Lipoprotein Lipase Gene Polymorphism and Lipid Profile in Coronary Artery Disease

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• ***Context.*—Lipoprotein lipase (LPL) plays a central role in lipid metabolism, hydrolyzing triglyceride in chylomicrons and very-low-density lipoproteins. The *Pvu*II polymorphic variant of LPL gene is common and might affect risk of coronary artery disease (CAD).**

***Objective.*—Our aim was to determine whether LPL–*Pvu*II polymorphism can be considered to be an independent risk factor or a predictor for CAD in Turkish subjects.**

***Design.*—We used polymerase chain reaction and restriction enzyme digestion to determine the distribution of the previously described C→T transition that causes a *Pvu*II polymorphism in intron 6 among healthy blood donors of Turkish origin and among angiographically confirmed CAD patients with comparable ethnic backgrounds.**

***Results.*—For the *Pvu*II genotypes, within the CAD group (n = 80), the +/− genotype was found in 39 individuals (48.8%), whereas 25 (31.3%) carried the +/+ genotype, and 14 (17.5%) carried the −/− genotype. Within the control group (n = 49), the −/− genotype was found in 19 individuals (38.8%), 16 (32.7%) carried the +/− genotype, and 14 (28.6%) carried the +/+ genotype. The genotype frequency distribution was significantly different (*P* = .049) in the CAD and control study groups. The most frequent genotype among CAD patients was +/−; this genotype was more frequent in patients than in control subjects. However, the −/− genotype was more prevalent in the control group. Lipoprotein lipase–*Pvu*II polymorphism was found to be associated with fasting total cholesterol and low-density lipoprotein cholesterol levels. The +/+ genotype was found to have higher levels of total cholesterol and low-density lipoprotein cholesterol in both the CAD and control groups.**

***Conclusion.*—There was a difference in the distribution of LPL–*Pvu*II genotypes between the healthy subjects and the patients with CAD. Lipoprotein lipase–*Pvu*II polymorphisms were not detected as independent risk factors for CAD in this study group, but had associations with lipid levels.**

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Lipoprotein lipase (LPL) plays a key role in lipid metabolism by hydrolyzing triglycerides in circulating lipoproteins, which constitutes the rate-limiting step in removal of triglyceride-rich lipoproteins, such as chylomicrons and very-low-density lipoproteins (VLDL) from the circulation.[1,2] Lipoprotein lipase is multifunctional and has been shown to serve as a ligand for low-density lipoprotein (LDL) receptor–related protein, to decrease the hepatic secretion, and to increase the uptake of VLDLs and LDL cholesterol.[3] Lipoprotein lipase also promotes the exchange of lipids between VLDL and high-density lipoproteins (HDLs).[1] Because of its key role in lipoprotein metabolism, LPL is likely to be an important factor in the development of dyslipidemia and, thus, atherosclerotic changes. Rare LPL mutations are known to cause marked dyslipidemia (eg, familial LPL deficiency with chylomicronemia)[2]; at least some of these mutations are known to be associated with premature atherosclerosis.[4]

The LPL gene is located on chromosome 8p22, spanning about 35 kilobases (kb) and containing 10 exons.[5] Several restriction fragment length polymorphisms (RFLPs) have been identified at the LPL gene. These include polymorphisms identified with *Bam*HI,[6] *Pvu*II,[6,7] *Hin*dIII,[8] *Bst*NI,[9] *Bst*I,[10] *Bgl*II,[11] and *Xba*I.[12] Those polymorphisms defined by the *Hin*dIII and *Pvu*II RFLP sites (located on introns 8 and 6, respectively, of the LPL gene) are the most common and may be associated with profound alterations in plasma lipids. *Pvu*II polymorphism is the result of C→T transition in the restriction site of the LPL gene intron 6, 1.57 kb from the SA site.[13] The region containing the *Pvu*II site resembles the splicing site in its homology to the consensus sequence required for 3′-splicing and the formation of the lariat structure, suggesting that C^{497}→T (CAG CTG ⇒ TAG CTG) change may interfere with correct splicing of messenger RNA.

Several trials have explored associations between LPL gene polymorphisms and lipoprotein phenotypes.[4,12,14–24]

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The *Hin*dIII (+) allele or the *Hin*dIII (+/+) genotype has been reported to be associated with an atherogenic profile (elevated triglycerides and/or decreased HDL cholesterol).[14–16,18–21] Some studies provided evidence for an association between genotypes identified by the *Pvu*II RFLP and triglyceride and HDL-cholesterol levels,[17,18,25–27] but others failed to find significant association with any lipid parameter.[28–30] The P+ allele of the *Pvu*II RFLP has been found to be associated with higher triglyceride[17] and lower HDL-cholesterol levels.[16] The LPL–*Pvu*II polymorphisms have been variably reported to be associated with coronary artery disease (CAD). While some investigators have shown an association between CAD and *Pvu*II genotypes,[18,31] other authors have found no significant association of *Pvu*II polymorphisms with CAD.[27–29,32]

Polymorphisms in genes related to CAD pathophysiology often have required multiple and large studies to clearly define attributable genetic risk. The allelic frequencies for the *Pvu*II (+) allele were 0.58, 0.64, 0.55, and 0.49 for Northern European,[31] Chinese,[33] white,[34] and North American[19] populations, respectively.

The aim of this study was to determine the potential relevance of the LPL–*Pvu*II variant over lipid parameters in patients with CAD and to assess whether the variant can act as an independent genetic risk factor for CAD in this study group.

MATERIALS AND METHODS

Patients and Control Subjects

The study group was composed of 80 individuals (60 men, 20 women) of Turkish descent with angiographically documented CAD. Additionally, 45 (14 men, 35 women) angiographically healthy inpatients at the Florence Nightingale Hospital (İstanbul, Turkey) were recruited as the control group to determine the carrier distribution of LPL–*Pvu*II polymorphism among the subjects. Both groups were matched for age (58.43 ± 0.96 years for CAD, 58.98 ± 1.69 years for control subjects [mean ± SE]), as well as for social and economic status.

Sample Collection and DNA Extraction

Ten milliliters of peripheral blood was collected, following informed consent, from all individuals who participated in this study. The blood was anticoagulated by collection in EDTA blood tubes. DNA extraction was performed using the PUREGENE DNA isolation kit from Gentra Systems (Minneapolis, Minn) and stored in aliquots at −20°C until required.

Criteria for CAD Risk Factors

Hypertension (blood pressure, >130/80 mm Hg or on drug therapy), diabetes mellitus (the diagnosis of type 2 diabetes mellitus was based on the criteria of the World Health Organization),[35] dyslipidemia (HDL cholesterol, <45 mg/dL; triglycerides, >150 mg/dL; and LDL cholesterol, >130 mg/dL), hypercholesterolemia (total cholesterol, >200 mg/dL; LDL cholesterol, >130 mg/dL or patients treated with antilipidemic agents), obesity (body mass index, >25), and smoking (current smokers), which are known as major CAD risk factors, were determined by viewing medical data and reviewing standardized questionnaires completed by the study subjects. All patients were admitted with a diagnosis of angina or myocardial infarction. Total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, and plasma glucose levels were measured after overnight fasting. Because it would not be ethically suitable in patients who had CAD with dyslipidemia, lipid-lowering drugs were not withheld before lipid testing.

Table 1. Risk Factors for Coronary Artery Disease in Patients and Control Subjects

	Patient (n = 80), No. (%)	Control Subjects (n = 49), No. (%)	P*
Dyslipidemia	56 (70)	17 (34.7)	<.001
Hypertension	49 (61.3)	12 (24.5)	<.001
Diabetes mellitus	28 (35)	4 (8.2)	<.001
Obesity	49 (61.3)	29 (59.2)	.71
Smokers	15 (18.8)	0 (0)	<.001

* The variables were compared with χ^2 test among groups. $P < .05$ was considered to be statistically significant.

Biochemical Measurements

The plasma glucose concentration was measured by the glucose oxidase method using a Biotrol kit on a Bayer opeRA analyzer. Serum total cholesterol was measured using the Biotrol commercial kit, HDL cholesterol was determined with a commercial Randox kit, LDL cholesterol was calculated by the formula of Friedewald, and triglyceride determination was made by the method of lipase/glycerol kinase UV endpoint on the opeRA analyzer.

Determination of the LPL–*Pvu*II Genotypes

The *Pvu*II genotypes were determined by polymerase chain reaction (PCR) amplification of the polymorphic regions found in intron 6, followed by digestion of these amplified fragments with *Pvu*II restriction endonuclease.[31]

The *Pvu*II-containing site was amplified using the following primers: forward primer 5′-ATG GCA CCC ATG TGT AAG GTG-3′ and reverse primer 5′-GTG AAC TTC TGA TAA CAA TCT C-3′. Each 25-μL PCR reaction contained 2.5 μL of 10× reaction buffer with magnesium chloride; 10 ρmol of each primer; 100 ρmol/μL each of deoxyadenosine triphosphate, deoxyguanosine triphosphate, deoxycytidine triphosphate, and deoxythymidine triphosphate in Tris-hydrochloride buffer; 1 unit Taq DNA polymerase; and 100 ng genomic DNA template. The mixture was amplified in a denaturing segment (94°C, 20 seconds), an annealing segment (50°C, 30 seconds), and an extension segment (72°C, 20 seconds), repeated for 30 cycles with a final extension of 7 minutes. The amplified product is 430 base pairs (bp). Polymerase chain reaction products were digested for 3 to 5 hours at 37°C, and digested products were resolved on a 4% Metaphore agarose gel (FMC BioProducts, Rockland, Me) for 3 hours in TE buffer containing 0.5 μg/mL ethidium bromide, and the sizes of the digested amplicons were determined using the 100-bp ladder (MBI Fermentas, Hanover, Md). The *Pvu*II restriction site yields 320- and 110-bp fragments.

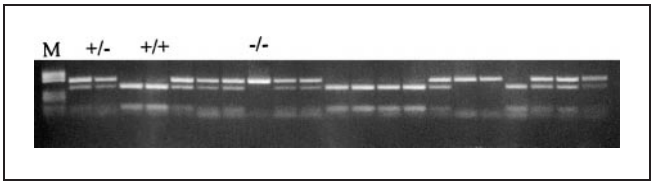
Statistical Analysis

Statistical analyses were conducted using Unistat 5.1 software. Data are expressed as numbers and percentages for discrete variables and as mean ± SE for continuous variables. Genotype frequencies between cases and control subjects were counted and compared by χ^2 analysis. Baseline differences between cases and controls were examined by a Student *t* test for continuous data. The variables across the LPL genotypes and groups were estimated by 2-way analysis of variance with an interaction term to test the influence of the *Pvu*II genotype on the lipid profile. A Bonferroni test was used to compare the lipid values of each LPL genotype (+/−, −/−, and +/+) with respect to each other. Obesity, hypertension, diabetes mellitus, dyslipidemia, smoking, and LPL–*Pvu*II genotypes were selected as potential risk factors for CAD. Independent predictors of CAD were determined using multivariate logistic regression analysis. Odds ratios with 2-tailed *P* values were calculated as a measure of the association of the LPL–*Pvu*II genotypes with CAD. Statistical significance was taken as $P < .05$.

Table 2. Lipid Profile of Patients and Control Subjects*				
	Patients (n = 80)	Control Subjects (n = 49)	P†	
			a	b
Total cholesterol, mg/dL	203.98 ± 5.51	190.14 ± 5.09	.09	.04
HDL cholesterol, mg/dL	43.5 ± 2.65	47.85 ± 1.26	.23	.09
LDL cholesterol, mg/dL	138.81 ± 4.4	129.80 ± 5.48	.21	.02
Triglycerides, mg/dL	145.35 ± 7.97	111.23 ± 6.43	.004	.29
Plasma glucose, mg/dL	117.50 ± 5.66	82.39 ± 1.54	<.001	.89

* Values are presented as mean ± SE in conventional units of measure. Conversion factors for SI units are as follows: glucose, multiply mg/dL by 0.055 to convert to mmol/L; total cholesterol, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol, divide mg/dL by 39 to convert to mmol/L; and triglycerides, divide mg/dL by 89 to convert to mmol/L.

† *P* values were calculated by 2-way analysis of variance. $P < .05$ was considered to be statistically significant. Column a displays the difference between case and control subjects; column b shows the lipoprotein lipase–*Pvu*II polymorphism effect.



Genotyping for the $C^{497}\rightarrow T$ polymorphism in the lipoprotein lipase (LPL) gene. M indicates the GeneRuler 100-bp DNA ladder. The presence of a T nucleotide at position 497 in the LPL gene creates a restriction site for the PvuII *enzyme. A 430-bp fragment of the gene encompassing the polymorphism was amplified by polymerase chain reaction and subjected to digestion with the* PvuII *enzyme. The individuals who were homozygous for the C allele (−/− genotype) were identified by the presence of a single 430-bp product (undigested). Those homozygous for the T allele (+/+ genotype) were identified by the presence of 2 products, of 320 bp and 110 bp (digestion products). The heterozygous individuals (+/− genotype) were identified by the presence of all 3 products, namely, 430, 320, and 110 bp.*

Table 3. Lipoprotein Lipase (LPL)–*Pvu*II Genotypes in Coronary Artery Disease Patients and Control Subjects			
	LPL–*Pvu*II Genotype Frequencies*		
	+/−, No. (%)	−/−, No. (%)	+/+, No. (%)
Patients	39 (48.8)†	14 (17.5)	25 (31.3)
Controls	16 (32.7)	19 (38.8)†	14 (28.6)

* The LPL–*Pvu*II genotype frequencies were compared between study groups with χ^2 analysis.

† $\chi^2 = 6.01176$; $P = .049$.

RESULTS

The frequencies of major CAD risk factors are summarized in Table 1. Dyslipidemia, hypertension, diabetes mellitus, obesity, and smoking were more frequent in the patient group than in controls, although obesity did not reach significance. The lipid profiles of CAD patients and healthy controls are presented in Table 2. The patients' triglyceride values were significantly higher than those of controls. The patients' total cholesterol and LDL-cholesterol levels were also higher than those of the control group, but the difference did not reach statistical significance. Levels of total cholesterol ($P = .04$) and LDL cholesterol ($P = .02$) were found to be effected by LPL–*Pvu*II polymorphism.

The LPL–*Pvu*II genotypes are demonstrated in the Figure. The genotype frequencies observed for *Pvu*II polymorphism both in the CAD and control subjects are shown in Table 3. We were not able to establish the genotype for 2 CAD patients successfully. The genotype frequencies in this study population were 48.8% for +/−, 17.5% for −/−, and 31.3% for +/+ genotypes in the CAD patients, and 32.7% for +/−, 38.8% for −/−, and 28.6% for +/+ genotypes in the control subjects. The χ^2 analysis showed significant differences between the genotypes ($\chi^2 = 6.01176$, $P = .049$).

The relationships between LPL gene *Pvu*II genotypes and lipid parameters of CAD and control subjects are displayed in Table 4. In control and CAD subjects, there were no significant differences in total cholesterol, HDL-cholesterol, and triglyceride levels among the common genotypes in *Pvu*II RFLPs. Although not significant, the triglyceride and total cholesterol levels were found to be highest in the +/+ genotype, both in patients and controls. Low-density lipoprotein cholesterol concentration was found to be lower ($P = .06$) in the atherosclerotic patients with +/− genotype compared to those carrying the +/+ genotype.

Major CAD risk factor frequencies for the LPL gene *Pvu*II genotypes in the CAD and control groups are presented in Table 5. Dyslipidemia, diabetes mellitus, and obesity were more frequent among patients with a +/− genotype than in controls of the same genotype.

The distribution of the *Pvu*II genotypes according to hypertension was not found to differ in patients and controls.

Obesity, hypertension, diabetes mellitus, dyslipidemia, smoking, and LPL genotypes were selected as conventional risk factors to be analyzed in multiple logistic regression analyses (Table 6). Dyslipidemia and hypertension were found to be independent risk factors for CAD, whereas no such association was observed for LPL–*Pvu*II genotypes.

COMMENT

Given the importance of LPL as a candidate gene for CAD risk, we evaluated independent, well-defined, angiographically controlled Turkish subjects to determine the possible association of the LPL–*Pvu*II polymorphisms to CAD in Turkish patients and to assess whether this LPL variant can act as a totally independent genetic risk factor for CAD in this population. We also investigated the genotype distribution of LPL polymorphism at a *Pvu*II polymorphic site and the lipid profile of different genotypes.

The results of various association studies of LPL–*Pvu*II polymorphisms with CAD have been inconsistent. In the available literature to date, an association between the extent of CAD and the LPL–*Pvu*II (+/+) genotype was reported by Wang et al.[18] Anderson et al[31] found the LPL–*Pvu*II (−/−) genotype to be moderately associated with

Table 4. Effects of Lipoprotein Lipase (LPL) Gene *Pvu*II Polymorphism Over Clinical Parameters in Patients With Coronary Artery Disease (CAD) and in Control Subjects*

	CAD Patients			
	+/− (n = 39)	−/− (n = 14)	+/+ (n = 25)	P†
Total cholesterol, mg/dL	197.27 ± 7.50	201.57 ± 11.21	217.88 ± 11.29	.26
HDL cholesterol, mg/dL	39.27 ± 1.84	46.14 ± 7.38	49.42 ± 6.76	.23
LDL cholesterol, mg/dL	129.61 ± 6.29	142.81 ± 7.06	153.22 ± 8.60	.06
Triglyceride, mg/dL	138.37 ± 10.16	145.00 ± 17.97	157.48 ± 16.99	.58
	Control Subjects			
	+/− (n = 16)	−/− (n = 19)	+/+ (n = 14)	P
Total cholesterol, mg/dL	184.12 ± 5.75	186.39 ± 9.47	202.77 ± 10.65	.31
HDL cholesterol, mg/dL	46.62 ± 1.94	50.39 ± 2.20	45.85 ± 2.34	.28
LDL cholesterol, mg/dL	124.31 ± 8.75	133.56 ± 10.70	131.38 ± 8.30	.77
Triglyceride, mg/dL	118.87 ± 10.47	94.83 ± 7.26	124.54 ± 15.81	.12

* Values are presented as mean ± SE in conventional units of measure. Conversion factors for SI units are as follows: glucose, multiply mg/dL by 0.055 to convert to mmol/L; total cholesterol, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol, divide mg/dL by 39 to convert to mmol/L; and triglycerides, divide mg/dL by 89 to convert to mmol/L.

† $P < .05$ was considered to be statistically significant. Lipoprotein lipase–*Pvu*II genotypes were compared with analysis of variance for the variables. The +/− and +/+ genotypes were not statistically significant ($P = .06$) for LDL-cholesterol levels with the Bonferroni test.

Table 5. The Association Between Conventional Risk Factors for Coronary Artery Disease and Lipoprotein Lipase (LPL)–*Pvu*II Genotypes

	LPL Genotypes		
	+/−	−/−	+/+
Dyslipidemia			
Patients, No. (%)	30 (58.8)	9 (17.7)	12 (23.5)
Control subjects, No. (%)	5 (29.4)	5 (29.4)	7 (41.2)
Hypertension			
Patients, No. (%)	21 (44.7)	10 (21.3)	16 (34)
Control subjects, No. (%)	5 (41.7)	3 (25)	4 (33.3)
Diabetes mellitus			
Patients, No. (%)	15 (57.7)	5 (19.2)	6 (23.1)
Control subjects, No. (%)	0	0	4 (100)
Obesity			
Patients, No. (%)	25 (52.1)	9 (18.7)	14 (29.2)
Control subjects, No. (%)	8 (27.6)	12 (41.4)	9 (31)

Table 6. Risk Factors Identified Independently Associated With Coronary Artery Disease by Multiple Logistic Regression Analysis*

	All			
	β	SE	OR	P
Obesity	−0.50	1.157	0.606	.66
Hypertension	3.017	1.024	20.439	.003†
Diabetes mellitus	1.027	1.035	2.793	.32
Dyslipidemia	3.233	1.286	25.348	.01†
Smoking	11.72	29.851	1.229	.70
LPL				
−/−	−0.887	1.145	0.412	.44
+/−	−0.310	1.308	0.733	.81
+/+	−0.577	0.941	0.562	.54

* The multivariate logistic regression model contained obesity, hypertension, diabetes mellitus, dyslipidemia, smoking, and lipoprotein lipase (LPL)–*Pvu*II genotype variables. β indicates estimated coefficient; OR, adjusted odds ratio.

† Statistically significant.

CAD. Other studies did not define any significant difference in the distribution of LPL–*Pvu*II polymorphism between the healthy group and the CAD group,[27–29,32] suggesting lack of association between any of the LPL–*Pvu*II genotypes and CAD. Furthermore, Jemaa et al[20] did not demonstrate any significant association of the *Pvu*II polymorphism with various biochemical traits examined in their control group, Peacock et al[14] did not observe differences in LPL–*Pvu*II allelic frequencies in young myocardial infarction survivors, and Mattu et al[16] found no association between the LPL–*Pvu*II (+/+) genotype and CAD. In summary, it appears that the relevance of the *Pvu*II genotypes may vary among different populations. In the present study, the distribution of the LPL–*Pvu*II genotypes was significantly different in CAD and control study groups; the frequency of the +/− genotype was higher in patients than in controls. Also, the +/− genotype rates observed in our study group were comparable to rates obtained for other populations,[32] which were higher in the CAD group compared to the −/− and +/+ genotypes.

Several genetic analyses have suggested that LPL genotypes causing decreased LPL activity correlate with the risk for CAD. For example, LPL activity was reported to be higher in the *Pvu*II −/− genotype compared to the +/− or +/+ genotypes.[34] Thus, LPL–*Pvu*II polymorphism is a possible marker for a functional mutation that is present in the LPL gene and that alters LPL-specific activity.

Studies concerning the association between LPL–*Pvu*II polymorphism and serum lipids in patients with CAD have produced contradictory results. While some investigators found significant associations of *Pvu*II genotypes with lipids,[17,18,25–27] others failed to show any significant intragenotype variances of mean lipid values.[28–30] An association between LPL–*Pvu*II polymorphism and triglycerides was investigated in white Japanese,[17] Australian,[18] and French[25] populations, and significantly higher triglyceride concentrations were observed in the *Pvu*II +/+ genotype than in the −/− genotype. These authors also found a significant decrease of HDL-cholesterol concentrations in *Pvu*II +/+ carriers. In contrast to these results, the *Pvu*II −/− genotype was observed to be associated with higher triglyceride concentrations compared to the +/+ genotype in Chinese subjects,[36] whereas Jemaa et al[20] and Wang et al[18] did not find any significant relationship between triglyceride concentration and *Pvu*II genotype in cardiac patients. Our results were in agreement with these findings, in that we found no significant relationship between elevated triglyceride or HDL-cholesterol levels and the *Pvu*II (+) allele. Minnich et al[26] showed a reduced HDL-cholesterol level in the heterozygotes for the LPL *Pvu*II gene mutation. The results of our study agree with those of Minnich et al, in that +/− genotype carriers of the CAD group had nonsignificantly lower HDL-cholesterol levels compared to the +/+ and −/− genotypes. Analysis of intragenotype variances of mean values of lipid levels showed that variability of *Pvu*II in LPL contributes to a certain extent to the level and variability of serum total cholesterol and LDL-cholesterol levels in this Turkish study group. For the LPL–*Pvu*II genotypes, the CAD subjects homozygous for the presence of the *Pvu*II site were associated with higher total cholesterol and LDL cholesterol compared with heterozygous +/− subjects or homozygotes for the absence of the *Pvu*II site. Furthermore, the heterozygous subjects did not have total cholesterol and LDL-cholesterol levels intermediate between those of the 2 homozygous genotypes, hence the association lacked a gene dosage trend.

In conclusion, the LPL–*Pvu*II polymorphism was not found to be an independent genetic risk factor for CAD in this selected study group. This genetic variation at the LPL locus has been found to affect plasma total cholesterol and LDL-cholesterol levels. Among other CAD risk factors, hypertension and dyslipidemia were found to influence CAD. Further studies performed on larger samples are needed to explore the biological pathways underlying coronary heart disease and to identify the functional variants.

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