



Research Article

The role of cholesteryl ester transfer protein Taq1B polymorphism in young atherosclerotic heart disease

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Abstract

Objectives: There is growing evidence that oxidative modification of low-density lipoprotein (LDL) plays a central role in the pathogenesis of atherosclerosis, which is increasingly seen at younger ages, and that high-density lipoprotein (HDL) levels are inversely associated with the risk of coronary artery disease (CAD). Cholesteryl ester transfer protein (CETP) has a role in the regulation of plasma HDL levels. The most studied polymorphism in the CETP gene is the Taq1B polymorphism, which has consistently been correlated with HDL levels. This case control study of a young (<50 years) Turkish population group with CAD was designed to assess whether there is a relationship between LDL oxidation and CETP Taq1B polymorphism.

Methods: A total of 97 patients with CAD and 43 healthy volunteers were included in the study. Traditional risk factors for CAD (age, gender, smoking, hypertension) were evaluated in the patient group. Oxidative markers of LDL were determined in both groups, as well as routine biochemical parameters. Following DNA extraction from white cells, CETP Taq1B polymorphism was determined using polymerase chain reaction amplification and restriction enzyme digestion. Fragments 174 and 361bp were identified as B1, and unrestricted 535 bp fragments as B2.

Results: There was no statistical significance between the B1B1, B1B2, B2B2 genotypes in the patient group in terms of body mass index, waist-to-hip ratio, or biochemical parameters. Though the HDL cholesterol levels were higher in the B2B2 genotype, there was no statistically significant difference in comparison with the control group.

Conclusion: The genetic polymorphism of CETP had no significant effect on CETP function and the CETP polymorphism should not be proposed as an independent risk factor for cardiovascular events.

Keywords: Atherosclerosis, cholesteryl ester transfer protein, polymorphism, oxidized low-density lipoprotein

According to World Health Organization data, approximately one-fourth of 56.9 million deaths worldwide in 2016 were caused by ischemic heart disease and stroke [1].

It is well established that oxidatively modified low-density lipoprotein (LDL) is an important risk factor for the initiation of atherosclerosis and that high-density lipoprotein (HDL) might

play a protective role against coronary artery disease (CAD) [2]. Cholesteryl ester transfer protein (CETP) is mostly bound to HDL in plasma; only 1% is free [3]. Previously, CETP has been shown to play a part in regulating plasma HDL levels. The role of CETP during the atherosclerotic process is still debated, since it can produce both antiatherogenic and proatherogenic con-

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sequences. It is thought to be proatherogenic as a result of transferring cholesteryl esters from HDL to atherogenic lipoproteins, very low-density lipoprotein (VLDL), and LDL, and to be antiatherogenic due to its role in reverse cholesterol transport [4, 5]. Animal studies have also shown a dual role of CETP with respect to CAD. The introduction of the human CETP gene into apolipoprotein E knock-out mice and LDL receptor knock-out mice resulted in a decrease in HDL levels, leading to increased susceptibility to atherosclerosis, supporting the notion that CETP is proatherogenic [3, 6, 7]. Expression of CETP demonstrated an antiatherosclerotic effect by lowering the VLDL level in transgenic mice expressing human lecithin cholesterol acyltransferase, as well as in a hypertriglyceridemic mouse model, and in mice overexpressing human apolipoprotein CIII [3, 8–10]. Several human studies have also yielded conflicting reports as to whether CETP is proatherogenic, antiatherogenic, or has no relation to atherosclerosis and CAD [3, 6, 11, 12].

Various mutations and polymorphisms have been identified in the CETP gene; however, the Taq1B polymorphism is one of the most studied. This is a polymorphism at 277 nucleotides at intron 1 in the CETP gene. There are two alleles, B1 and B2 [15]. There have been studies investigating the plasma concentration as well as the activity of CETP and it has consistently been found to be associated with HDL levels [13, 14]. The B2B2 allele has been shown to be closely related to increased HDL levels and decreased CETP levels in normolipidemic subjects [15, 16]. This was a case control study designed to investigate any relationship between well-known risk factors, such as oxidized-LDL and paraoxonase (PON) activity, in a young Turkish population with CAD that comprised different genotypes of CETP Taq1B.

Materials and Methods

A total of 97 patients (<50 years) with angina pectoris, myocardial infarction (MI), and/or a positive exercise test were included in the study, as well as 43 healthy control group participants (<50 years). Blood samples were taken after 8-10 hours of fasting. Plasma and serum were separated immediately after centrifugation and then stored at -80°C until analysis. Whole blood samples were used for DNA isolation.

Serum lipid analyses were performed using standard laboratory methods. PON activity was measured using a previously defined method [17].

LDL isolation and determination of in vitro oxidation of LDL

Plasma samples were incubated with a precipitating reagent (Merck KGaA, Darmstadt, Germany) for 30 minutes at room temperature [18]. Following centrifugation at 1600g for 10 minutes, LDL samples were solubilized with 0.15 N NaOH. LDL oxidation was determined using thiobarbituric acid reactive substances (TBARS). TBARS was analyzed with TBARS solution

and the protein was measured in accordance with Lowry's method [19]. Conjugated diene levels in LDL samples containing 200 µg protein were analyzed with a spectrophotometer by monitoring the absorbance change at a wavelength of 234 nm at 5 minute intervals for 3 hours and calculations were performed using the extinction coefficient of 29.500 L/mol·cm. In vitro oxidation of LDL was stimulated with 5µM CuSO₄. The conjugation diene peak was measured at the 110th minute after the induction of in vitro oxidation of LDL. TBARS levels were also determined at the same time interval.

CETP Taq polymorphism

After isolation of genomic DNA from leukocytes using the phenol-ethanol extraction method, CETP gene amplification by polymerase chain reaction (PCR) was performed. Each amplification used 100 ng genomic DNA in a volume of 25 µL containing 5 pmol of the primers forward 5'-CACTAGCCCAGAGAGAGAGTGCC-3' and reverse 5'-CTGAGCCCAGCCGCACACTAAC-3', and 0.5 mM dNTPs, 1.75 mM MgCl, 10 mM Tris, and 0.5U Taq polymerase. Following denaturation at 94 °C for 5 minutes, PCR reaction was exposed to 30 cycles (94 °C for 1 minute, 60°C for 30 seconds, 72 °C for 45 seconds) and then was finalized with an extension at 72 °C for 5 minutes. The PCR products were restricted with TaqI endonuclease (5U) at 65 °C for 3 hours. The resulting fragments (174bp and 361bp) were visualized with a UV transilluminator following 2% agarose gel electrophoresis. Fragments 174 and 361bp were identified as B1, and unrestricted 535 bp fragments as B2.

Statistical analysis

Statistical analysis was performed using SPSS for Windows, Version 11.0 (SPSS Inc., Chicago, IL, USA). The results were examined using analysis of variance for intergroup comparisons followed by one-way analysis of variance, and the Mann-Whitney U test was used for non-parametric variables. Genotypes and Allelic frequencies were determined by chi-square test. P<0.05 was considered statistically significant.

This study was approved by the Research Ethics Committee of the Medical Faculty of Ege University (No: 05-5.1/2; 2005-05). The study complied with the principles set out in the Helsinki Declaration.

Results

The baseline characteristics and data related to the oxidant-antioxidant status of both groups are summarized in Table 1. The CAD group had more conventional risk factors (smoke, family history, obesity, hypertension). Age (p<0.01) and body mass index (BMI) values (p<0.01) were higher in the patient group than the control group. Additionally, the patients had higher triglyceride (p<0.01) and apolipoprotein B (Apo-B) (p<0.01) levels, and lower HDL (p<0.01) and apolipoprotein A (Apo-A) levels (p<0.01) than the control group.

Table 1. Baseline characteristics and data related to the oxidant-antioxidant status of the study groups

Parameters	Control (n=43)	Patients (n=97)	p
Male/female (n)	14/29	78/19	<0.001
Age (years)	33.4±6.48	38.3±5.23	<0.001
Smoker (n)	8	55	
Hypertension (n)	0	26	
Family history (n)	0	46	
Diabetes (n)	0	5	
Obesity (n)	0	29	
BMI (kg/m ²)	22.3±2.9	26.9±3.8	<0.001
Waist/hip ratio	0.81±0.13	1.10±1.23	0.222
Apo-A (mg/dL)	151±23.9	119±21.6	<0.001
Apo-B (mg/dL)	78.7±19.7	103±31.8	<0.001
TG (mg/dL)	97.8±45	231±204	<0.001
TC (mg/dL)	186±32	203±4	0.10
HDL (mg/dL)	51.1±8.38	41.3±9.3	<0.001
LDL (mg/dL)	115±29.2	119±48.1	0.711
Basal LDL-diene (μmol/mg pr)	159±49.5	192±74.5	0.030
Stimulated LDL-diene (μmol/mg pr)	203±47.8	213±66.7	0.703
Basal LDL-TBARS (nmol/mg pr)	0.34±0.11	0.39±0.19	0.278
Stimulated LDL-TBARS (nmol/mg pr)	3.54±1.43	4.72±2.57	0.025
PON (U/mL)	45.2±17.6	30.7±19.3	<0.001

Data are presented as mean±SD; Apo-A: Apolipoprotein A; Apo-B: Apolipoprotein B; BMI: Body mass index; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; PON: Paraoxonase; TBARS: Thiobarbituric acid reactive substances; TC: Total cholesterol; TG: Triglyceride.

The antioxidant-oxidant status parameters of the study group are summarized in Table 1. Indicators of LDL oxidation (LDL-diene and TBARS levels, $p<0.01$ and $p<0.05$, respectively) and LDL susceptibility to oxidation (copper-stimulated LDL-TBARS levels, $p<0.01$) were higher in the CAD patients compared with the control group. PON activity, an antioxidant parameter, was lower in the patient group.

Genotype frequencies

The B1B2 genotype frequency was higher in the patient group ($p=0.005$, chisquare test). The genotype distribution of CETP Taq1B gene polymorphism is shown in Table 2. The B1 and B2 allele frequencies were 0.60 and 0.40, respectively, in the controls and 0.53 and 0.47 in the patients.

When the baseline characteristics and the data related to oxidant-antioxidant status among the genotypes (B1B1, B1B2, and B2B2) were compared in the control group, it was found that only the BMI was higher in subjects with the B2B2 genotype (data not shown). Comparison of the data of baseline characteristics and oxidant-antioxidant status revealed no statistically significant difference in any parameters between genotypes (B1B1, B1B2, B2B2) in the patient group (data not shown). The biochemical parameters of the patients and the controls with the B1B1 genotype are shown in Table 3. The patient group had higher triglyceride levels ($p<0.01$) and age ($p<0.01$), and lower HDL ($p<0.01$) and Apo-A ($p<0.01$) levels than the control group. Biochemical analyses of the patients and controls who had the B1B2 genotype are shown in Table 3. The patients had higher

Table 2. Genotype frequencies of CETP Taq1B gene polymorphism in the study groups

	Control n (%)	Patient n (%)
B1B1 genotype	17 (39.5)	28 (28.9)
B1B2 genotype	18 (41.9)	47 (48.5)
B2B2 genotype	8 (18.6)	22 (22.7)
B1 allele	52 (60.4)	103 (53.1)
B2 allele	34 (39.5)	91 (46.9)

triglyceride levels ($p<0.01$), age ($p<0.01$), and Apo-B levels ($p<0.01$), and lower HDL ($p<0.01$) and Apo-A levels ($p<0.01$) than the control group. In addition, the patients had higher levels of basal LDL-diene and stimulated LDL-TBARS, which are indicators of LDL oxidation, and lower activity antioxidant parameter PON activity than the control group.

Biochemical analyses of the patients and controls who had the B2B2 genotype are shown in Table 3. The patients had higher triglyceride levels ($p<0.01$), and lower Apo-A levels ($p<0.01$) than the control group. In addition, the patients had lower levels of basal LDL-TBARS and PON activity than the control group.

Discussion

One of the most common causes of death worldwide is atherosclerosis. The incidence of atherosclerosis at a younger age and deaths due to MI have increased with increasing industrialization.

Table 3. Biochemical analysis of 3 genotypes of CETP Taq1B polymorphism in the study groups

Parameters	B1B1			B1B2			B2B2		
	Control (n=17)	Patients (n=28)	p	Control (n=18)	Patient (n=47)	P	Control (n=8)	Patient (n=22)	p
Age (years)	33.2±6.00	39.0±5.55	0.002	31.9±6.46	37.5±5.46	<0.001	36.9±6.93	39.3±6.34	0.533
TC (mg/dL)	193±34.3	201±55.8	0.940	179±29.6	203±54.7	0.107	185±32.1	206±53.3	0.177
TG (mg/dL)	104±43.9	251±283	<0.001	83.3±27.0	187±102	<0.001	121±70.7	297±225	0.043
HDL (mg/dL)	51.4±8.86	40.8±9.32	<0.001	51.6±8.98	40.2±8.29	<0.001	49.1±6.12	44.2±11.1	0.406
LDL (mg/dL)	121±30.5	114± 40.5	0.623	111±25.5	122±54.1	0.522	121±36.7	121±45.4	0.836
Apo-A (mg/dL)	151±29.0	118±22.9	<0.001	153±23.2	121±22.3	<0.001	148±13.0	120±19.1	0.002
Apo-B (mg/dL)	81.7±21.8	95.5±32.4	0.291	76.1±18.8	112±33.4	<0.001	78.7±19.0	94.8±22.8	0.131
Basal LDL-diene (µmol/mg pr)	171±39.4	189±60.8	0.462	143±55.8	197±85.5	<0.001	172±47.4	187±67.9	0.961
Stimulated LDL-diene (µmol/mg pr)	213±36.5	217±67.9	0.874	191±56.4	213±68.3	0.911	211±46.4	206±64.3	0.354
Basal LDL-TBARS (nmol/mg pr)	0.33±0.12	0.45±0.24	0.829	0.31±0.10	0.37±0.16	0.136	0.41±0.77	0.35±0.19	0.026
Stimulated LDL-TBARS (nmol/mg pr)	3.93±1.02	4.54±1.59	0.991	3.38±1.82	4.69±2.73	0.018	3.12±1.14	5.01±3.23	0.051
PON (U/mL)	41.6±18.0	32.3±21.7	0.106	48.4±19.8	32.2±19.3	0.003	45.2±17.6	25.5±15.5	0.002

Data are presented as mean±SD or median (25th-75th percentile). Apo-A: Apolipoprotein A; Apo-B: Apolipoprotein B; BMI: Body mass index; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; PON: Paraoxonase; TBARS: Thiobarbituric acid reactive substances; TC: Total cholesterol; TG: Triglyceride.

Oxidized LDL and low HDL levels play a central role in the development of atherosclerosis. The suggested antioxidant properties of HDL are related to PON as well as Apo-A and platelet activating factor acetylhydrolase. As expected, our study results showed that the conventional risk factors were higher in the CAD group. The lipoprotein profile of the patients was compatible with CAD. Only intermediate-density lipoprotein and LDL particles can enter the subintimal space of the vasculature where the atherosclerotic process begins through modified LDL, and LDL molecules are then absorbed by macrophages [20]. Therefore, LDL and modified LDL molecules, especially oxidized LDL, have an important role during the atherosclerotic process. In our study, we examined the ox-LDL levels and the sensitivity of LDL to oxidation in young patients with CAD. The LDL-diene and TBARS levels and the LDL-oxidation sensitivity (copper-induced LDL-TBARS levels) of the patients were higher than those of the control group. Consistent with our data, it has been reported that LDL was more susceptible to oxidation in patients with diabetes mellitus and CAD [21, 22]. In our study, PON activity was lower and the basal LDL-diene level was higher in patients, once again demonstrating the role of PON in preventing LDL oxidation [23].

More genetic studies are being conducted to identify possible genes and polymorphisms in CAD formation. Genome-wide association (GWA) studies have so far found 150 gene loci associated with plasma lipids [24]. Among these genetic variants, ZNF259, CETP, LPA, LPL and PSRC1 have been reported associated with dyslipidemia and CAD [25]. Recently CETP has been shown to play a role in regulating plasma HDL levels. The presence of B2

allele has been suggested to be associated with lower plasma CETP concentration, higher HDL-C level. B2 allele frequencies of the individuals in the study were 0.40 in controls and 0.47 in patients. These results are consistent with previous studies in the Turkish population [26-28]. Tanrikulu-Kucuk S et al. [26] reported that B2 allele frequency was 0.46. Yilmaz et al. [27] found that B2 allele frequency was 0.40. Ozsait et al. [28] reported that B2 allele frequency was 0.43 in men and 0.43 in women. Differences in frequency of the B2 allele was reported in other ethnic groups. B2 allele frequency was found 0.396, 0.40, 0.37, 0.49 in Italy, Chinese, Malaysians and Indians living in Singapore, respectively [29, 30]. Although the distribution of alleles displays similarities between societies, the question of whether certain polymorphisms have more risk for CAD has not yet been clarified.

While the distribution of 3 CETP genotypes in the control group did not demonstrate a statistically significant difference, the B1B2 genotype frequency was 10.536 times higher in our patient group.

In the control group, BMI was found to be higher only in subjects with the B2B2 genotype. There was no significant difference in waist-to-hip ratio, BMI, or other biochemical and oxidant-antioxidant parameters among the 3 genotypes in the patient group.

Patients with the B1B1 allele had higher triglyceride levels with lower HDL and Apo-A levels compared with the control group. There was no significant difference in LDL oxidation products or PON enzyme activity between the patient and the control groups. It may be that high triglyceride levels, especially low HDL and Apo-A levels, are more important than total chole-

terol and LDL levels with respect to atherosclerosis development in those with the B1B1 genotype.

Patients with B1B2 had higher triglyceride, Apo-B, stimulated LDL-TBARS, and basal LDL-diene levels compared with the control group, whereas the levels of HDL, Apo-A and PON activity were lower. It can be concluded that patients in this group are more vulnerable to oxidation and other risk factors.

Patients with the B2B2 polymorphism had high triglyceride levels, low Apo-A and basal LDL-TBARS levels, and low PON activity. PON protects HDL and LDL from oxidation [32] by inhibiting lipid peroxide formation up to 90% in stimulated LDL [23]. Although HDL levels were higher in those with the B2B2 genotype, there was no significant difference in comparison with the control group (Fig. 1). It has been reported that patients with B2B2 polymorphism had low CETP activity, a high HDL, and a decreased risk of CAD [31]. Freeman et al. [13] observed that high HDL and low CETP activity in normolipidemic subjects with the B2 allele may be caused by environmental factors, such as smoking and alcohol use. It has also been reported that the first MI occurred later in people with the B2B2 allele [33]. In a meta-analysis, the B2 allele was reported to be a protective factor for ischemic stroke [34]. In a Framingham study group of 1411 males and 1505 females, B1B1 subjects had higher CETP and lower HDL levels than B1B2 or B2B2 individuals, and men with the B2 allele were reported to have a lower risk of developing CAD [14]. This result was thought to be the result of a reduced atherogenic lipid profile in the B2 allele. In an experimental study, the authors reported that cardiovascular mortality decreased by 30% in B1B2 carriers and by 68% in B2B2 carriers [35]. Yilmaz et al. [27] found that MI patients with the B1B1 genotype had lower HDL levels than patients with the B2B2 genotype. They reported that Taq1B polymorphism may be responsible for low HDL levels in both patients and healthy individuals in the Turkish population. In our study, we found that HDL levels in patients with B2B2 genotype were higher than the other two genotypes but it was not statistically significant. This may be due to the low number of the patient groups.

The results of our study revealed that LDL oxidation and oxidative stress indicators were higher in young CAD patients. However, there was no significant difference between the baseline characteristics and oxidant-antioxidant status parameters in CAD patients with different genotypes of CETP Taq1B polymorphism.

Conclusion

We concluded that the genetic polymorphism of CETP had no significant effect on CETP function and that CETP polymorphism cannot be proposed as an independent risk factor for cardiovascular events.

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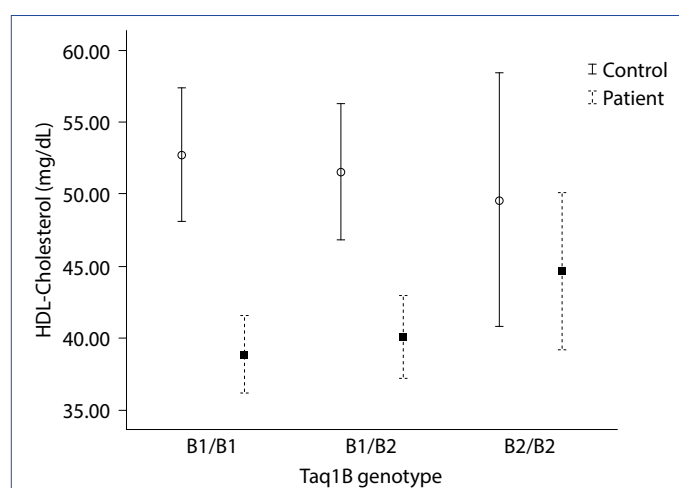


Figure 1. HDL-cholesterol levels in control and patients according to Taq1B polymorphisms.

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