Regulation of Plasma HDL Cholesterol and Subfraction Distribution by Genetic and Environmental Factors

Associations Between the TaqI B RFLP in the CETP Gene and Smoking and Obesity

Dilys J. Freeman, Bruce A. Griffin, Andrew P. Holmes, Grace M. Lindsay, Dairena Gaffney, Christopher J. Packard, James Shepherd

Abstract
This study investigated in a healthy population (n=220) the association of the TaqI B restriction fragment length polymorphism (RFLP) in the cholesteryl ester transfer protein (CETP) gene with plasma high-density lipoprotein (HDL) cholesterol concentration and subfraction distribution. A raised HDL cholesterol level was found in B2B2 homozygotes (B2 cutting site absent) and was associated specifically with a 45% increase in HDL2 compared with B2B2 homozygotes (B1B1, 77±39 mg/100 mL, mean±SD; B2B2, 112±59 mg/100 mL; P<0.01). Total plasma, very-low-density lipoprotein, and HDL triglyceride levels did not differ among the genotype groups, nor did plasma apolipoprotein AI levels with a 45% increase in HDL2 compared with other mechanisms, independent of the aforementioned triglyceride exchange, may be responsible for the reduced HDL level. In this regard, it has been reported that HDL concentration and subfraction distribution are altered by environmental factors, including cigarette smoking, alcohol intake, physical activity, and obesity. In a study of normal individuals, we provided evidence that the marked difference in plasma HDL levels between smokers and nonsmokers was apparently the result of both triglyceride-dependent and triglyceride-independent mechanisms.

Several restriction fragment length polymorphisms (RFLPs) in the cholesteryl ester transfer protein (CETP) gene have been detected. One of these, a site polymorphic for the presence or absence of a TaqI restriction site, has been shown by us to be associated with high plasma HDL concentrations. Individuals homozygous for the absence of a TaqI restriction cutting site, ie, B2B2 individuals, have a higher HDL concentration than those homozygous for the presence of the restriction site. This was found in both a random sample of normal subjects and a population selected according to whether they had high or low HDL levels. In the latter group, higher HDL
levels were also associated with a lower plasma CETP activity, an enzyme that is thought to be involved in the metabolism of plasma HDL, raising the possibility that variation in the enzyme was the underlying effect. A further observation from the study of Kondo et al.18 was that the association of the TaqI B polymorphism with apolipoprotein AI levels was present in nonsmokers but not in smokers. They found no significant effect of TaqI B RFLP on HDL cholesterol in nonsmokers, but they did note a trend.

The aim of this cross-sectional study was to establish the nature of the association of the TaqI B polymorphism with plasma HDL cholesterol, subtraction distribution, and apolipoprotein AI in a healthy population. We addressed specifically the potential role of plasma CETP activity in the mechanism. Cigarette smoking and other environmental factors were assessed to determine whether there was an interaction between environment and the genetic influence on plasma HDL concentration.

Methods

Subjects

The population was drawn from normal, healthy volunteers attending three health centers in Strathclyde and from healthy hospital staff at Glasgow Royal Infirmary. Subjects, under nurse supervision, completed a lifestyle questionnaire and donated a 50-mL blood sample after a 12-hour fast. The questionnaire asked for information on smoking status and alcohol consumption. At the time of blood donation, height and weight (without shoes) were measured, as was blood pressure. Body mass index (BMI) was calculated as weight (kg)/height (m) squared. Routine analyses for urea, electrolytes, and glucose; liver function tests; and thyroid hormone and HDL cholesterol were measured, and hyperlipidaemia. Total cholesterol, VLDL cholesterol, LDL cholesterol, and HDL subfraction concentrations were assessed using the Lipid Research Clinics Program protocol.18 The HDL subfraction distribution was determined by two techniques: analytical ultracentrifugation (Beckman L-8 ultracentrifuge unit; Beckman, High Wycombe, UK), which gave concentration values for HDL 2 and HDL 1; and a modification of the Lipid Research Clinics Program protocol,12 which was a gift from Dr D. Drayna (Genentech Inc, San Francisco, Calif.). Plasma triglycerides, VLDL triglycerides, HDL cholesterol, HDL 2, apolipoprotein AI, HDL 3, apolipoprotein B, and BMI values were transformed to their log 10 values, whereas VLDL cholesterol and alcohol consumption were normalized by taking the square root. Differences among genotypes were tested on transformed data using one-way ANOVA. Differences between groups at the extremes of the distribution were tested with a two-sample t test. Difference in distribution was assessed by χ 2 test. The independent contributions of genotype, age, sex, BMI, smoking, alcohol consumption, and triglyceride to HDL and HDL subfraction concentrations were assessed using the general linear model within MINITAB. Adjustment of HDL concentration for confounding factors was carried out using the general linear model. For the multivariate analysis, smoking status was defined as either current smoker or nonsmoker, which comprised those who had never smoked as well as exsmokers who had stopped smoking for more than 1 year. The bimodality of HDL distributions was assessed using a test for clusters.24 25

Results

Population Description

Demographic data for the population (n=220) studied are shown in Table 1. The frequencies for the TaqI B1 allele and the TaqI B2 allele were 0.54 and 0.46, respectively. These values are in good agreement with previously reported frequencies,12 16 and the polymorphism was shown to be in Hardy-Weinberg equilibrium. The genotype groups were well matched with respect to age, sex, and lifestyle factors (BMI, alcohol consumption, cigarette smoking, and degree of exercise). Individuals were categorized as "actively engaged in exercise" if they intimated on the lifestyle questionnaire that they participated in sports and were conscientious exercisers.

Lipid Parameters

Plasma total cholesterol and total, VLDL, and HDL triglycerides were similar in the different genotypes (Table 2). HDL cholesterol levels were significantly higher in B2B2 individuals than in B1B1 individuals (P<.005), while B1B2 heterozygotes had an intermediate HDL cholesterol level. There was also a nonsignificant trend toward higher apolipoprotein AI in the B2B2 homozygotes. There was no overall difference in

The assay was based on the exchange of cholesteryl ester between exogenous substrates, radiolabeled 3H-CE-HDL 2, and an LDL/VLDL mixture. Concentrations of donor (0.5 mg/dL protein) and acceptor (0.8 mg/dL protein) particles were such as to reveal maximal transfer activity. Activity was expressed as percent transfer of cholesteryl ester per microliter plasma (in 2.5 hours). The interassay coefficient of variation was <10%.

Southern Blot Analyses

DNA was prepared from blood lymphocytes by the method of Kunkel et al.22 RFLP detection was performed using standard methods.23 TaqI A and TaqI B restriction polymorphisms were detected using a 1581-bp complementary DNA CETP clone,12 which was a gift from Dr D. Drayna (Genentech Inc, San Francisco, Calif.). Statistical Analyses

Statistical manipulations and sample difference testing were carried out using the MINITAB computer program (version 8, 1991, Minitab Inc, State College, Pa). Data were tested for normality using normal probability plots and, if necessary, transformed to produce a normal distribution. Plasma triglycerides, VLDL cholesterol, LDL cholesterol, HDL 3, apolipoprotein AI, HDL 3, HDL 2, and BMI values were transformed to their log 10 values, whereas VLDL cholesterol and alcohol consumption were normalized by taking the square root. Differences among genotypes were tested on transformed data using one-way ANOVA. Differences between groups at the extremes of the distribution were tested with a two-sample t test. Difference in distribution was assessed by χ 2 test. The independent contributions of genotype, age, sex, BMI, smoking, alcohol consumption, and triglyceride to HDL and HDL subfraction concentrations were assessed using the general linear model within MINITAB. Adjustment of HDL concentration for confounding factors was carried out using the general linear model. For the multivariate analysis, smoking status was defined as either current smoker or nonsmoker, which comprised those who had never smoked as well as exsmokers who had stopped smoking for more than 1 year. The bimodality of HDL distributions was assessed using a test for clusters.24 25

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plasma CETP activity among the genotypes (ANOVA), but activity was marginally higher in the B1B2 heterozygotes ($P<.05$, two-sample $t$ test) than in B1B1 homozygotes and the same as that seen in B2B2 homozygotes.

**HDL Subtractions**

The difference in plasma HDL cholesterol levels was associated with a specific increase in HDL$_2$ in the B2B2 group as detected by analytical ultracentrifugation (Table 3). B2B2 homozygotes had 45% higher HDL$_2$ levels than B1B1 homozygotes ($P<.01$), with the heterozygous group showing intermediate levels. When HDL subtraction distribution was assessed using gradient gel electrophoresis, the HDL$_2$ subtraction was significantly raised (54%) in the B2B2 group ($P<.05$). There were no differences among the groups in HDL3 subtraction concentrations detected by analytical ultracentrifugation, but HDL$_{38}$ was marginally reduced (11%) in B1B2 heterozygotes.

A number of lifestyle factors had an impact on HDL cholesterol and HDL$_2$ levels. Multivariate analysis revealed that age, sex, BMI, alcohol consumption, and smoking were independent explanatory variables whose association with HDL cholesterol and HDL$_2$ persisted even when plasma triglyceride was included in the model (Table 4). The TaqI B polymorphism also had an independent effect on plasma HDL cholesterol ($P<.01$) and HDL$_2$ ($P<.02$) that was similar in magnitude to that of smoking.

**Interaction of Smoking and TaqI B Genotype**

The subjects were divided into groups according to their TaqI B genotype and smoking status (Table 5).

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**Table 1. Demographic Data for the Population**

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>58</td>
<td>121</td>
<td>41</td>
<td>220</td>
</tr>
<tr>
<td>Age, y</td>
<td>38±9</td>
<td>39±11</td>
<td>41±12</td>
<td>39±11</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>28/32</td>
<td>52/69</td>
<td>18/23</td>
<td>96/124</td>
</tr>
<tr>
<td>BMI, kg/m$^2$</td>
<td>24.6±3.9</td>
<td>25.2±3.7</td>
<td>25.1±3.9</td>
<td>25.0±3.8</td>
</tr>
<tr>
<td>(24.4±1.2)</td>
<td>(25.0±1.2)</td>
<td>(24.8±1.2)</td>
<td>(24.8±1.2)</td>
<td></td>
</tr>
<tr>
<td>Alcohol consumption, units/wk</td>
<td>9±11</td>
<td>8±11</td>
<td>9±11</td>
<td>8±11</td>
</tr>
<tr>
<td>(6±3)</td>
<td>(5±3)</td>
<td>(6±3)</td>
<td>(5±3)</td>
<td></td>
</tr>
<tr>
<td>Actively engaged in exercise, %</td>
<td>16</td>
<td>20</td>
<td>17</td>
<td>18</td>
</tr>
</tbody>
</table>

BMI indicates body mass index.

Means±SDs are shown; (geometric means±SDs) are shown for transformed data. Differences among the three groups were tested by ANOVA (on transformed data if required). The difference in sex and exercise distribution among the groups was tested using a $\chi^2$ test.

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**Table 2. Lipid Parameters, Cholesteryl Ester Transfer Protein Activity, and Apolipoprotein Al for the Population**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total-C, mmol/L (n=220)</td>
<td>5.36±0.97</td>
<td>5.60±1.16</td>
<td>5.61±0.96</td>
<td>5.54±1.08</td>
</tr>
<tr>
<td>Total-TG, mmol/L (n=220)</td>
<td>1.15±0.56</td>
<td>1.21±0.59</td>
<td>1.15±0.55</td>
<td>1.18±0.57</td>
</tr>
<tr>
<td>(1.04±1.55)</td>
<td>(1.08±1.61)</td>
<td>(1.03±1.60)</td>
<td>(1.06±1.59)</td>
<td></td>
</tr>
<tr>
<td>LDL-TG, mmol/L (n=145)</td>
<td>0.84±0.79</td>
<td>0.86±0.65</td>
<td>0.81±0.65</td>
<td>0.85±0.68</td>
</tr>
<tr>
<td>(0.59±2.32)</td>
<td>(0.67±2.05)</td>
<td>(0.59±2.32)</td>
<td>(0.63±2.17)</td>
<td></td>
</tr>
<tr>
<td>HDL-TG, mmol/L (n=115)</td>
<td>0.21±0.07</td>
<td>0.20±0.06</td>
<td>0.23±0.07</td>
<td>0.21±0.06</td>
</tr>
<tr>
<td>HDL-C, mmol/L (n=219)*</td>
<td>1.36±0.31</td>
<td>1.42±0.30</td>
<td>1.60±0.43†</td>
<td>1.44±0.34</td>
</tr>
<tr>
<td>(1.32±1.26)</td>
<td>(1.39±1.24)</td>
<td>(1.54±1.32)</td>
<td>(1.40±1.27)</td>
<td></td>
</tr>
<tr>
<td>CETP, % transfer/µL (n=216)</td>
<td>41±15</td>
<td>46±17†</td>
<td>46±19</td>
<td>45±17</td>
</tr>
<tr>
<td>Apo Al, mg/mL (n=203)</td>
<td>1.45±0.35</td>
<td>1.54±0.38</td>
<td>1.56±0.33</td>
<td>1.51±0.36</td>
</tr>
<tr>
<td>(1.41±1.24)</td>
<td>(1.50±1.26)</td>
<td>(1.53±1.21)</td>
<td>(1.53±1.25)</td>
<td></td>
</tr>
</tbody>
</table>

C indicates cholesterol; TG, triglyceride; VLDL, very-low-density lipoprotein; HDL, high-density lipoprotein; CETP, cholesteryl ester transfer protein; and Apo Al, apolipoprotein Al.

Means±SDs are shown; (geometric means±SDs) are shown for transformed data.

*P<.01, by ANOVA.
†P<.05, different from B1B1.
‡P<.005, different from B1B1.

Differences among the three groups were tested by ANOVA (on transformed data if required). Differences among genotypes were tested by two-sample $t$ test.
Smokers were individuals who were currently smoking, and nonsmokers were those who had never smoked. Exsmokers were a heterogeneous group who had smoked in the past but at the time had stopped for a period ranging from less than 1 year up to 25 years. The association of the TaqI B2 restriction site with high-density lipoprotein (HDL) levels was only observed in the nonsmoker (P < .05) and exsmoker (P < .05) groups. There was no difference between B1B1 smokers' and B2B2 smokers' HDL cholesterol. This pattern was maintained after plasma HDL concentrations were corrected for sex, age, BMI, alcohol consumption, and plasma triglyceride using a general linear model (Table 5) (smokers B1B1 versus B2B2, not significant; nonsmokers B1B1 versus B2B2, P < .005). Within each genotype, there was a trend toward higher HDL cholesterol levels in nonsmokers, but this was only significant for the B2B2 group.

### Interaction of Other Lifestyle Factors With TaqI B Genotype

The population was divided according to sex, tertile of age, tertile of BMI, tertile of alcohol consumption, oral contraceptive use, or exercise habit. Each factor was assessed for its interaction with the TaqI B-associated influence on HDL. Only BMI showed an effect in the whole group (data not shown), and it was similar to that observed for smoking. Because there was no effect of genotype on HDL levels in smokers, the analysis was also restricted to nonsmokers (Table 6), comprising those who had never smoked as well as exsmokers who had stopped smoking for more than 1 year. When nonsmokers were divided into groups according to TaqI B genotype and tertile of BMI, it was seen that homozygotes for the B2 site had significantly higher HDL than B1B1 homozygotes if they were relatively lean (lowest BMI tertile) but not if they had a higher BMI. A substantial (0.36 mmol/L) difference in the lowest BMI tertile, but not if they had a higher BMI. A substantial difference between smokers' and exsmokers' HDL cholesterol levels was only observed in the nonsmoker (P < .05) and exsmoker (P < .05) groups. There was no difference between B1B1 smokers' and B2B2 smokers' HDL cholesterol. This pattern was maintained after plasma HDL concentrations were corrected for sex, age, BMI, alcohol consumption, and plasma triglyceride using a general linear model (Table 5) (smokers B1B1 versus B2B2, not significant; nonsmokers B1B1 versus B2B2, P < .005). Within each genotype, there was a trend toward higher HDL cholesterol levels in nonsmokers, but this was only significant for the B2B2 group.

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### Table 3. High-Density Lipoprotein Subfractions in the Population and TaqI B Genotype Groups

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL3 mass, mg/100 mL (n=215)*</td>
<td>77±39</td>
<td>87±48</td>
<td>112±59</td>
<td>89±50</td>
</tr>
<tr>
<td>(66±1.8)</td>
<td>(75±1.8)</td>
<td>(95±1.9)†</td>
<td>(76±1.8)</td>
<td></td>
</tr>
<tr>
<td>HDL4 mass, mg/100 mL (n=215)</td>
<td>86±66</td>
<td>68±68</td>
<td>57±72</td>
<td>61±68</td>
</tr>
<tr>
<td>HDL5 mass, mg/100 mL (n=114)*</td>
<td>67±26</td>
<td>77±33</td>
<td>103±57†</td>
<td>79±39</td>
</tr>
<tr>
<td>(62±1.6)</td>
<td>(70±1.6)</td>
<td>(88±1.8)</td>
<td>(70±1.6)</td>
<td></td>
</tr>
<tr>
<td>HDL6 mass, mg/100 mL (n=114)</td>
<td>100±24</td>
<td>95±28</td>
<td>106±30</td>
<td>98±28</td>
</tr>
<tr>
<td>HDL7 mass, mg/100 mL (n=114)</td>
<td>130±30</td>
<td>116±33†</td>
<td>136±39</td>
<td>123±34</td>
</tr>
<tr>
<td>(56±1.4)</td>
<td>(50±1.6)</td>
<td>(50±1.4)</td>
<td>(52±1.5)</td>
<td></td>
</tr>
</tbody>
</table>

HDL indicates high-density lipoprotein.

Mean and SDs are shown; (geometric means±SDs) are shown for transformed data.

### Table 4. Multivariate Analysis of the Influence of TaqI B Genotype, Lifestyle Factors, and Triglycerides on High-Density Lipoprotein Cholesterol and High-Density Lipoprotein

<table>
<thead>
<tr>
<th>HDL (log), %</th>
<th>HDL2 (log), %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>12.5±1</td>
</tr>
<tr>
<td>Age</td>
<td>2.4±5</td>
</tr>
<tr>
<td>BMI (log)</td>
<td>4.3±1</td>
</tr>
<tr>
<td>Alcohol consumption (square root)</td>
<td>5.9±1</td>
</tr>
<tr>
<td>Triglyceride (log)</td>
<td>3.2±1</td>
</tr>
<tr>
<td>Smoking</td>
<td>1.9†</td>
</tr>
<tr>
<td>TaqI B RFLP</td>
<td>1.5±1</td>
</tr>
</tbody>
</table>

HDL indicates high-density lipoprotein; BMI, body mass index; and RFLP, restriction fragment length polymorphisms.

*P<.05; †P≤.01; ‡P<.005; and §P≤.001.

For multivariate analysis, the general linear model was used to examine the independent effects of sex, age, BMI (log), alcohol consumption (square root), plasma triglyceride (log), smoking, and TaqI B RFLP on plasma HDL cholesterol (log) and HDL2 (log). Percent contribution to the variation (adjusted means squared, divided by the total means squared, expressed as a percentage) is quoted along with the level of significance. Individuals were categorized as smokers or nonsmokers. Exsmokers who had stopped smoking for more than 1 year were classified as nonsmokers. HDL and HDL2 were higher in women than in men and negatively associated with BMI, plasma triglycerides, and smoking. HDL was marginally positively associated with alcohol consumption and age, whereas HDL2 was marginally negatively associated with these variables.
TABLE 5. High-Density Lipoprotein Concentrations in TaqI B Genotype Groups Subdivided by Smoking Status

<table>
<thead>
<tr>
<th>Genotype</th>
<th>B1B1, mmol/L</th>
<th>B1B2, mmol/L</th>
<th>B2B2, mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smokers</td>
<td>1.30±0.35</td>
<td>1.39±0.27</td>
<td>1.34±0.37</td>
</tr>
<tr>
<td></td>
<td>(1.28)</td>
<td>(1.36)</td>
<td>(1.32)</td>
</tr>
<tr>
<td></td>
<td>n=22</td>
<td>n=39</td>
<td>n=11</td>
</tr>
<tr>
<td>Exsmokers</td>
<td>1.29±0.29</td>
<td>1.43±0.26</td>
<td>1.62±0.36*</td>
</tr>
<tr>
<td></td>
<td>(1.31)</td>
<td>(1.38)</td>
<td>(1.56)*</td>
</tr>
<tr>
<td></td>
<td>n=10</td>
<td>n=23</td>
<td>n=13</td>
</tr>
<tr>
<td>Nonsmokers</td>
<td>1.43±0.28</td>
<td>1.44±0.34</td>
<td>1.76±0.47†</td>
</tr>
<tr>
<td></td>
<td>(1.36)</td>
<td>(1.43)</td>
<td>(1.61)††</td>
</tr>
<tr>
<td></td>
<td>n=26</td>
<td>n=58</td>
<td>n=17</td>
</tr>
</tbody>
</table>

High-density lipoprotein (HDL) concentrations shown are means±SDs and (adjusted HDL). HDL was adjusted for sex, age, body mass index, alcohol consumption, and triglyceride using a general linear model (MINITAB). Differences among the three groups were tested by ANOVA (on transformed data if required). HDL cholesterol levels differed among the genotype groups in exsmokers (HDL, P<.05) and nonsmokers (HDL, P<.01; adjusted HDL, P<.05). HDL cholesterol levels differed among the smoking groups in the B2B2 individuals (HDL, P<.05; adjusted HDL, P<.05). Differences between specific groups were tested by using a two-sample t test (on transformed data if required).

*P<.05, B1B1 vs B2B2.
†P<.05, smoker vs nonsmoker.

those in the highest BMI tertile, but this difference was no longer significant after correction of HDL for confounding factors.

The general linear model (MINITAB, version 8) was used to test for interaction between TaqI B genotype, smoking, and obesity. There was no significant interaction between TaqI B genotype and smoking when subjects were divided into the three categories of smokers, exsmokers, and nonsmokers. However, if exsmokers who had stopped smoking for more than 1 year were reclassified as nonsmokers and exsmokers who had stopped smoking for less than 1 year (n=4) were excluded, then there was weak evidence (P=.06) for an interaction between smoking and TaqI B genotype. The smoker B1B1/B2B2 difference in plasma HDL concentration was 0.01 mmol/L and the nonsmoker difference

TABLE 6. Nonsmoker High-Density Lipoprotein Concentrations in TaqI B Genotype Groups Subdivided by Body Mass Index Tertiles

<table>
<thead>
<tr>
<th>Genotype</th>
<th>B1B1, mmol/L</th>
<th>B1B2, mmol/L</th>
<th>B2B2, mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tertile 1, 18.3 to 23.5 kg/m²</td>
<td>1.52±0.31</td>
<td>1.55±0.30</td>
<td>1.96±0.52*</td>
</tr>
<tr>
<td></td>
<td>(1.41)</td>
<td>(1.50)</td>
<td>(1.77)*</td>
</tr>
<tr>
<td></td>
<td>n=14</td>
<td>n=24</td>
<td>n=9</td>
</tr>
<tr>
<td>Tertile 2, 23.5 to 26.2 kg/m²</td>
<td>1.45±0.23</td>
<td>1.47±0.37</td>
<td>1.70±0.36</td>
</tr>
<tr>
<td></td>
<td>(1.39)</td>
<td>(1.43)</td>
<td>(1.59)</td>
</tr>
<tr>
<td></td>
<td>n=8</td>
<td>n=28</td>
<td>n=11</td>
</tr>
<tr>
<td>Tertile 3, 26.2 to 40.4 kg/m²</td>
<td>1.23±0.23†</td>
<td>1.27±0.23‡</td>
<td>1.45±0.27†</td>
</tr>
<tr>
<td></td>
<td>(1.29)</td>
<td>(1.32)</td>
<td>(1.46)</td>
</tr>
<tr>
<td></td>
<td>n=13</td>
<td>n=25</td>
<td>n=9</td>
</tr>
</tbody>
</table>

High-density lipoprotein (HDL) concentrations shown are means±SDs (adjusted HDL). HDL was adjusted for sex, age, alcohol consumption, and triglyceride using a general linear model (MINITAB). Differences among genotype groups for each tertile of body mass index (BMI) were carried out using ANOVA. There was a significant difference in plasma HDL concentrations among the genotype groups in BMI tertile 1 (HDL, P<.05; adjusted HDL, P<.05). Similarly, ANOVA was used to test for differences among BMI tertiles within each genotype group. HDL, but not adjusted HDL, differed among BMI tertiles for each TaqI B genotype group (B1B1, P<.05; B1B2, P<.01; B2B2, P<.05).

*P<.05, difference from B1B1.
†P<.05, tertile 1 vs tertile 3.
‡P<.001, tertile 1 vs tertile 3.
was 0.29 mmol/L. Using the general linear model, there was no evidence for interaction between Taq1 B genotype and BMI tertile.

**Frequency Distributions of HDL Cholesterol by Genotype**

The frequency distribution of HDL cholesterol in the three Taq1 genotypes was determined in a combined group of nonsmokers plus those exsmokers who had stopped for more than 1 year. The Figure (A) shows that the distribution did not depend on the sex of individuals in the group, and it was also independent of BMI (B). Distributions of men and women or individuals with BMI greater than or less than the median were not different among the genotypes (not significant by \( \chi^2 \) test). It can be seen from these histograms that the distribution of HDL cholesterol in B1B1, B1B2, and B2B2 individuals is different. HDL cholesterol in B1B1 homozygotes is distributed between 0.9 mmol/L and 1.8 mmol/L, with a median of 1.38 mmol/L. Only three individuals have an HDL cholesterol level of \( >1.6 \) mmol/L. The distribution of the B1B2 individuals covered the whole range of HDL cholesterol but appeared to be bimodal, with one population distributed around 1.4 mmol/L and the other distributed around 1.8 mmol/L. In this genotype, 75% of individuals had an HDL cholesterol \( \leq1.6 \) mmol/L, and 25% had HDL cholesterol \( >1.6 \) mmol/L. The numbers of individuals in the B2B2 group were too low to discern whether the distribution was bimodal; however, 52% of individuals had HDL cholesterol \( \leq1.6 \) mmol/L, and 48% had an HDL cholesterol of \( >1.6 \) mmol/L. A \( \chi^2 \) test demonstrated that there was a significant difference (\( P<.005 \)) in the distribution of HDL cholesterol levels above and below 1.6 mmol/L across the Taq1 B genotypes.

To test whether the population demonstrated a bimodal distribution of HDL cholesterol, a test for clusters was used with critical values for the population size in question computed by simulation. Using this analysis, we were unable to demonstrate whether the B1B2 nonsmoking population was bimodal.

**Discussion**

In the population under study, the Taq1 B RFLP of the CETP gene was associated with variation in plasma HDL cholesterol concentrations. This confirms the trend reported by Kondo et al in Norwegian individuals and by our own previous study where subjects were selected on the basis of a high or low HDL level. Two studies found weak or no evidence for an association between Taq1 B genotype and HDL. However, in one study, an association did become apparent when nonsmokers only were analyzed. In the other study, no information on smoking was available for the control population, and in the hyperlipidemic population, accounting for smoking did not reveal any association between Taq1 B genotype and HDL. It is possible that any association between the two parameters may not be evident in all genetic populations and may be affected by hyperlipidemia. In the present study, individuals homozygous for the absence of the Taq1 B restriction site (B2B2) had significantly and substantially higher HDL cholesterol than those homozygous for its presence (B1B1). The 0.24-mmol/L difference (Table 2) represents 30% of the reference range for our local population (1.0 to 1.8 mmol/L). The difference in HDL concentration was associated specifically with a higher HDL, and when subfractions were analyzed on the basis of their particle size distribution, it could be seen that the 35 mg/100 mL difference in plasma HDL, was explained predominantly by a 36 mg/100 mL difference in the largest HDL subfraction, HDL3 \( \beta \) (\( P<.05 \)).

Our initial hypothesis was that the Taq1 B-dependent variation was mediated by changes in plasma CETP activity. However, in contrast to our previous study in highly selected populations, we saw no association between the Taq1 B genotype, plasma CETP activity, and HDL cholesterol. In the present study, CETP activity was found to be marginally lower in B1B1 individuals, the group of individuals associated with low plasma HDL concentration. This effect is opposite to that which might be expected from previous reports of an inverse relation between HDL and plasma CETP activity. However, we and other investigators have shown that there is no correlation between plasma HDL concentration and CETP activity within a normal population, although a weak correlation has been found with CETP mass. It is possible that a strong relation between these two parameters is only evident in extreme cases of CETP variation, eg, familial hyperalphalipoproteinemia, or between species. In our previous study, in which differences in genotype and plasma CETP activity between two groups selected according to high or low HDL levels were studied, our high-HDL group may have contained a subpopulation of individuals who were hyperalphalipoproteinemic with accompanying low CETP activity; consequently, this lowered the mean CETP activity for the high-HDL population as a whole. On the basis of the current data, we surmise that CETP activity per se is not responsible for the association between the Taq1 B RFLP and HDL. It is possible that mutations in the CETP gene may affect substrate specificity, and such a phenomenon has been identified for the lipoprotein lipase gene. The CETP assay used here measured CETP activity and would have been unable to detect differences in substrate specificity.

In an attempt to investigate this further, we studied the possible role of variation in plasma triglycerides, which are known to be important in determining HDL2 and HDL3 concentrations. No significant difference in plasma total, VLDL, or HDL triglycerides was observed among the Taq1 B genotype groups. Furthermore, multivariate analysis using a general linear model demonstrated that the contribution of the polymorphism to variation in HDL cholesterol was independent of triglyceride; after accounting for the confounding effects of age, sex, BMI, alcohol consumption, and plasma triglyceride, the Taq1 B RFLP was still a significant \( (P<.01) \) explanatory variable for plasma HDL cholesterol and HDL3 (\( P<.02 \)).

The previous report of Kondo et al indicated that smoking may interfere with the Taq1 B association with plasma HDL. We studied smoking and other lifestyle factors to determine whether this was a generalized effect of lifestyle on HDL or a specific smoking phenomenon. Alcohol consumption, oral contraceptive use, sex, age, and exercise did not disrupt the relation between the Taq1 B2 restriction site and HDL concentration. However, smoking very clearly did interact with this association (Table 5) both before and after the data
Bar graphs show distribution of high-density lipoprotein (HDL) cholesterol in TaqI B genotypes. Distributions of nonsmokers' (nonsmokers plus exsmokers who had stopped > 1 year) HDL concentration are plotted for each of the three TaqI B genotypes. HDL concentrations were adjusted for sex, age, body mass index (BMI), alcohol consumption, and plasma triglyceride using a general linear model (Minitab). The antilog of the adjusted HDL concentration was plotted. A, HDL concentrations of men and women are plotted separately. B, HDL concentrations of individuals with a BMI < median and a BMI > median are plotted separately.*

*χ² test for male/female distribution between TaqI B genotypes:

<table>
<thead>
<tr>
<th>Male</th>
<th>Female</th>
<th>Critical value</th>
<th>Not significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1B1</td>
<td>13</td>
<td>22</td>
<td>0.746</td>
</tr>
<tr>
<td>B1B2</td>
<td>33</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>B2B2</td>
<td>10</td>
<td>19</td>
<td></td>
</tr>
</tbody>
</table>

χ² test for BMI < median/BMI > median distribution between TaqI B genotypes:

<table>
<thead>
<tr>
<th>BMI &lt; median</th>
<th>BMI &gt; median</th>
<th>Critical value</th>
<th>Not significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1B1</td>
<td>18</td>
<td>17</td>
<td>0.317</td>
</tr>
<tr>
<td>B1B2</td>
<td>36</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>B2B2</td>
<td>13</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

were adjusted for the effects of other confounding factors. There was no difference in HDL cholesterol between B1B1 and B2B2 homozygotes in the smoking group (0.04 mmol/L; not significant). In the exsmoking group, there was a significant difference in HDL (0.25 mmol/L; P < .05), equivalent to the difference observed in the nonsmoking group (0.25 mmol/L; P < .005). A similar but less evident interaction was seen with BMI in nonsmokers. In the top BMI tertile (26.2 to 40.4 kg/m²), there was a 0.17-mmol/L difference in HDL between B1B1 and B2B2 homozygotes after correction for sex, age, smoking status, alcohol consumption, and plasma triglyceride.
triglyceride, whereas in the bottom BMI tertile (18.3 to 23.5 kg/m²), the difference was larger and statistically significant (0.29 mmol/L; P < 0.05).

Previously, we have suggested that smoking affects plasma lipids via multiple mechanisms. Part of the influence of smoking on HDL cholesterol, and more specifically HDL₂, could be explained by triglyceride-mediated mechanisms. This includes the neutral lipid exchange process that generates triglyceride-enriched HDL that can be lipolyzed to smaller, denser species, which are rapidly cleared from the circulation. However, our earlier data also provided strong evidence for the existence of a triglyceride-independent mechanism by which smoking lowered HDL. We postulate that the TaqI CETP gene RFLP effect identified here contributes at least in part to this mechanism. As noted above, we surmise that an effect on CETP activity is not responsible because activities of CETP were not different between groups. Changes in CETP substrate specificity could be potentially responsible for this effect. Alternatively, we postulate that the TaqI B RFLP is in linkage disequilibrium with an adjacent critical site. The LCAT gene, which is proximal to the CETP gene on chromosome 16, is a likely candidate. Variation in its activity would be expected to lead to altered HDL formation, which would not be affected by the prevailing plasma triglyceride concentration. However, the genetic distance of approximately 16 centiMorgans between the CETP gene and the LCAT gene is probably too large for the LCAT gene to demonstrate allelic association with the TaqI B RFLP. In addition, in our previous study of a selected population, LCAT activity did not differ between the high- and low-HDL groups.

The frequency distribution of HDL cholesterol shown in the Figure indicates that not all B2B2 individuals have high HDL cholesterol. Statistical models can be used to test whether a population demonstrates bimodality. Collingrum analysis has been used to study the distribution of apolipoprotein B levels in familial combined hyperlipidemia. This involves fitting a mixture of two normal densities and comparing the fit to that of a single normal distribution via a generalized likelihood ratio test. It is usual to fit a mixture of two normals with equal variance, to avoid singularities in the likelihood function. This model was thought unsuitable for analysis of HDL distribution, as the assumption that the two subpopulations have the same variance is not necessarily true for two populations of HDL. The test for clusters is sensitive to the alternative hypothesis of two subpopulations with different variances. It might be expected from the skewed distribution of HDL that the population with the higher mean HDL would have a larger variance. There are also theoretical problems with likelihood ratio tests in this context that cast doubt on their validity. The small group size and inherent variation in HDL leave the statistical tests with low power to detect bimodality, and in this case the apparent bimodality failed to reach statistical significance. The data suggest that only one half of the B2B2 homozygotes have a substantially raised HDL cholesterol (>1.6 mmol/L) whereas 25% of the B1B2 heterozygotes fall into this category. From the observations, we postulate that the HDL-raising effect is present on one half of the B2 alleles detected in our population.

We are presently seeking through family studies better genetic markers that are in closer association with the HDL-raising effect, in the anticipation that these will be in linkage disequilibrium with a true HDL-raising allele.

Acknowledgments

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References


Regulation of plasma HDL cholesterol and subfraction distribution by genetic and environmental factors. Associations between the TaqI B RFLP in the CETP gene and smoking and obesity.

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