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Research Article

Endothelial lipase 584C/T gene polymorphism in coronary artery disease in Elazig population: a pilot study

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Abstract

Objectives: Coronary artery disease (CAD) is one of the major causes of death in the world. It can be a result of environmental or genetic factors. A single nucleotide polymorphism (SNP) is a variation of 1 nucleotide sequence in any region of the genome, which may cause susceptibility to the disease. Therefore, SNPs have been proposed as ideal markers in disease association studies. Endothelial lipase (EL) is a protein of the triglyceride lipase family that plays an important role in high-density lipoprotein (HDL) metabolism. The EL gene has a common 584C/T polymorphism, but it is unclear whether this polymorphism is associated with HDL-cholesterol levels or CAD. The purpose of this study was to investigate the relationship between the EL 584C/T gene polymorphism, HDL level, and the risk of CAD in residents of Elazig province.

Methods: The population of this study consisted of 78 patients with angiographically confirmed CAD and 81 healthy controls. Genotyping for the 584C/T polymorphism was performed using the polymerase chain reaction-restriction fragment length polymorphism technique.

Results: The frequency of the CT, CC, and TT genotypes was 40.74%, 56.8%, and 2.46%, respectively, in the control group, and 66.67%, 30.37%, and 2.56%, respectively, in the CAD group. The Hardy-Weinberg value was $p<0.05$ for the control group and $p>0.05$ for the CAD group. No significant association was found between the 584C/T variant and HDL level. The T allele frequency was higher in the CAD group than among the controls.

Conclusion: It was concluded that the T allele was associated with a risk of CAD in Elazig province, independent of the HDL-cholesterol level.

Keywords: Coronary artery disease, endothelial lipase, polymorphism

Cardiovascular diseases, particularly coronary artery disease (CAD), remain the major cause of mortality worldwide [1]. Current evidence suggests that a positive family history of CAD, high levels of low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG), and decreased levels of high-density lipoprotein cholesterol (HDL-C) are important risk factors for CAD [2]. Many prospective randomized and epidemiological studies have demonstrated that a strong inverse relationship between HDL-C level and the risk of CAD. Population studies have also indicated a decrease in cardiovascular mortality of 2% to 3% for every 1 mg/dL increase in HDL-C level [3]. Recent research has recommended that the biological functions of HDL are important in defining the role of HDL in CAD and major cardiovascular events [4]. HDL particles have atheroprotective features, including reverse cholesterol transport, anti-inflammatory, anti-thrombotic, and anti-oxidant effects [5]. Hence, it is generally accepted that HDL-C levels are inversely associated with the risk of CAD [6]. It is thought that greater understanding of the factors contributing to HDL homeostasis will be important for public health.

One of the most important factors affecting HDL metabolism is the endothelial lipase (EL) enzyme, which prefers HDL

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as a substrate [7]. EL is a new member of the TG lipase gene family, which includes lipoprotein lipase (LPL) and hepatic lipase (HL), which is synthesized by endothelial cells and expressed in a variety of tissues, including the coronary arteries [8]. EL is the key enzyme regulating HDL-C levels to produce free fatty acids and low-fat apolipoprotein A1 and hydrolyze HDL-C [9]. Several genetic variants have been recognized in the LIPG gene. Among these, 584C/T is the most frequently studied variant that can change the amino acid threonine, converting it into isoleucine at codon 111 [10]. Some studies have supported the idea that the 584C/T variant was inversely correlated with serum HDL level [11, 12], but other studies did not find the same association [13, 14]. So far, the relationship between the 584C/T variant and the risk of CAD and HDL level has not been examined in Elazig province. Therefore, the objective of this study was to investigate whether 584C/T was associated with HDL level or the risk of CAD in the population of Elazıg province.

Materials and Methods

Study subjects

A total of 159 patients were enrolled in this hospital-based case-control study. CAD was defined as angiographic evidence of >50% organic stenosis in at least 1 segment of a major coronary artery, including the 3 main arteries: the left anterior descending artery, left circumflex artery, and the right coronary artery. Myocardial infarction (MI) history was described by integrating clinical history data, including details of the typical MI sequelae, enzyme changes, and electrocardiogram on ventricular angiography. The angiograms were reviewed by 2 cardiologists who were aware that the patients were to be included in this study. The controls, who were selected from individuals admitted to the hospital to rule out CAD, were diagnosed with a luminal stenosis of <50% of the major coronary arteries and without typical chest pain. Those with concomitant diseases, such as congenital heart disease, renal failure, and malignancies were excluded. Patients younger than 18 years were also excluded from the study. Whole blood from subjects participating in this research was obtained with the appropriate institutional review and informed consent documentation, which defined the study design and provided an assessment of the risks and benefits associated with participation, was also obtained. The DNA sample and relevant clinical data were anonymized prior to performing any genetic analysis. The medical ethics committee of Firat University Medical Faculty approved the study. (Project No: TF12.85, Decision No: 12/04).

Genotyping

Genomic DNA was isolated from peripheral blood leukocytes according to a Zymo Research kit (Catalog No: D 3073; Zymo Research, Irvine, CA, USA). Genotypes were determined using the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method. The sequences of the forward and backward primes used for 584C/T were 5′-CATGAGCTGAGATTGTTGTCAGTGC-3′ and 5′CAGTCAACCACAACT ACATTGGCGTCTTTCTCTCAT-3′, respectively. A total volume of 25 µL of reaction mix included 1-10 µg genomic DNA, 0.2 µm of each primer solution, 0.5 mM of each deoxynucleotide, 2.5 mM magnesium chloride, 150 mM trisaminomethanes–Hydrochloride, pH 8.0, 500 mM potassium chloride buffer and 1.0 U Taq polymerase.

The amplification protocol consisted of the following conditions: initial denaturation at 95°C for 2 minutes, amplification for 35 cycles at 95°C for 30 seconds, at 59°C for 45 seconds, at 72°C for 45 seconds, followed by a final elongation step at 72C° for 7 minutes. The PCR products were digested overnight with NdeI at 37°C. The 254 bp PCR product was split into 2 fragments of 217 and 37 bp for the T homozygote, 3 fragments of 254, 271, and 37 bp for the CT heterozygote, and the C homozygote remained whole, a 254 bp PCR product.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 22.0. (IBM Corp., Armonk, NY, USA). The Kolmogorov-Smirnov test was used to evaluate normal distribution of the variables. When there was a normal distribution, an independent samples t-test was used, and when the variables were not normally distributed, the Mann-Whitney U test was used. Numerical variables were expressed as mean±SD or as median and upper-lower limits.

Categorical variables were expressed as n (%) and Pearson's chi-square test was used to compare the groups according to gender. A chi-square test was used to analyze whether the distribution of observed and expected genotypic frequencies were within the Hardy-Weinberg equilibrium. A p value <0.05 was accepted as statistically significant.

Results

Baseline Characteristics

The baseline characteristics of the 2 groups are presented in Table 1. There was a statistically significant difference in the mean total cholesterol and the HDL-C level ($p<0.05$). The frequency of hypertension and the LDL-C level was higher in the CAD group when compared with the controls ($p<0.05$). Statistically significant differences were not observed in analysis of gender, age, mean of systolic and diastolic pressure, very low-density lipoprotein (VLDL), or TG levels.

Genotype Frequencies

The distribution of endothelial lipase gene (LIPG) 584C/T genotypes in the patient group was: CC 24 (30.37%), CT 52 (66.67%), and TT 2 (2.56%), whereas in the control group it was CC 46 (56.8 %), CT 33 (40.74 %), and TT 2 (2.46 %). According to the Hardy-Weinberg principle, the alleles, genotypes, and

Table 1. Baseline characteristics of the controls and patients

Risk factors	Controls (n=81)	CAD (n=78)	p value
Gender (female/male)	39 (48.1%)/42 (51.9)	30 (38.5%)/48 (61.5%)	0.218
Age (years)	57.98±8.62	60.57±9.32	0.071
Systolic pressure (mmHg)	110 (90-170)	120 (90-180)	0.087
Diastolic pressure (mmHg)	70 (60-110)	80 (60-110)	0.064
Hypertension: Yes/no (%)	13 (16%)/68 (84%)	26 (33.3%)/52 (66.7%)	0.011*
Total cholesterol (mg/dL)	162 (79-332)	200 (108-417)	0.000**
HDL cholesterol (mg/dL)	43 (28-73)	40 (16.5-92.9)	0.019*
LDL cholesterol (mg/dL)	115.34±45.12	132.69±40.86	0.012*
VLDL cholesterol (mg/dL)	24 (10-215)	27 (7-182)	0.632
Triglyceride (mg/dL)	121 (43-730)	136 (37-911)	0.419

*p<0.05, compared with the control group. **p<0.01, compared with the control group.
Categorical variables are expressed as n (%), numerical variables are expressed as mean±SD or median and upper-lower limits.
CAD: Coronary artery disease; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; VLDL: Very low-density lipoprotein.

Table 2. Hardy-Weinberg equilibrium of the study groups

Study groups	Genotypes	Observed frequency	Expected frequency	Chi Square	HWE p value	(CC+TC) C allele	(TT+TC) T allele
Control	CT	33	38.7	8.859	0.002*	125(77.16%)	37(22.84%)
	CC	46	34.6				
	TT	2	7.7				
CAD	CT	52	52.5	0.155	0.693	100(64.10%)	56(35.89%)
	CC	24	23				
	TT	2	2.5				

CAD: Coronary artery disease; HWE: Hardy-Weinberg equilibrium.
*p<0.05.

Table 3. Effects of endothelial lipase gene polymorphism variants on lipid parameters in the study groups

	Control group			
	CT (n=33)	CC (n=46)	TT (n=2)	p value
Total cholesterol (mg/dL)	172 (79-332)	157 (79-304)	247 (238-256)	0.758
HDL cholesterol (mg/dL)	43 (32-73)	43 (28-73)	36.5 (32-41)	0.477
LDL cholesterol (mg/dL)	113 (42-201.1)	103.9 (36.2-221)	197.5 (181-214)	0.490
Triglyceride (mg/dL)	110 (66-730)	123 (43-348)	226.5 (124-329)	0.808
VLDL (mg/dL)	22 (13-146)	25 (10-215)	45.5 (22-66)	0.929
	CAD group			
	CT (n=52)	CC (n=24)	TT (n=2)	p value
Total cholesterol (mg/dL)	195.5 (108-417)	206.5 (161-385)	172 (130-214)	0.183
HDL cholesterol (mg/dL)	41.35 (20.07-92.9)	39 (16.5-63)	30 (22-38)	0.134
LDL cholesterol (mg/dL)	121.5 (57.9-226)	135.5 (82-282)	97.5 (58-137)	0.325
Triglyceride (mg/dL)	136 (37-439)	152 (68-911)	161 (107-215)	0.492
VLDL (mg/dL)	27 (7-88)	29.39 (14-182)	32.5 (21-44)	0.619

Numerical variables are expressed as median and upper-lower limits.
CAD: Coronary artery disease; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; VLDL: Very low-density lipoprotein.

frequencies of generations remain constant in the gene pool of the population if there are no factors influencing gene frequency. It was determined that the control group was not well balanced, as the Hardy-Weinberg p value was less than 0.05. This inconsistency of genotype distribution may have been due to factors such as consanguineous marriage and migra-

Table 4. Relationship between high-density lipoprotein and age, blood pressure, and lipid parameters

	HDL	Total cholesterol	LDL	VLDL	TG	Age	Systolic pressure	Diastolic pressure
HDL	n=78 r=1	n=78 r=-0.027 p=0.818	n=78 r=-0.074 p=0.517	n=78 r=-0.042 p=0.713	n=78 r=-0.050 p=0.667	n=78 r=0.001 p=0.990	n=78 r=-0.082 p=0.474	n=78 r=0.019 p=0.867

The Spearman correlation test was performed and a p value of <0.05 was considered statistically significant.
HDL: High-density lipoprotein; LDL: Low-density lipoprotein; TG: Triglyceride; VLDL: Very low-density lipoprotein.

tion rates, which affect the frequency of genes. Firat University Hospital, where the samples were collected, accepts many patients from the eastern and southeastern Anatolia region. Genotype frequencies may have changed due to the gene flow generated by the presence of individuals originally from other geographical regions (Table 2).

The effects of EL polymorphism variants on lipid parameters in the study groups are illustrated in Table 3. There was no statistically significant difference in the total lipid parameters ($p>0.05$).

A Spearman correlation test was performed to determine if there was any correlation between HDL and other parameters and no statistically significant difference was observed ($p>0.05$). The results are provided in Table 4.

There was no statistically significant association between HDL and hypertension or genotype. The p values were 0.589 and 0.140, respectively.

Discussion

Cardiovascular diseases are one of the most important causes of mortality and morbidity, and large-scale research findings indicate that worldwide coronary mortality may increase from 28.9% to 36.3% between 1990 and 2020 [15]. Hypertension, smoking, oxidant-antioxidant factors, familial predisposition, diabetes, and changes in total cholesterol, HDL-C, LDL-C, VLDL-C, and TG levels may cause the development of atherosclerosis [16, 12]. The genetic diversity of several genes within this pathway has been investigated, but data on the genetic contribution of more recently identified genes, such as LIPG, are limited. Studies of the 584C/T polymorphism have had different and conflicting results about the effect of human serum/plasma HDL level [10, 11, 17, 18]. Hutter et al. [19] found a weak association between the LIPG 584C/T polymorphism and HDL-C level in their study. Tang et al. [20] also found a weak correlation between Thr111 replacement and HDL level. In another study, a significant association was also found between 584C/T and HDL level [21].

In contrast, Shimizu et al. [22] reported that the 584C/T gene polymorphism was associated with acute MI, but this association was independent of plasma HDL level. De lemos et al. [23] conducted a study of 3 groups: 176 black control participants, 165 white controls, and 123 white individuals with a high HDL-cholesterol level. They demonstrated that the T allele was not significantly associated with plasma HDL level. In another study, Yamakawa-Kobayashi et al. [12] found that there was no correlation between serum HDL level and the T allele in Japanese school-aged children. In our study, no statistically significant association was found between the LIPG 584C/T polymorphism and HDL-cholesterol level. The relationship between the 584C/T polymorphism and HDL varies in different studies; this may be the result of study groups selected from different races and ethnic groups.

Conclusion

This study was carried out in the province of Elazig, Turkey, to investigate the frequency of the EL 584C/T polymorphism in healthy subjects and CAD patients who had different nutritional conditions and social status, and to investigate the distribution of alleles and genotypes. In our study, the CT genotype was more prevalent than the CC genotype. These data suggest that people with a mutant T allele may have an increased risk of developing coronary artery atherosclerotic disease. It is suggested that the EL 584C/T gene polymorphism may be an important parameter in terms of CAD independent of HDL level and it is important to investigate the genetic basis of the disease and to follow up the prognosis. For this reason, follow-on study of this research examining other parameters and subgroups may be important related to the pathogenesis of CAD in larger populations.

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