men with the B1B1 genotype, who had the lowest levels of HDL-C at baseline, were found to have the most favorable response to gemfibrozil therapy in terms of HDL elevation and triglyceride lowering. The mean percent increase in HDL-C was 7.5±18.0% for the B1B1 group, relative to 5.0±19.4% and 5.4±20.5% for the B1B2 and B2B2 groups, respectively. Although the differences in HDL-C were not statistically significant because of the large variability seen within each genotype, the differences observed in plasma triglyceride lowering were highly significant, with B1B1 men having the greatest reduction in response to gemfibrozil therapy (−34%, P=0.006).

Association Between CETP TaqIB Genotype and CHD End Points

Because VA-HIT consisted solely of men with established CHD, we also evaluated our data for associations between CETP TaqIB genotype and CHD end points, defined as death due to CHD or nonfatal myocardial infarction. The total number of end points observed for carriers of the B2 allele...
was 32 versus 72 for noncarriers. After adjustment for age, diabetes, hypertension, and smoking status, homozygous carriers of the B2 allele in VA-HIT had a 48% reduction in risk for CHD end points (95% CI 0.25 to 1.08, \( P=0.08 \)). Although this result did not quite reach statistical significance, it is consistent with our results from men in FOS,\textsuperscript{34} in which the odds ratio for prevalent CHD associated with the B2 allele was 0.696 (95% CI 0.50 to 0.98, \( P=0.035 \)).

**Discussion**

HDL deficiency is the most common lipid abnormality observed among patients with premature CHD.\textsuperscript{45} It has been reported that >50% of the variation in HDL-C levels in humans is genetically determined,\textsuperscript{46} with gene products that influence the amount and nature of lipid contained within HDL particles having important effects on the metabolism of HDL and apoA-I. Included among these gene products is CETP. We have previously reported that genetic variation at the CETP \emph{Taq}\textsubscript{IB} locus was associated with plasma lipoprotein levels and CHD risk in men from FOS.\textsuperscript{34} To further explore the contribution of this variant to the modulation of CHD risk at the population level, we examined its influence on plasma lipoproteins, response to gemfibrozil therapy, and CHD end points in 852 men from the VA-HIT. Because VA-HIT consists solely of men having established CHD and HDL deficiency, it provides us with a unique population in which to identify those common genetic variants that have the greatest influence on HDL-C levels and CHD risk.

In VA-HIT, the frequency of the CETP \emph{Taq}\textsubscript{IB} B2 allele was 0.396, whereas that of the B1 allele was 0.604. These allele frequencies were significantly different from those of 0.444 and 0.556, respectively, which we had observed for CHD-free males in FOS. Although this comparison should be interpreted with caution, the fact that the frequency of the B2 allele was significantly reduced in men with CHD, whose primary lipid abnormality was a low level of HDL-C, supports the concept that the B2 allele is associated with increased HDL-C levels and reduced CHD risk. Moreover, our results are very similar to those reported by Kuivenhoven et al\textsuperscript{33} in 807 men with CHD from REGRESS, a study in which allele frequencies were 0.594 for B1 men and 0.406 for B2 men. Taken together, the preceding data not only suggest that the CETP \emph{Taq}\textsubscript{IB} B2 allele is associated with atheroprotection but also imply that the B1 allele may be associated with increased CHD risk.

Similar to our results in the FOS population, we observed a gene-dosage relationship between the CETP \emph{Taq}\textsubscript{IB} B2 allele and HDL-C concentrations in VA-HIT. In VA-HIT, men with the B2B2 genotype had the highest mean level of HDL-C, followed by men with the B1B2 genotype, and, last, by men with the B1B1 genotype. Thus, despite being a relatively homogeneous population with respect to plasma lipid concentrations, CETP \emph{Taq}\textsubscript{IB} genotype was a significant predictor of plasma HDL-C levels in this group of men. In view of the significant relationship that has been shown to exist between CETP \emph{Taq}\textsubscript{IB} genotype and CETP activity,\textsuperscript{21,33,34,47} it is not unreasonable to surmise that the relatively elevated levels of HDL-C observed in carriers of the B2 allele in VA-HIT were due, at least in part, to decreased CETP activity.

In addition to plasma lipid parameters, we also explored the relationships between CETP \emph{Taq}\textsubscript{IB} genotype and subpopulations of LDL and HDL, as determined by NMR. To our knowledge, the present study is the first to report that a significant association exists between CETP \emph{Taq}\textsubscript{IB} genotype and the concentration of large-sized HDL in men with CHD. Specifically, plasma concentrations of the H4 subclass of HDL were significantly elevated (\( P=0.02 \)) in heterozygous (22%) and homozygous (18%) carriers of the B2 allele relative to noncarriers. This effect is consistent with the concept that the B2 allele is associated with reduced CETP activity, which, in turn, results in an increase of cholesteryl ester–enriched, large-sized HDL in the plasma. It is this fraction of HDL that has been proposed to be protective with regard to CHD risk.\textsuperscript{48,49}

Compatible with a report that has shown linkage of the CETP gene locus to LDL particle size,\textsuperscript{50} we observed trends between CETP \emph{Taq}\textsubscript{IB} genotype and the amount of cholesterol in the large and small subclasses of LDL. Homozygous carriers of the B2 allele had the highest levels of LDL3 cholesterol, followed by heterozygous carriers, and, last, by noncarriers, with the opposite seen for levels of LDL1 cholesterol. Thus, as was the case for men in FOS, the B2 allele was associated with a relatively beneficial LDL subclass distribution profile in VA-HIT, consisting of increased levels of the less atherogenic large LDL subclass and reduced levels of the more atherogenic small LDL subclass.\textsuperscript{48,51,52} Taken together with the HDL subclass data, these results indicate that CETP \emph{Taq}\textsubscript{IB} genotype is associated not only with total plasma lipid concentrations but also with those of lipoprotein subclasses as well.

In addition to testing for associations between CETP \emph{Taq}\textsubscript{IB} genotype and plasma lipid levels at baseline, we also examined whether this genetic marker was correlated with the plasma lipid response to gemfibrozil therapy in VA-HIT. Our analysis revealed that homozygous carriers of the B1 allele had a significantly greater response to gemfibrozil therapy in terms of triglyceride lowering than did carriers of the B2 allele, suggesting that this marker may allow one to identify those subjects who may benefit most from a given lipid-altering agent. Further support for this hypothesis is provided by the fact that pravastatin therapy was associated with beneficial angiographic effects only in men having the B1B1 genotype in REGRESS.\textsuperscript{33}

Finally, in the present study, we investigated the relationship between CETP \emph{Taq}\textsubscript{IB} genotype and CHD outcomes. Consistent with our earlier work in FOS,\textsuperscript{31} the B2B2 genotype was associated with a reduced risk of CHD end points in VA-HIT. Moreover, this finding is in agreement with those of 2 other studies, which were conducted in men with CHD.\textsuperscript{25,30} Specifically, Eiriksdottir et al\textsuperscript{32} reported that the occurrence of a first myocardial infarction was significantly delayed in men with CHD having the B2B2 genotype, whereas Kuivenhoven et al\textsuperscript{33} noted the least progression of coronary artery disease in B2B2 men on placebo.

In summary, our data demonstrate that the CETP \emph{Taq}\textsubscript{IB} variant is associated with plasma HDL-C levels and CHD
outcomes in men with established CHD in whom HDL deficiency is the primary lipid abnormality. Moreover, subjects having the B1B1 genotype in VA-HIT were found to have the greatest reduction in plasma triglyceride levels during gemfibrozil therapy, suggesting that this marker may provide insight into which patients may have a favorable response to this lipid-lowering agent. Together with our earlier results from FOS, the present data support the concept that increased HDL-C levels, resulting from reduced CETP activity, are beneficial regarding CHD risk reduction and, furthermore, suggest that drugs aimed at HDL elevation via CETP inhibition are attractive candidates for CHD risk reduction in patients with HDL deficiency, for whom optimal therapies are lacking.

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Cholesteryl Ester Transfer Protein TaqI B2B2 Genotype Is Associated With Higher HDL Cholesterol Levels and Lower Risk of Coronary Heart Disease End Points in Men With HDL Deficiency. Veterans Affairs HDL Cholesterol Intervention Trial

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