CLINICAL RESEARCH

Association of the cholesteryl ester transfer protein Taq1 B2B2 genotype with higher high-density lipoprotein cholesterol concentrations and lower risk of coronary artery disease in a Tunisian population

Association du génotype B2B2 du polymorphisme Taq1B de la protéine de transfert des esters de cholestérol avec l’augmentation de la concentration du cholestérol des lipoprotéines de haute densité et avec la diminution du risque coronarien dans une population Tunisienne

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KEYWORDS
Cholesteryl ester transfer protein; Taq1B polymorphism; Coronary artery disease;

Summary
Background. — The role of cholesteryl ester transfer protein (CETP) in the development of atherosclerosis is undergoing debate.
Aims. — In this prospective study, we sought to explore the role of the CETP Taq1B variant in coronary artery disease risk, and its association with plasma lipid and apolipoprotein concentrations.

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Methods. — DNA was extracted from 316 patients undergoing coronary angiography. The Taq1B polymorphism was genotyped using polymerase chain reaction—restriction fragment length polymorphism analysis. Lipid and apolipoprotein concentrations were measured by enzymatic and nephelometric assays.

Results. — In our study population, the B2 allele frequency was 0.29. B2 allele carriers had a significantly higher high-density lipoprotein cholesterol (HDL-C) concentration than those with the B1B1 genotype (1.041 ± 0.294 versus 0.995 ± 0.277; p = 0.039). After adjusting for age, sex, smoking status, diabetes, hypertension and dyslipidaemia, the odds ratio (OR) for significant stenosis associated with the B2 allele was 0.82 (95% confidence interval (CI) 0.60—0.97; p = 0.039), suggesting that the B2 allele is associated with an 18% lower risk of significant stenosis. This protective effect seemed to be more significant in male non-smokers (38% lower risk; OR 0.62, 95% CI 0.29—0.92; p = 0.029). No significant protective effects were observed in women or male smokers.

Conclusion. — Our data suggest that the B2 allele is associated with higher concentrations of HDL-C, which confer a protective effect with regard to coronary atherosclerosis. This effect seems to be more significant in men than in women and in non-smokers than in smokers.

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The CETP gene is highly polymorphic; several common single nucleotide polymorphisms in the CETP gene have been described. The most studied among these is the Taq1B polymorphism, which has been shown to be a silent base change affecting the 277th nucleotide in the first intron of the gene [9,10]. A polymorphism detected using Taq1 endonuclease has been shown, in various population studies, to be associated with plasma HDL-C concentration. The allele containing the restriction site for the Taq1 endonuclease is called B1, while the allele without the restriction site is called B2. Individuals with the B2 allele have been found to have higher plasma HDL-C concentrations [11—16] and investigators have suggested an association between the polymorphism and coronary artery disease [15,16]. A recent meta-analysis, which included 92 studies but did not include a North African population, reported higher mean concentrations of HDL-C and ApoA-I and a weak reduction in coronary risk in individual carriers of the B2 allele [17]. However, other similar studies have reported conflicting results [18—20], and few data have been reported on the screening of this single nucleotide polymorphism in the CETP gene in Tunisia [21]. It has been suggested that the association may be population specific [22] and highly influenced by environmental factors, such as alcohol consumption and tobacco smoking [12,23].

The aim of our study was to determine the effects of the Taq1B polymorphism on lipid and apolipoprotein concentrations, and on coronary artery disease, in a Tunisian population.

**Methods**

**Study population**

In this prospective study, we recruited patients undergoing coronary angiography because of myocardial infarction, angina, thoracic pain or heart failure in the Cardiology Department at Sahliou University Hospital, Sousse, Tunisia. All patients were recruited between 2003 and 2007. Patients were subdivided into two groups: those with coronary artery disease and controls. Coronary artery disease patients were those who had significant coronary artery stenosis, which was defined as a luminal narrowing of more or equal to 50% in at least one major coronary artery, as judged by coronary angiography. Controls were those who had normal or insignificant coronary angiographic findings (<50%).

**Data collection**

Data on lifestyle factors were collected using an interviewer—administered questionnaire. The questionnaire included details of personal history, presence of disease, drug intake if any, cigarette smoking and alcohol consumption. Patients taking lipid-lowering drugs were excluded. Informed consent for the study was obtained from all the patients. The study was approved by the local medical ethics committee.

**Definition of cardiovascular risk factors**

Cardiovascular risk factors were evaluated. Diabetes mellitus was defined as fasting glucose more than 7 mmol/L or currently receiving antidiabetic medication. The smoking status of an individual was assigned ‘yes’ if they were smoking currently or had given up less than 3 months previously. Hypertension was defined as greater than 140/90 mmHg or currently on antihypertensive medication. Dyslipidaemia was defined as LDL-C concentration more or equal to 4.1 mmol/L and/or HDL-C concentration less or equal to 1 mmol/L and/or triglyceride concentration more or equal to 1.71 mmol/L.

**Measurement of plasma lipids and apolipoproteins**

Blood samples were collected from patients after a 12 h overnight fast and before coronary angiography. Serum total cholesterol, triglyceride and HDL-C concentrations were determined by standard assays using the Synchro CX7 Clinical System (Beckman, Fullerton, CA, USA). ApoA-I and ApoB were assayed using the IMMAGE Immunochemistry System (Beckman, Fullerton, CA, USA), based on immunonephelometric quantitation. LDL-C concentrations were calculated with the Friedewald formula [24].

**DNA analysis**

Genomic DNA was isolated from peripheral blood leucocytes by the salting out method [25]. CETP genotyping was performed as described by Fumeron et al. [12], with some modifications. A 535 base pair fragment in intron of the CETP gene was amplified by polymerase chain reaction in a DNA thermal cycler (LP × 2 Thermal Cycler, Thermo Electron Corporation, Milford, NE, USA). The oligonucleotide primers used for amplification were: forward 5′—CACTAGCCAGAGAGAGGAGTGCC-3′ and reverse 5′—CTGAGCCAGCCGCACACTAC-3′. Each amplification was performed by using 100 ng of genomic DNA in a volume of 30 μL containing 8 pmol of each oligonucleotide, 100 μM deoxyribonucleotide triphosphate and 1 U of Taq DNA polymerase (Promega, Madison, WI, USA). DNA templates were denatured at 95 °C for 3 min and then each polymerase chain reaction was subjected to 30 cycles with a temperature cycle consisting of 95 °C for 30 s, 60 °C for 30 s, 72 °C for 45 s, and, finally, an extension at 72 °C for 5 min. The 535 base pair polymerase chain reaction products were digested with 2U of Taq1 (Promega, Madison, WI, USA) at 65 °C for 2 h. Digested products were resolved by gel electrophoresis (2% agarose gel) and visualized by ethidium bromide staining. The digestion resulted in 174 base pair and 361 base pair fragments for the B1 allele and 535 base pair fragments for the uncut B2 allele.

**Statistical analyses**

Statistical analyses were performed with Epi Info™ software distributed by the World Health Organization. The frequencies of the allele genotypes among the patients were counted and compared by the chi-square test. The biological variables were compared with one-way analysis of variance then by Student’s t test or Fisher’s exact test and their values were reported as means ± S.D. OR were calculated as a measure of the association of the CETP Taq1B genotypes with the phenotypes. For each OR, two-tailed p-values and 95% CI were calculated; p was considered to be significant when it was less than 0.05. Adjusted OR for potential confounders were determined
using logistic regression analysis and corresponding p-values were reported.

**Results**

**Population characteristics**

The study comprised 316 patients (205 men and 111 women) undergoing coronary angiography because of myocardial infarction (n = 113), angina (n = 169), thoracic pain (n = 18) or heart failure (n = 16). The causes of thoracic pain were valvulopathy (n = 3), hypertensive cardiomyopathy (n = 2), severe aortic disease and mitral insufficiency (n = 2), dilated cardiomyopathy (n = 2) and psychiatric problems such as anxiety or depression (n = 9). The causes of heart failure were ischemic cardiopathy (n = 6), acute pulmonary oedema (n = 3), chronic pulmonary heart disease or chronic obstructive pulmonary disease (n = 3) and hypertensive crisis (n = 4). The clinical and biochemical characteristics of the patients are presented in Table 1. Compared with the control group, the coronary artery disease group had higher proportions of smokers, patients with dyslipidemia, patients with a history of myocardial infarction and patients with diabetes. In addition, triglyceride concentrations and apoB/apoA-I ratios were also significantly higher in coronary artery disease patients than in controls. Conversely, HDL-C and apoA-I concentrations were significantly lower in coronary artery disease patients than in controls.

**Genotype frequencies**

The genotype frequencies of B1B1, B1B2 and B2B2 are shown in Table 2. The calculated frequencies of the Taq1 B2 allele in coronary artery disease patients and controls were 0.29 and 0.34, respectively.

**Association of the CETP Taq1B allele with lipids and apolipoproteins**

Table 3 shows the association between the CETP Taq1B allele and lipid and apolipoprotein concentrations. The mean apoB/apoA-I ratio was significantly lower in carriers of the B2 allele than in patients with the B1B1 genotype. In carriers of the B2 allele compared with those with the B1B1 genotype, there was a statistically significant increase in HDL-C concentration (1.041 ± 0.294 mmol/L versus 0.995 ± 0.277 mmol/L, respectively; p = 0.039) and in ApoA-I concentration (1.27 ± 0.472 g/L versus 1.182 ± 0.334 g/L, respectively; p = 0.020). No other associations were detected between the CETP Taq1B allele and lipids or apolipoproteins in our study population.

We carried out a sex-based subgroup analysis to further investigate the effect of the two alleles on lipids and apolipoproteins. The increase in HDL-C was greater in men (p < 0.001) than in women (p = 0.034). Male carriers of the B2 allele had a significantly higher HDL-C concentration than men with the B1B1 genotype (1.009 ± 0.27 mmol/L versus 0.894 ± 0.20 mmol/L, respectively; p < 0.001) and a higher apoA-I concentration (1.186 ± 0.30 g/L versus 1.081 ± 0.31 g/L, respectively; p = 0.005). Male carriers of

| Table 1 Clinical and biochemical characteristics of the study population. |
|-----------------------------|--------------------------|--------------------------|---|
| Characteristics             | Coronary artery disease patients (n = 212) Mean ± SD or n (%) | Controls (n = 104) Mean ± SD or n (%) | p  |
| Sex ratio                   | 1.97                     | 1.26                     | 0.010 |
| Age (years)                 | 60.6 ± 10.6              | 59.4 ± 11.9              | 0.380 |
| Smoker                      | 120 (56.6)               | 47 (45.2)                | 0.013 |
| Diabetic                    | 73 (34.4)                | 23 (22.1)                | 0.001 |
| Hypertension                | 97 (45.7)                | 46 (44.2)                | 0.353 |
| History of myocardial infarction | 91 (42.9)            | 13 (12.5)                | < 0.001 |
| Dyslipidemia                | 33 (15.6)                | 9 (8.6)                  | 0.027 |
| Total cholesterol (mmol/L)  | 5.029 ± 1.192            | 5.025 ± 1.087            | 0.970 |
| Triglyceride (mmol/L)       | 1.614 ± 1.117            | 1.340 ± 0.647            | 0.022 |
| HDL-C (mmol/L)              | 0.961 ± 0.283            | 1.030 ± 0.276            | 0.040 |
| LDL-C (mmol/L)              | 3.440 ± 1.167            | 3.330 ± 1.039            | 0.370 |
| ApoA-I (g/L)                | 1.160 ± 0.379            | 1.290 ± 0.437            | 0.009 |
| ApoB (g/L)                  | 1.153 ± 0.376            | 1.110 ± 0.413            | 0.460 |
| ApoB/ApoA-I                 | 1.057 ± 0.493            | 0.920 ± 0.290            | 0.036 |
| Triglyceride/HDL-C          | 5.630 ± 2.040            | 5.410 ± 1.890            | 0.430 |

ApoA-1: apolipoprotein A-1; ApoB: apolipoprotein B; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; S.D.: standard deviation.

| Table 2 Frequency of Taq1B among study participants. |
|-----------------------------|--------------------------|---|
| Genotypes      | Frequency, n (%) | ---|
| B1B1          | 104 (49)          | 45 (42.9)         |
| B1B2          | 93 (44)           | 47 (45.4)         |
| B2B2          | 15 (7)            | 12 (11.7)         |
| B2 allele frequency | 0.29 | 0.34 |
the B2 allele also had a lower apoB/apoA-I ratio than men with the B1B1 genotype (0.883 ± 0.30 versus 1.056 ± 0.31, respectively; \( p = 0.030 \)). These differences in lipids and apolipoproteins between genotypes were not observed for women.

Our data on smoking were limited to men, because all the women in our study population were nonsmokers. Men were divided into two groups according to smoking status (Table 4). The mean concentration of HDL-C in smokers was lower than that in nonsmokers (0.95 and 1.11 mmol/L, respectively). Smokers with the B2 allele had significantly higher HDL-C and apoA-I concentrations compared with smokers with the B1B1 genotype; similarly, nonsmokers with the B2 allele had significantly higher HDL-C and apoA-I concentrations compared with nonsmokers with the B1B1 genotype. The difference in HDL-C concentration between carriers of the B2 allele and those with the B1B1 genotype was more statistically significant within the non-smoking group (\( p < 0.001 \) versus \( p = 0.006 \) among smokers).

Association of CETP Taq1B polymorphism with the presence of stenosis

Among the non-smoking men in this study, there were 16 coronary artery disease patients and 15 controls. Table 5 shows the crude and adjusted OR for significant stenosis in carriers of the B2 allele compared with those with the B1B1 genotype. After adjustment for age, sex, smoking status, diabetes, hypertension and dyslipidaemia, carriers of the B2 allele had an 18% reduced risk of significant stenosis (OR 0.82, 95% CI: 0.6–0.97; \( p = 0.039 \)). The B2 allele frequency was 0.315 in men and 0.298 in women. After adjustment for age, smoking status, diabetes, hypertension and dyslipidaemia, the OR for significant stenosis associated with the B2 allele in men was 0.77 (95% CI: 0.57–0.91; \( p = 0.037 \)). Among non-smoking men, those with the B2 allele had a 38% lower risk of significant stenosis (OR 0.62, 95% CI: 0.29–0.92; \( p = 0.029 \)). There was no significant reduction in risk of significant stenosis among female carriers of the B2 allele (OR 0.90, 95% CI: 0.08–1.19; \( p = 0.067 \)).

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Association of CETP Taq1B allele with lipids and apolipoproteins.</th>
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<tbody>
<tr>
<td></td>
<td>B1B1 (( n = 149 )) Mean ± S.D.</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.943 ± 1.189</td>
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<tr>
<td>HDL-C (mmol/L)</td>
<td>0.995 ± 0.277</td>
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<tr>
<td>LDL-C (mmol/L)</td>
<td>3.354 ± 1.174</td>
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<tr>
<td>Triglyceride (mmol/L)</td>
<td>1.474 ± 0.728</td>
</tr>
<tr>
<td>ApoA-I (g/L)</td>
<td>1.182 ± 0.334</td>
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<tr>
<td>ApoB (g/L)</td>
<td>1.14 ± 0.420</td>
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<tr>
<td>ApoB/ApoA-I</td>
<td>0.964 ± 0.322</td>
</tr>
<tr>
<td>Triglyceride/HDL-C</td>
<td>5.353 ± 2.04</td>
</tr>
</tbody>
</table>

ApoA-1: apolipoprotein A-1; ApoB: apolipoprotein B; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; S.D.: standard deviation.


Discussion

The role played by genetic polymorphisms in coronary atherosclerosis is far from being resolved. CETP polymorphism is one of the most studied polymorphisms in atherosclerosis. The impact of the gene/environmental interaction is difficult to determine and genetic studies are
needed in various population contexts. The study of CETP polymorphism in northern Africa provides a good opportunity to focus attention on unknown aspects of coronary atherosclerosis. The key finding of this study is that the B2 allele of the CETP gene Taq1B polymorphism is associated significantly with an increase in HDL-C concentration and a reduction in the prevalence of coronary artery disease.

The frequencies of the genotypes B1B1, B1B2 and B2B2 in our population with coronary artery disease were 49, 44 and 7%, respectively, with a B2 allele frequency of 0.29. These results are similar to those reported elsewhere in other populations, such as African–Americans, where the B2 allele frequency was 0.26 [20]. However, there have been reports of differences in the frequencies of the B2 allele in other ethnic groups: the frequency of the B2 allele was found to be 0.43, 0.44, 0.43 and 0.42 in Europeans, Americans, Israelis and Taiwanese, respectively [13,16,26,27].

In our study, carriers of the B2 allele had a significantly higher HDL-C concentration than those with the B1B1 genotype (p = 0.039). In a sex-based analysis, the effect of the B2 allele on HDL-C seemed to be more significant in men (p < 0.001). Studies have shown that the B2B2 genotype is associated with increased HDL-C concentrations and decreased risk of atherosclerosis and coronary artery disease [15,28]. The mechanism by which Taq1B polymorphism may affect HDL-C concentrations is not known. Taq1B polymorphism cannot be responsible solely for the change in HDL-C concentration, as it is a silent mutation and does not influence CETP transcriptional regulation or sequence directly. The slight increase in HDL-C concentration may be due to its linkage with other functional polymorphisms in the promoter area of the gene, which may cause decreased activity of CETP and hence, an increase in HDL-C concentration [29].

Human and transgenic mouse experiments have shown that environmental factors play an important role in the modulation of CETP gene expression [30]. Various studies in human populations have analysed the possible interaction between environmental factors and the effect of the CETP Taq1B polymorphism on plasma HDL-C concentrations. In this regard, we considered only the smoking habits of patients, and observed that, in men, the correlation between the presence of the B2 allele and an increased HDL-C concentration was stronger among nonsmokers than smokers. In the west of Scotland coronary prevention (WOSCOP) study [28], the association of the Taq1B genotype with coronary artery disease was seen only in nonsmokers. In contrast with our data, Fumeron et al. [12] did not find such an interaction with tobacco smoking.

We also found that the B2 allele was associated significantly with a 14% lower risk of significant stenosis (OR 0.86, 95% CI: 0.7—0.96; p = 0.042), and that the association was more statistically significant after adjustment for multiple risk factors (age, sex, smoking status, diabetes, hypertension and dyslipidaemia).

The results of our study support the concept that increased HDL-C concentrations appear to be associated with a lower risk of significant stenosis in men. In fact, the B2 allele was associated with a 33% reduced risk of significant stenosis in men (OR 0.77, 95% CI: 0.57—0.91; p = 0.037), and this potential protective effect was more notable in male nonsmokers (38% reduced risk). Thus, the results of our study support the protective role of this genetic variant against atherosclerosis and are concordant with other studies reporting a lower risk for coronary heart disease among carriers of the B2 allele [17,31].

Our data are in agreement with the regression growth evaluation statin study (REGRESS), the Framingham study, the WOSCOP study and the veterans affairs HDL cholesterol intervention trial (VA-HIT) [15,16,28,32]. The B1B1 genotype was associated with greater progression of coronary atherosclerosis in REGRESS and more cardiovascular endpoints in VA-HIT than the B2B2 genotype. In each study, B1B1 individuals had lower HDL-C concentrations than B2B2 individuals. These data are inconsistent with those derived from the physicians’ health study where no association between the Taq1B polymorphism and myocardial infarction was found [33].

There are some limitations to our study. Unfortunately, CETP activity was not measured. Although many large-scale studies have reported an association between CETP activity and the Taq1B polymorphism [13—15], some have not found such an association [34]. In addition, we did not take alcohol consumption into account because almost all of our study population declared that they did not drink alcohol. Various studies have reported that the association between the CETP Taq1B polymorphism and HDL-C concentration was

| Table 5 | Odds ratios for significant stenosis (B2 allele compared with B1B1 genotype). |
|---------|-------------------------------|----------------|----------|-----------------|-----------------|
|         | OR   | 95% CI      | p       | OR   | 95% CI      | p       |
| All patients (n = 316) | 0.86 | 0.7—0.96 | 0.042 | 0.82 | 0.60—0.97 | 0.039 |
| Men (n = 205) | 0.80 | 0.59—0.89 | 0.036 | 0.77 | 0.57—0.91 | 0.037 |
| Women (n = 111) | 0.90 | 0.08—1.19 | 0.067 | 0.89 | 0.12—1.22 | 0.089 |
| Male smokers (n = 173) | 0.96 | 0.70—1.31 | 0.165 | 0.94 | 0.65—1.42 | 0.172 |
| Male nonsmokers (n = 32) | 0.64 | 0.32—0.97 | 0.032 | 0.62 | 0.29—0.92 | 0.029 |

CI: confidence interval; OR: odds ratio.

a Adjusted for age, sex, smoking status, diabetes, hypertension and dyslipidaemia.
b Adjusted for age, smoking status, diabetes, hypertension and dyslipidaemia.
c Adjusted for age, diabetes, hypertension and dyslipidaemia.
more evident in alcohol consumers than in those who did not drink alcohol [35,36].

In summary, the CETP Taq1B genotype was associated with HDL-C concentration and the risk of coronary artery disease in a Tunisian population. The B2 allele has a protective effect on coronary artery disease by increasing HDL-C concentration. This protective effect seems to be more significant in male nonsmokers. CETP is expected to be a potential target for the development of new pharmacological agents that may raise serum HDL-C concentrations. It will therefore be interesting to examine the relationship between responses to such drugs and the genotypes of the CETP gene.

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**Conflict of interest**

None.

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