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Endothelial Lipase Gene Polymorphism (584 C/T) in Coronary Artery Patients Among a Turkish Population

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Abstract. *Background/Aim: The endothelial lipase gene (LIPG) has a major role in regulating high density lipoprotein cholesterol (HDL-C), therefore this study investigated whether LIPG is associated with coronary artery disease (CAD) in a Turkish population. Materials and Methods: The LIPG (584 C/T) mutation was analyzed in 74 CAD patients and 73 controls. Results: There was a significant difference between the two groups regarding the mutant T allele frequencies (χ^2=0.456, p=0.020; 26.7% and 41.8% in patient and control groups, respectively) for 584 C/T. Even though the TT genotype was not significantly different, it had p=0.054 which supported our results. Conclusion: The endothelial lipase gene (584 C/T) T allele might be protective in association with coronary artery disease. Therefore, LIPG gene is related to risk for CAD in the Turkish population probably through altering HDL-C metabolism.*

Coronary artery disease (CAD) affects millions of people around the world and is known to be the most common type of heart disease. It is known to be a multifactorial, having both genetic and environmental contributes, disease which has been the focus of many studies (1). Certain studies point out that low levels of high-density lipoprotein cholesterol (HDL-C) and increased triglyceride (TG) and are the main risk factors for the pathogenesis of the condition (2). Regarding the genetic contribution of the disease, variations in members of gene families that effect the HDL metabolism including, lecithin cholesterol acyltransferase (LCAT), hepatic lipase (LIPC), cholesteryl ester transfer protein (CETP) are being studied among some ethnic groups (3).

The endothelial lipase gene (*LIPG*, 584 C/T) is located on 18q21.1; the protein product, which is a member of the TG lipase family, has substantial phospholipase activity and most probably functions in lipoprotein metabolism and associated systems such as vascular structures. It has been shown that overexpression of the gene may lead to reduction of the plasma concentrations of HDL-C and, on the contrary, under-expression leads to increased levels (4-6). Previous works that focused on the human LIPG and its dynamics were able to present an association between the inflammation process and LIPG (7-9). In addition, plasma LIPG levels have been of critical significance in the inflammatory state of the metabolic syndrome. In overweight subjects, LIPG levels have shown a positive correlation with hypertension, TG levels and obesity, whereas a negative association has been observed with HDL-C levels (7).

In light of these data, studying LIPG is a promising way to better understand the nature of CAD and it may be a way for the prevention and treatment of the disease. Investigation of the associations of *lipg* gene variants with CAD was the main purpose of this study.

Materials and Methods

Study population. In the Marmara University Cardiovascular Surgery Department, a group of patients who had coronary artery disease was selected from cardiovascular surgery department. A control group was also selected from Marmara University Hospital. From a total of 147 subjects, 74 were CAD patients (20 females and 54 males, mean age: 63.04±8,922 years) and 73 were healthy control patients (25 females and 48 males, mean age: 66.01±8,035 years). Diagnosis of CAD was confirmed according to the criteria of WHO from 1978. The main criterion is observing >%50 luminal stenosis in angiography of at least one major coronary vessel. None of the patients took hypolipidemic drugs prior to the lipid

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measurement and angiography. The coronary vessels taken into consideration were; left anterior descending (LAD), left main coronary artery (LCA), left circumflex (LC), right coronary artery (RCA) and other large branches. The control group was selected *via* health examinations and group members' health confirmed *via* clinical and biochemical tests and examinations. There were no signs or symptoms of CAD in any one of them. All members of groups were selected randomly between March 2015 and July 2016.

Biochemical analysis and genotyping. Peripheral blood samples were collected after overnight fasting in EDTA containing tubes (purple) and serum separating gel containing tubes (yellow), after getting informed consent from each person in the patient and control groups. Yellow tubes were centrifuged to separate the plasma and cells and plasma were collected. Collected plasmas were used immediately for biochemical analysis. Total cholesterol, triglyceride, HDL (high density lipoprotein), LDL (low density lipoprotein) levels were measured. 350 μl whole peripheral blood samples were used for DNA isolation which was performed by Invitrogen iPrep purification instrument and Invitrogen iPrep PureLink gDNA blood kits (Invitrogen, Life Technologies, Carlsbad, CA, USA). 100 μl DNA was isolated by iPrep purification instrument. Isolated DNA samples were measured by NanoDrop 2000 (Thermo Scientific, Waltham, MA, USA) for concentrations and optical density. Applied biosystems fast real-time polymerase chain reaction (RT-PCR) instrument and TaqMan reagents primer-probe sets (Applied Biosystems, Foster City, CA, USA) was preferred for genotyping. Genotyping was performed for *LIPG*(584C/T) genes. The PCR reaction mixture's ingredients were: 10 μl X Genotyping master mix, 0.5 μl 40X TaqMan genotyping assay (TaqMan Reagents; Applied Biosystems), 8.5 μl PCR grade water and 1 μl of sample DNA. This mixture was prepared and used as recommended by the manufacturer. PCR was applied according to the following procedure: Hold stage at 95˚C for 10 min, denaturation at 92˚C for 15 sec and 40 cycles and annealing/extension at 60˚C for 60 sec as suggested by supplier. Allelic discrimination of samples was done by collecting and interpreting the fluorescent data of hybridizing probes by the software of 7500 fast real-time PCR instrument.

Statistical analyses. SPSS Version 23 software (SPSS Inc. Chicago, IL, USA) was used for statistical analyses. Student's *t*-test was used to determine the significance of differences between groups and demographics were compared by χ^2 and Fisher's exact tests. Binary logistic regression analysis as odds ratio (OR) at 95% confidence interval (CI). $p<0.05$ was denoted as statistically significant.

Results

The demographic characteristics of control and patient groups are given in Table I. The mean age of patients with coronary artery disease and healthy controls was 63.04±8.92 years and 66.01±8.035 years, respectively. There is a significant difference between patients and controls in terms of median age ($p=0.036$), however the older group was the control group. Mean LDL level of patient group was 117.52±44.159 mg/dl and of the control group was 85.99±40.927 mg/dl. The patient group had significantly higher LDL ($p=0.000$) compared to the control group and *vice versa* on HDL levels ($p=0.000$) of groups. The allelic and genotypic frequencies for LIPG (584 C/T) in patients with coronary artery disease and controls are given Table II. There was a significant difference in genotypic variations between the groups, the CC genotype had a *p*-value equal to 0.020 and odds ratio(OR)=1.191. Even though TT genotype did not have significant value it had $p=0.054$ which supported our results. The frequencies of CC, TT, and CT genotypes among the patients with coronary artery disease were 54.8%, 8.2% and 37.0%, respectively, and among the control subjects were 35.6%, 19.2%, and 45.2%, respectively. The frequency of LIPG (584 C/T) wild-type C allele was 73.3% in patient group and 58.2% in control group and there was no statistically significant difference between the groups ($\chi^2=2.650$; $p=0.054$). Mutant T allele frequencies were 26.7% and 41.8% in patient and control groups, respectively and there was a statistically significant difference between the groups. ($\chi^2=0.456$; $p=0.020$). Also, there was a significant difference between the groups in the frequency of T allele; $p=0.020$, or $p=0.456$ and again without significant difference in the C allele frequency with $p=0.054$. As we expected, there were also significant differences between the control and patient group regarding diabetes mellitus type 2, smoking, tage, gender, BMI, Triglyceride levels and hypertension which shows stereotypic correlations with coronary artery disease.

Discussion

Recently an increasing attention on genetic studies regarding coronary artery disease (CAD) has been recorded, which is not surprising if one considers the effect of CAD on the health of World's population (1). There are 50 genetic variations which were found to be related with CAD (10). However, there are very few articles addressing the correlation of lipoprotein lipase gene (LIPG) with CAD than expected. Our research group aimed to focus on the relation between CAD and polymorphic variations in *LIPG* (also known as endothelial lipase gene) at position 584 C/T. Therefore, we determined the genetic frequencies in CAD patients and control groups.

In a similar study which was performed in Han Chinese population, the results showed that there was no significant difference in the polymorphic distribution between the groups of patients and controls (11, 12). Also, in a meta-analysis investigating the associations between LIPG and CAD, the results were controversial however, T allele carriers have higher HDL-C levels in Caucasian populations (13). Nevertheless, our results suggest that the T allele might be protective for CAD which was also shown in studies on Fars province and Chinese population.

Endothelial Lipase (EL) is a member of Triglyceride Lipase family and is produced by the endothelial cells, which are abundant in tissues of the coronary arteries, liver, lung, kidney,

Table I. *Demographic characteristics of the population in our study.*

	Control (n=73)	CAD (n=74)	*p*-Value
Gender			
Male	%65.8 (n=48)	% 73.0 (n=54)	0.0375
Female	%34.2 (n=25)	% 27.0 (n=20)	
Age (Year)	66.01±8.035	63.04±8.922	0.036
COPD	% 8.2 (n=6)	% 8.3 (n=6)	1.000
Diabetes Mellitus	% 17.4 (n=8)	% 52.1 (n=38)	0.000
Chronic Renal Failure	% 4.1 (n=3)	% 6.9 (n=5)	0.494
Hypertension			
Systolic blod pressure >140			
Diastolic blod pressure >90			
+	% 19.2 (n=14)	% 64.4 (n=47)	0.000
–	% 80.8 (n=59)	% 35.6 (n=26)	
Smoking	% 32.9 (n=24)	%83.8 (n=62)	0.000
Body Mass Index (BMI) (kg/m^2)	26.88±2.80	28.47±4.09	0.010
Plasma Cholesterol (mg/dl)	184.66±31.014	193.73±55.164	0.225
TG (mg/dl)	122.33±76.554	162.31±93.537	0.006
HDL (mg/dl)	75.18±37.697	43.13±10.442	0.000
LDL (mg/dl)	85.99±40.927	117.52±44.159	0.000

n: Number of individuals; ±SD: standard deviation; TG: triglyceride; LDL: low-density lipoprotein; COPD: chronic obstructive pulmonary disease; HDL: high density lipoprotein; *statistically significant difference.

Table II. *Genotype and allele frequencies in our study group.*

	CAD n (%)	Control n (%)	*p*-Value	OR	95%CI
LIPG (584 C/T) genotype					
CC	40 (54.8%)	26 (35.6%)	0.020	2.191	1.127-4.260
CT	27 (37.0%)	33 (45.2%)	0.313	0.711	0.136-1.045
TT	6 (8.2%)	14 (19.2%)	0.054	0.377	0.367-1.379
LIPG (584 C/T) allele					
C	107 (73.3%)	85 (58.2%)	0.054	2.650	0.957-7.336
T	39 (26.7%)	61 (41.8%)	0.020	0.456	0.235-0.887

N: Number of individuals; OR: odds ratio; CI: confidence interval. *p*-Values less than 0.05 denoted statistical significance.

ovary, thyroid gland, testis, and placenta (14). It shows 45% homology with lipoprotein lipase (LPL), 40% with hepatic lipase (HPL) and 27% with pancreatic lipase (PL); plus, it shares some typical properties such as catalytic triad, heparin binding sites, lipid-binding regions and cysteine residues with LPL and HL. On the other hand, it differs from those two in having high phospholipase and low TG lipase activity; it regulates the HDL-C levels with mainly its high phospholipase activity by hydrolyzing the HDL molecule in to smaller pieces (14). It has been shown that overexpression of the gene may lead to the reduction of the plasma concentrations of HDL-C (4-6, 13) and on the contrary its under-expression leads to increased levels (15, 16).

The metabolism of HDL is a complex process to precisely understand and control. However, it is a very important biochemical pathway, which is thought be one of the significant factors of CAD. Therefore, it has the potential to become an important target for the resolution of this disease. Results of studies on human and mouse have shown that EL has major influence on the regulation of HDL metabolism (5, 6, 17-19) and over expression of EL correlates with decreased levels of HDL-C (20). Also, it has been shown that HDL-C levels in human were negatively correlated with plasma EL levels (21) and impairment in EL results in high levels of HDL-C (22). Our study is the first to show the effect and correlation of polymorphic variations in the *LIPG* with CAD in Turkish people living in Turkey.

Our present study revealed that the CC genotype increases the risk of CAD approximately 2-folds (p=0.020). Also, the TT genotype is protective with a risk ratio of 0.377. The allele

frequencies results support our hypothesis. Carriers of the C allele have about 2,6 times increased risk of CAD *p*-value was 0.054. Although the difference was not statistically different, in our own consideration it was close enough to not discard it from our discussion. Also, carriers of the T allele were protected from CAD (p=0.020) with the risk ratio of 0,456 (23, 17).

Although, the majority of our results are significant and supporting each other, there are some differences compared with other articles (1, 11, 12). The differences may be due to different ethnicities with varied genetical features. Perhaps, these controversies may show the potential importance of pharmacogenetics and might eventually relate to it.

Conclusion

As mentioned, this study was the first to investigate the *LIPG* polymorphism (584 C/T) – CAD correlation study in Turkish people living in Turkey. Results of this study suggest that the CC genotype may be a genetic risk factor for CAD and carrying T allele may be protective against CAD.

Conflicts of Interest

The Authors declare that they have conflicts of interest regarding this study.

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