Cholesteryl Ester Transfer Protein B1B1 Genotype Is Associated with a Parental History of Cardiovascular Diseases in Taiwanese People

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Key Words
Cholesteryl ester transfer protein \textit{Taq}I\textit{B} polymorphism · Cholesteryl ester transfer protein B1B1 genotype · Cardiovascular disease, family history

Abstract
Objective: To investigate the association between family history of cardiovascular disease (CVD) and cholesteryl ester transfer protein (CETP) \textit{Taq}I\textit{B} polymorphism in Taiwanese subjects. Subjects and Methods: In this cross-sectional study, 240 subjects (115 men and 125 women) were divided into two groups based on whether or not they had a parental history of CVD. Polymerase chain reaction/restriction fragment length polymorphism was used to analyze the genotype of the subjects for the \textit{Taq}I\textit{B} polymorphism of CETP in intron 1. Results: The frequency of the B1B1 genotype was significantly higher in Taiwanese subjects with a family history of CVD than in those without it (31.2 vs. 18.8\%, odds ratio = 1.97, 95\% confidence interval = 1.084–3.579, \(p = 0.035\)). Siblings with the B1B1 genotype had lower levels of serum high-density lipoprotein cholesterol (HDL-C) than siblings with either B1B2 (46.7 ± 11.0 vs. 52.5 ± 11.1 mg/dl, \(p = 0.034\)) or B2B2 genotypes (46.7 ± 11.0 vs. 55.2 ± 9.6 mg/dl, \(p = 0.01\)). Conclusion: CETP \textit{Taq}I\textit{B} polymorphism is associated with plasma HDL-C levels. The CETP B1B1 genotype may influence the susceptibility to CVD in Taiwan.
Plasma high-density lipoprotein cholesterol (HDL-C) level is widely recognized as a powerful predictor for the development of coronary artery disease [12, 13]. Cholesteryl ester transfer protein (CETP) is a protein that plays a central role in HDL-C metabolism. CETP can modify the lipid composition of plasma by transferring triglycerides and cholesterol esters between lipoproteins [14, 15]. Recent studies have found CETP inhibitors to markedly increase HDL-C in individuals with low HDL-C levels [16, 17]. Several polymorphisms have been found in the CETP gene locus [18–20]. The most studied polymorphism to date, TaqIB, has been shown to be a silent base change affecting the 227th nucleotide in the first intron of the gene [21]. The B2 allele of this polymorphism has been associated with increased HDL-C levels [22, 23]. Recently, Elousa et al. [24] evaluated the association of 12 variants in 10 lipoprotein-related genes with carotid intimal medial thickness and found that, in men, only the CETP TaqIB polymorphism could be associated with carotid intimal medial thickness. Ordovas et al. [25] reported that the CETP TaqIB genotype played a significant role in determining HDL-C variability and this association translated into a lower CHD risk in men but not in women. Still, while many studies have been done to investigate the relationship between the TaqIB genotype and the risk of CVD, the results have not been consistent [26–29]. Since no study of CETP TaqIB gene polymorphism and the risk of CVD has been performed in Taiwan, we wanted to investigate the association between family history of CVD and CETP TaqIB gene polymorphism in people of Han Chinese descent in Taiwan.

Subjects and Methods

Subjects

Between May 2003 and March 2004, a total of 240 Taiwanese subjects (115 men and 125 women) were recruited at the Kaohsiung Municipal Hsiao-Kang Hospital. Exclusion criteria were: (a) a previous diagnosis of acute myocardial infarction and/or cerebrovascular disease; (b) a definite history of angina pectoris with the following oligonucleotide primers: 5'-CTGAGCC-CAGAGAGAGGAGTGCC-3' (forward) and 5'-CTGAGCC-CAGCGCACACTAAC-3' (reverse) [31]. In PCR cycling, one denaturation cycle (94 °C for 5 min) was followed by 35 cycles (94, 60, and 72 °C for 30 s each). The PCR products were then digested with Taq IB restriction endonuclease (GIBCO-BRL; 65 °C for 2 h) and restriction enzyme digestion. Genomic DNA was extracted from peripheral blood leukocytes with either a QIAamp mini kit (Qiagen) or a Generation Capture Column kit (Gentra Systems). A 535-bp fragment in intron 1 of the CETP gene was PCR amplified with the following oligonucleotide primers: 5'-CAGAGAGAGGAGTGCC-3' (forward) and 5'-CTGAGCC-CAGCGCACACTAAC-3' (reverse) [31]. In PCR cycling, one denaturation cycle (94 °C for 5 min) was followed by 35 cycles (94, 60, and 72 °C for 30 s each). The PCR products were then digested with Taq restriction endonuclease (GIBCO-BRL; 65 °C for 2 h) and the fragments were separated by electrophoresis in a 2% agarose gel. The resulting DNA fragments were 174 and 361 bp for the B1 allele, and 535 bp for the undigested B2 allele.

Detection of CETP TaqIB Genotypes

CETP TaqIB genotypes were determined by polymerase chain reaction (PCR) amplification of genomic DNA, followed by restriction enzyme digestion. Genomic DNA was extracted from peripheral blood leukocytes with either a QIAamp mini kit (Qiagen) or a Generation Capture Column kit (Gentra Systems). A 535-bp fragment in intron 1 of the CETP gene was PCR amplified with the following oligonucleotide primers: 5'-CAGAGAGAGGAGTGCC-3' (forward) and 5'-CTGAGCC-CAGCGCACACTAAC-3' (reverse) [31]. In PCR cycling, one denaturation cycle (94 °C for 5 min) was followed by 35 cycles (94, 60, and 72 °C for 30 s each). The PCR products were then digested with Taq restriction endonuclease (GIBCO-BRL; 65 °C for 2 h) and the fragments were separated by electrophoresis in a 2% agarose gel. The resulting DNA fragments were 174 and 361 bp for the B1 allele, and 535 bp for the undigested B2 allele.

Statistical Analysis

The hospital’s Human Research Ethics Committee approved the design of this study, and informed consent was obtained from each participant. Individual interviews were held with participants about their disease and smoking history. They received a complete physical examination. Routine blood analyses were performed. Measurements were taken to calculate the body mass index (BMI).

The 240 subjects were divided into two groups based on their family history of CVD: group 1 consisted of 112 subjects with a family history of CVD (57 coronary artery disease and 55 strokes) and group 2 consisted of 128 subjects with no family history of CVD. An individual was considered to have a family history of CVD when he or she reported having a parent(s) with CVD before the age of 55 years.

The information regarding an individual’s family history of CVD was obtained from both hospital records and a questionnaire completed by the subject and confirmed verbally by interviewing the subject’s parent(s). Individuals with an unclear or unknown family history of CVD were excluded.

Biochemical Analyses

Total cholesterol and triglyceride (TG) were determined by a Beckman Coulter biochemical analyzer (Synchron CX-5CE, Fullerton, Calif., USA). The total cholesterol and TG levels were analyzed by the cholesterol oxidase/peroxidase method and the lipase/glucose oxidase/peroxidase method (Beckman reagent kit, Fullerton, Calif., USA), respectively. The HDL-C and low-density lipoprotein cholesterol (LDL-C) fractions were determined with an electrophoresis analyzer (Helena REP). The Helena REP electrophoresis system separates very-low-density lipoprotein (VLDL), HDL-C and LDL-C by agarose gel electrophoresis [32]. In our study, the specimen was applied to an agarose gel. The lipoprotein fractions were separated by electrophoresis and stained with Fat Red 7B. The stained bands were quantified with a scanning densitometer (rapid electrophoresis analyzer) using a 525-nm filter. The control was used as a marker for the location of the lipid bands and was quantified to verify the accuracy of the measurements of lipoprotein fractions.

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SBP = Systolic blood pressure; DBP = diastolic blood pressure; Chol = cholesterol. Results are means ± SD unless noted otherwise.

1 Median with interquartile range (Q1–Q3) in parentheses.

these variables were natural log-transformed for all other analyses. Odds ratios and 95% confidence intervals were determined to assess the level of differences in distributions for allelic and genotype frequencies between the two groups. The observed frequencies of the genotypes were compared with the frequencies under Hardy-Weinberg equilibrium by χ² tests [34]. The t test and one-way ANOVA were used to detect the mean differences between the biochemical data and in groups or CETP genotype. All p values were calculated based on two-sided tests. Significance was defined as p value less than 0.05.

Results

The clinical characteristics of all subjects are listed in table 1. There were no significant differences between groups 1 and 2 with regard to age, BMI, waist circumference, blood pressures, fasting plasma glucose levels, serum cholesterol, TG, HDL-C or LDL-C and the frequency of smoking. Genotype distribution and allele frequencies were in Hardy-Weinberg equilibrium and were comparable between the two groups. The CETP TaqIB genotype was determined unequivocally in all of them. The CETP TaqIB genotype and allele frequencies in both groups are shown in table 2. The CETP B1B1 genotype occurred more frequently in group 1 (31.2%) than group 2 (18.8%). Individuals having the CETP B1B1 genotype were associated with having a family history of CVD (group 1; odds ratio = 1.970, 95% confidence interval = 1.084–3.579, p = 0.035).

Table 2. CETP genotype distribution and allele frequency

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Group 1 (n = 112)</th>
<th>Group 2 (n = 128)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotypes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1B1</td>
<td>35 (31.2)</td>
<td>24 (18.8)</td>
</tr>
<tr>
<td>B1B2</td>
<td>60 (53.6)</td>
<td>72 (56.2)</td>
</tr>
<tr>
<td>B2B2</td>
<td>17 (15.2)</td>
<td>32 (25.0)</td>
</tr>
<tr>
<td>Alleles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td>130 (58)</td>
<td>120 (46.9)</td>
</tr>
<tr>
<td>B2</td>
<td>94 (42)</td>
<td>136 (53.1)</td>
</tr>
</tbody>
</table>

Figures in parentheses are percentages. a Odds ratio = 1.970, 95% confidence interval = 1.084–3.579, p = 0.035.

Table 3. Clinical characteristics of subjects according to CETP genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Group 1 (n = 112)</th>
<th>Group 2 (n = 128)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>59</td>
<td>132</td>
</tr>
<tr>
<td>Age, years</td>
<td>50.2 ± 11.6</td>
<td>51.2 ± 13.0</td>
</tr>
<tr>
<td>BMI</td>
<td>26.7 ± 4.3</td>
<td>26.1 ± 3.4</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>80.2 ± 9.9</td>
<td>79.3 ± 10.6</td>
</tr>
<tr>
<td>Fasting sugar, mg/dl</td>
<td>92.0 ± 12.7</td>
<td>89.1 ± 10.7</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>123.1 ± 11.0</td>
<td>122.6 ± 15.4</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>79.3 ± 10.0</td>
<td>78.1 ± 10.2</td>
</tr>
<tr>
<td>Chol, mg/dl</td>
<td>192.8 ± 22.1</td>
<td>191.7 ± 28.3</td>
</tr>
<tr>
<td>HDL-C, mg/dl</td>
<td>46.7 ± 11.0</td>
<td>52.5 ± 11.1</td>
</tr>
<tr>
<td>LDL-C, mg/dl</td>
<td>120.3 ± 24.1</td>
<td>118.2 ± 25.2</td>
</tr>
<tr>
<td>TG, mg/dl</td>
<td>106 (79–190)</td>
<td>102 (72–180)</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>18.6</td>
<td>19.7</td>
</tr>
</tbody>
</table>

Data are means ± SD unless noted otherwise. SBP = Systolic blood pressure; DBP = diastolic blood pressure; Chol = cholesterol. a B1B1 vs. B1B2, p = 0.034; B1B1 vs. B2B2, p = 0.010.
1 Median with interquartile range (Q1–Q3) in parentheses.

The characteristics of the subjects categorized by CETP genotype are shown in table 3. There were no significant differences with respect to age, BMI, waist circumference, blood pressures, blood levels of cholesterol, LDL-C, TG and the frequency of smoking. The CETP genotypes were found to be significantly associated with HDL levels using ANOVA. After a multiple comparison test (Bonferroni), siblings with the B1B1 genotype had lower serum HDL-C than siblings with B1B2 (46.7 ± 11.0 vs. 52.5 ± 11.1 mg/dl, p = 0.034) and B2B2 genotypes (46.7 ± 11.0 vs. 55.2 ± 9.6 mg/dl, p = 0.01).
Discussion

CVDs make up some of the major causes of mortality and disabilities in the world. Although screening for traditional risk factors (e.g., elevated blood pressure, plasma glucose and lipids) has been shown to identify persons at increased risks for CVD, most CVD events occur to persons with risk factor measurements in the middle of the distribution rather than at the extremes. Therefore, the cost-effectiveness of screening the populations to identify persons with abnormal traditional risk factors has been questioned [35]. Meanwhile, family history of CVD remains an independent risk factor for CVD even after correcting for traditional risk factors for CVD and has been used in previous studies [36, 37]. Family history of CVD does not always connote genetic susceptibility, but it is the most effective, efficient and low-cost way of identifying subgroups at risk for CVD in the population as evidenced by the new guidelines for primary prevention of CHD and stroke issued by the American Heart Association [38]. Using family history we were able to identify individuals at high risk for CVD and our data revealed that the frequency of the B1B1 genotype of CETP is significantly higher in Taiwanese subjects with a family history of CVD than in those without. CVDs were more common among parents of homozygous B1 carriers than among parents of homozygous B2 carriers, suggesting that the B1 allele was transmitted from the parent with CVD to the child. Many studies investigated the relationship between the TaqIB genotype and the risk of CVD, but their results have been inconsistent [26–29]. Recently, we found that the presence of the CETP B1B1 genotype could predict coronary artery disease in Taiwanese subjects with type 2 diabetes [39]. In this study, our results suggest that the CETP TaqIB polymorphism might play some role in CVD of Taiwanese people. Further study is necessary to confirm whether or not it does.

In this study, CETP TaqIB gene polymorphism is associated with serum HDL-C levels. This finding is consistent with the results of previous studies [25, 40, 41]. Although other factors such as sex, smoking and BMI have been reported to be associated with CETP genotype [42, 43], there was no such association here.

How CETP isoforms might influence the development of CVD is unclear. CETP may mediate cholesterol redistribution by reducing the amount of cholesterol ester extracted from atherosclerotic lesions as a result of reduced HDL function. However, CETP regulates one of the steps in reverse cholesterol transport, an anti-atherogenic process. CETP is involved in modulating the concentration of HDL [44, 45] and may, therefore, alter susceptibility to CVD. Since the CETP TaqIB polymorphism is located in an intron, it may not be a functional mutation. The results of the present study show that the CETP TaqIB polymorphism can be related with a family history of CVD and HDL-C levels. Further studies are needed to determine if this polymorphism is a nonfunctional marker in linkage disequilibrium with functional variants of the CETP gene or other closely linked genes.

There are limitations inherent in the design of this study. This is a cross-sectional study. The effect of CETP genotype with plasma CETP levels was not determined because plasma samples that had not been freeze-thawed were unavailable. However, the association between CETP TaqIB genotype and CETP levels has previously been investigated in many studies [22–25], although with conflicting results. These inconsistencies may be a result of differences among study samples or differences among the complex CETP activity assays that were used.

Conclusion

Family history of CVD was used to identify individuals at high risk for CVD. Our results show that the CETP B1B1 genotype may influence the susceptibility to CVD in Taiwan, as parents of offspring with the B1B1 genotype reported an increased prevalence of CVD.

References


