Associations of three lipoprotein lipase gene polymorphisms, lipid profiles and coronary artery disease

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Abstract. Lipoprotein lipase (LPL) plays a central role in lipoprotein metabolism by hydrolyzing the core triglycerides (TGs) of circulating chylomicrons and very-low-density lipoprotein (VLDL). The effects of LPL polymorphisms on lipid levels and coronary artery disease (CAD) have been inconsistent among studies and populations. To assess the lipid profiles and distributions of three LPL gene polymorphisms in Saudi patients with CAD, the *Hin*dIII, *Pvu*II and Ser447Ter polymorphisms in the LPL gene were analyzed in 226 patients with CAD and 110 controls. Polymerase chain reaction-restriction fragment length polymorphism was used to detect LPL gene polymorphisms. The plasma lipid profiles of the patients were determined using standard enzymatic methods. Patients in the CAD group had significantly higher triglyceride (TG), total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels than controls irrespective of the *Hin*dIII, *Pvu*II or Ser447Ter genotype. Compared to the findings in controls, the *Hin*dIII TT, *Pvu*II TC and Ser447Ter CC genotypes were associated with significantly reduced high-density lipoprotein cholesterol (HDL-C) levels in patients with CAD ($P<0.0001$). In summary, there are associations between LPL gene variants and high plasma TG, TC and LDL-C levels as well as low HDL-C levels.

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Introduction

Lipoprotein lipase (LPL) is a glycoprotein that is synthesized in the parenchymal cells of different tissues. LPL hydrolyzes circulating core triglycerides (TGs) of exogenous (chylomicron) and endogenous [very-low-density lipoprotein (VLDL)] origins to provide free fatty acids for oxidation and utilization in the heart and other tissues and for storage in adipose tissue (1). LPL affects circulating triglyceride (TG) levels by generating lipoprotein remnants, which are processed by hepatic lipase. Following secretion, LPL attaches to the luminal surface of endothelial cells, in which it has a significant role in the catabolism of lipoproteins in circulation and interacts with lipoproteins locally (2). It was previously reported that LPL increases the retention of low-density lipoprotein (LDL) and VLDL particles by the subendothelial matrix of the arterial wall, which enhances the conversion of these lipoproteins into more atherogenic forms (3). These localized and deleterious effects cannot be assessed by measuring either circulating LPL or TG levels but can be explored by identifying genetic variants of LPL and their relationships with the presence and extent of atherosclerotic lesions. Findings of previous studies revealed that plasma lipoprotein concentrations are significant predictors for the risk of coronary heart disease (CHD) (4). Thus, genes with essential roles in lipoprotein metabolism are excellent candidates for inter-individual variation in the susceptibility to CHD (5), including the LPL gene. The LPL gene spans over 30 kb on chromosome 8p22, and is divided into 10 exons (6). The major products of LPL activity are free fatty acids and glycerol for energy utilization and storage (1) and LDL activity is accompanied by the formation of intermediate-density lipoprotein and chylomicrons remnants (CRs). Independent of its lipolytic activity, LPL binds to and travels with CRs to the liver, where it enhances the clearance of these lipoproteins via LDL receptors (7). Due to the pivotal role of LPL in lipid metabolism, genetic defects

in the LPL gene can affect lipoprotein metabolism, resulting in an atherogenic lipid profile. A number of mutation have been identified in this locus. Additionally, several rare mutations in the LPL gene have been associated with markedly reduced enzyme activity and a number of common variants have been associated with moderate changes in LPL catalytic function (8). However, there is controversy with regard to the effects of these variants on LPL activity. For example, Emi *et al* (9) reported that the G188E mutation leads to the expression of an inactive enzyme, which explains the manifestation of LPL deficiency. However, Hallman *et al* (10) did not find any association between the *Hin*dIII(-) allele and higher LPL activity. The associations of certain polymorphic loci in the LPL promoter, introns or exons with lipid disorders and coronary artery disease (CAD) have been reported by some groups (rs285, rs1800590, rs320, rs268, rs1801177) (6-8,11-14) but contested by other authors (15-17). To the best of our knowledge, no data on the screening of such specific polymorphisms in the Saudi population have been reported. Therefore, the aim of the present investigation was to determine and address the lipid profile and distribution of the *Hin*dIII, *Pvu*II and Ser447Ter polymorphisms of LPL in Saudi patients with CAD.

Materials and methods

Study subjects. The study comprised 226 patients (157 males and 69 females, aged 42-82 years) who were admitted to the Department of Cardiology, King Khalid University Hospital, Riyadh, Saudi Arabia and 103 healthy subjects (58 males and 45 females, aged 20-78 years) who had no history of CAD as controls. The subjects included in this study were of unrestricted age and gender. All the subjects provided written informed consent prior to participation as well as to having blood drawn at the time of angiography or time of screening for DNA extraction. The study was reviewed and approved by the Institutional Review Board of the King Khalid University Hospital. This study was conducted in accordance with the guidelines set by the Ethics Committee of the College of Medicine and Research Centre of King Saud University, Riyadh, Saudi Arabia. All subjects enrolled in this study were Saudi residents with similar dietary patterns. The key demographic data of the subjects were recorded including age, gender and lipid profiles. CAD was assessed by review of the patients' angiograms by their treating cardiologists.

Sample collection and lipid analysis. Blood samples for the glucose and lipid measurements were drawn from the patients and controls subsequent to an overnight fast. The plasma glucose concentration was measured by the glucose oxidase method using a Biotrol kit (Biotrol, Earth City, MO, USA) on a Bayer Opera analyzer [Bayer Diagnostics (Siemens), Munich, Germany]. Serum total cholesterol (TC) was measured using a Biotrol kit, and high-density lipoprotein cholesterol (HDL-C) was measured using a commercial Randox kit (Randox Laboratories Ltd., London, UK). LDL cholesterol [low-density lipoprotein cholesterol (LDL-C)] levels were calculated using the Friedewald formula and TG levels were measured using the lipase/glycerol kinase UV endpoint method of the Opera analyzer.

DNA extraction. Genomic DNA was extracted from peripheral blood specimens, which were drawn into tubes containing ethylenediaminetetraacetic acid, using the QIAamp DNA isolation kit (Qiagen, Hilden, Germany).

Genotyping. The presence of three common polymorphisms of the LPL gene was determined by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) analysis using genomic DNA. The primer sets were selected on the basis of previously published information as follows (18): *Hin*dIII forward (H1): 5'-TGA AGC TCA AAT GGA AGA GT-3', and reverse (H2): 5'-TAC AAG CAA ATG ACT AAA-3'; *Pvu*II forward (P1): 5'-ATG GCA CCC ATG TGT AAG GTG-3', and reverse (P2): 5'-GTG AAC TTC TGA TAA CAA TCT C-3'; and Ser447Ter forward: 5'-TAC ACT AGC AAT GTC TAG GTG A-3', and reverse: 5'-TCA GCT TTA GCC CAG AAT GC-3'. In each reaction, 3 μl (150 ng) of the genomic DNA template was added to the PCR reaction mixture, which consisted of 12.5 μl of 2X Promega master mix (Promega Corporation, Madison, WI, USA), 2 μl of each primer and distilled water to a final volume of 25 μl. The PCR conditions were as follows: an initial denaturation at 94˚C for 2 min was followed by 40 cycles of denaturation at 94˚C for 15 sec, annealing at 50˚C for 30 sec and extension at 72˚C for 1 min, with a final extension at 72˚C for 2 min. PCR was performed in a MyCycler (Bio-Rad, Hercules, CA, USA).

LPL gene polymorphism analysis. Digestion of the PCR products was performed by adding 1 μl of the respective restriction enzyme (*Hin*dIII and *Pvu*II, both Promega Corporation; *Mnl*I: New England Biolabs, Ipswich, MA, USA) to 10 μl of the PCR product containing 2 μl of 10X buffer solution (final reaction volume of 20 μl). The mixtures were centrifuged for 2 min at 3,913 x g and incubated in a water bath at 37˚C overnight. The resulting fragments were resolved by electrophoresis (80 V, 60 min) on a 2.5% agarose gel and visualized using UV light. The *Hin*dIII site (intron 8) produced a 600 bp fragment following digestion. The *Pvu*II restriction site (intron 6) yielded 330 and 110 bp fragments. Genotypes were scored by an experienced reader blinded to the clinical and angiographic results. The polymorphic allele with the restriction site was designated as 'T' and the allele without the site as 'G' for *Hin*dIII. For *Pvu*II, the allele with the restriction site was designated as 'T' and that without the site as 'C'. The 488 bp PCR product contained two *Mnl*I restriction sites, one of which is a polymorphic site indicating the Ser447Ter mutation. Digestion of the PCR product with *Mnl*I resulted in three fragments of 290, 250 and 200 bp (18,19). The identified genotypes were named according to the presence or absence of the enzyme restriction sites; for example, Ser447Ter GG, GC and CC indicated homozygosity for the presence of the site, and heterozygosity and homozygosity for the absence of the site, respectively.

Statistical analysis. Measurement data were presented as the mean ± standard deviation (SD), and compared using the two-sample Student's t-test. Enumeration count data were summarized as numbers (%) and compared using the χ^2 test. Two analyses were used to evaluate the allelic and genotypic frequencies that were calculated from the observed genotypic

Table I. Characteristics of the controls and patients.

Characteristic	Controls (n=103)	CAD group (n=226)	P-value
Age, years (mean ± SD, range)	46.60±16.69 (20.0-78.0)	61.62±9.89 (42.0-82.0)	<0.0001
Gender: male (%), female (%)	58 (56.3), 45 (43.7)	157 (69.50), 69 (30.50)	<0.0001
Fasting blood sugar, mmol/l (mean ± SD, range)	4.48±0.66 (3.21-7.10)	8.0±3.48 (3.3-20.6)	<0.0001
TG, mmol/l (mean ± SD, range)	1.11±0.28 (0.53-1.94)	2.79±0.99 (1.84-8.7)	<0.0001
TC, mmol/l (mean ± SD, range)	3.81±0.56 (3.01-7.11)	5.04±0.83 (3.10-8.3)	<0.0001
HDL-C, mmol/l (mean ± SD, range)	1.24±0.38 (0.76-2.15)	1.11±0.38 (0.53-3.12)	0.004
LDL-C, mmol/l (mean ± SD, range)	1.65±0.61 (0.86-4.50)	2.72±0.83 (1.12-5.89)	<0.0001

The Student's t-test and the χ^2 test were used to compare the values of the controls and patients. SD, standard deviation; CAD, coronary artery disease; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

Table II. Risk factors for CAD in patients and controls.

Parameter	CAD (n=226)	Control (n=103)	OR	95% CI	P-value
Diabetes mellitus					
Diabetic	148 (66%)	8 (7%)			
Non-diabetic	78 (34%)	95 (93%)	22.53	10.41-48.75	<0.0001
Dyslipidemia					
Positive	128 (57%)	13 (12%)			
Negative	98 (43%)	90 (88%)	9.04	4.77-17.11	<0.0001
Hypertension					
Hypertensive	169 (75%)	11 (11%)			
Normotensive	57 (25%)	92 (89%)	24.79	12.39-49.61	<0.0001
Smoking					
Smoker	90 (40%)	16 (15%)			
Non-smoker	136 (60%)	87 (85%)	3.59	1.98-6.53	<0.0001

CAD, coronary artery disease; OR, odds ratio; CI, confidence interval.

counts and to assess the Hardy-Weinberg equilibrium expectations. The same methodology was applied to comparisons between allelic and genotypic frequencies. Associations were determined as odds ratios (ORs) and 95% confidence intervals (CIs). The likelihood of carrying a specific allele was defined as the number of subjects who carried the allele divided by the number of subjects in who did not carry the allele. The OR for the LPL genotype distribution was determined using χ^2 analysis. The CAD was defined as the odds of allelic carriage in the diseased (CAD) group divided by the odds of allelic carriage in the healthy (control) group. Statistical analysis was performed using the Statistical Package for Social Sciences for Windows, version 20.0 (SPSS, Inc., Chicago, IL, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

Patient data. Details regarding the clinical and biochemical characteristics of the study population (226 patients with CAD and 103 controls) are included in Table I. Patients with CAD had significantly lower HDL-C concentrations compared to the control group and significantly higher plasma levels of fasting blood sugar, TG, TC and LDL-C (P<0.0001 for each) compared to the control subjects.

Risk factors for CAD. Diabetes mellitus, dyslipidemia, hypertension and smoking were selected as major risk factors. The frequencies of the major CAD risk factors are shown in Table II. Diabetes mellitus, dyslipidemia, hypertension and smoking were more frequently present in the patient group than in the control group and diabetes mellitus, dyslipidemia, hypertension and smoking were identified as risk factors for CAD (OR=22.53, 9.04, 24.79 and 3.59, respectively, P<0.0001 for each).

The entire study population was genotyped for three LPL gene polymorphisms: *Hin*dIII (alleles designated as T and G), *Pvu*II (alleles designated as T and C) and Ser447Ter (alleles designated G and C). Table III shows the genotypes and frequencies for the observed alleles. For all polymorphisms in the patients and controls, the distribution of genotypes yielded from the Hardy-Weinberg equilibrium were as expected. Allele frequencies did not

Table III. Genotype distributions and allele frequencies of the *Hind*lll, *Pvu*ll and Ser447Ter polymorphisms.

	Genotype			Allele frequency	
	Mutant	Normal	Heterozygote		
*Hind*lll	TT	GG	TG	T allele	G allele
Controls (n=103)	42.0 (40.8%)	26.0 (25.2%)	35.0 (34%)	119 (57.77%)	87 (42.23%)
Patients (n=226)	102.0 (45.1%)	43.0 (19%)	81.0 (35.8%)	285 (63.05%)	167 (36.95%)
P-value	0.460	0.200	0.743	0.196	0.196
*Pvu*ll	TT	CC	TC	T allele	C allele
Controls (n=103)	46.0 (44.7%)	13.0 (12.6%)	44.0 (42.7%)	136 (66.02%)	70 (33.98%)
Patients (n=226)	89.0 (39.4%)	35.0 (15.5%)	102.0 (45.1%)	280 (61.95%)	172 (38.05%)
P-value	0.376	0.495	0.682	0.315	0.315
Ser447Ter	GG	CC	GC	G allele	C allele
Controls (n=103)	0	92.0 (89.3%)	11.0 (10.7%)	11 (5.34%)	195 (94.66%)
Patients (n=226)	0	185 (81.9%)	41.0 (18.1%)	41 (9.07%)	411 (90.93%)
P-value	-	0.088	0.088	0.103	0.103

The χ^2 test was used to compare the allele frequencies between the control and coronary artery disease groups.

differ significantly between the CAD and control groups. Within the CAD group (n=226), the TT *Hin*dIII genotype was identified in 102 patients (45.1%), whereas 81 (35.8%) and 43 patients (19%) carried the TG and GG genotypes, respectively. Within the control group (n=103), the TT genotype was identified in 42 subjects (40.8%), whereas 35 (34%) and 26 subjects (25.2%) carried the TG and GG genotypes, respectively. Regarding *Hin*dIII, patients with CAD were less likely to carry the GG genotype than the control group and patients with CAD had higher frequencies of the TT and TG genotypes. For the *Pvu*II genotype, the TT genotype was identified in 89 patients (39.4%) within the CAD group, whereas 44 (42.7%) and 35 patients (15.5%) carried the TC and CC genotypes, respectively. Within the control group, the TT genotype was identified in 46 patients (44.7%), while 44 (42.7%) carried the TC genotype and 13 patients (12.6%) carried the CC genotype. Concerning *Pvu*II, patients with CAD had higher frequencies of the TC and CC genotypes but a lower frequency of the TT genotype compared to the controls. For the Ser447Ter genotype, the CC genotype was found in 185 patients (81.9%) within the CAD group, whereas 41 patients (18.1%) carried the GC genotype. Within the control group (n=103), the CC genotype was identified in 92 patients (89.3%) and 11 patients (10.7%) carried the GC genotype. We did not find any GG genotypes in either group for this gene. A higher frequency of the GC genotype and a lower frequency of the CC genotype were observed in the CAD group compared to the control group.

Data are presented as the observed number of cases and the expected number of cases in parentheses. The χ^2 test was used for comparisons between positive and negative risk factors.

Relationships between the frequencies of major CAD risk factors and LPL gene polymorphisms. The relationships between the frequencies of major CAD risk factors and those of LPL gene polymorphisms in the patients with CAD are presented in Table IV. The results revealed associations between LPL *Hin*dIII genotypes and hypertension (χ^2=6.68, P=0.03) and smoking (χ^2=5.80, P=0.05). Additionally, a positive relationship between *Pvu*II genotypes and smoking (χ^2=6.964, P=0.03) was noted as well as a relationship between Ser447Ter genotypes and diabetes (χ^2=6.74, P=0.009).

Relationships between the HindIII, PvuII and Ser447Ter genotypes and lipid parameters of CAD and the control groups. The relationships between the *Hin*dIII, PvuII and Ser447Ter genotypes and the lipid parameters of the CAD and control groups are shown in Table V. TG, TC and LDL-C levels were significantly higher in the CAD group compared to the control group irrespective of the *Hin*dIII, *Pvu*II or Ser447Ter genotype (P<0.0001 for each), whereas compared to the levels in the control group, the *Hin*dIII TT (P=0.03), *Pvu*II TC (P=0.006) and Ser447Ter CC genotypes (P=0.003) were associated with decreased HDL-C levels in the CAD group.

Association among the HindIII, PvuII and Ser447Ter genotypes. As shown in Table VI, no association was observed between the *Hin*dIII and *Pvu*II genotypes. However, an association between the *Hin*dIII and Ser447Ter genotypes was noted (χ^2=7.33, P=0.03). A significant relationship was observed between the *Pvu*II and Ser447Ter genotypes (χ^2=7.3, P=0.02).

Haplotype frequencies for the three LPL polymorphisms. Haplotype reconstruction for the three LPL polymorphisms under study revealed 18 haplotypes possessing *Hin*dIII (intron 8 T481G), *Pvu*II and Ser447Ter polymorphisms. The GGTTCC and TGCCCC haplotypes were significantly more common in the control group compared to the CAD group (P=0.003 and 0.007, respectively, Table VII).

Table IV. Relationships between lipoprotein lipase (LPL) genotypes and the presence of risk factors (diabetes, dyslipidemia, hypertension and smoking).

	Genotype					
	TT	GG	TG	Total	χ^2	P-value
LPL-*Hind*III						
Diabetes						
Diabetic	69 (66.79)	27 (28.15)	52 (53.04)	148	-	-
Non-diabetic	33 (35.02)	16 (14.84)	29 (27.96)	78	-	-
Total	102	43	81	226	0.41	0.82
Dyslipidemia						
Positive	59 (57.77)	30 (24.35)	39 (45.88)	128	-	-
Negative	43 (44.23)	13 (18.64)	42 (35.12)	98	-	-
Total	102	43	81	226	5.46	0.07
Hypertension						
Hypertensive	68 (76.27)	34 (32.15)	67 (60.57)	169	-	-
Normotensive	34 (25.73)	9 (10.84)	14 (20.42)	57	-	-
Total	102	43	81	226	6.68	0.03
Smoking						
Smoker	38 (40.61)	24 (17.12)	28 (32.25)	90	-	-
Non-smoker	64 (61.38)	19 (25.87)	53 (48.74)	136	-	-
Total	102	43	81	226	5.80	0.05

	Genotypes					
	TT	CC	TC	Total	χ^2	P-value
LPL-*Pvu*II						
Diabetes						
Diabetic	57 (58.28)	25 (22.92)	66 (66.79)	148	-	-
Non-diabetic	32 (30.71)	10 (12.07)	36 (35.20)	78	-	-
Total	89	35	102	226	0.66	0.72
Dyslipidemia						
Positive	48 (50.41)	24 (19.82)	56 (57.76)	128	-	-
Negative	41 (38.59)	11 (15.17)	46 (44.23)	98	-	-
Total	89	35	102	226	2.42	0.29
Hypertension						
Hypertensive	61 (66.55)	25 (26.17)	83 (76.27)	169	-	-
Normotensive	28 (22.44)	10 (8.82)	19 (25.72)	57	-	-
Total	89	35	102	226	4.39	0.11
Smoking						
Smoker	26 (35.44)	17 (13.94)	47 (40.62)	90	-	-
Non-smoker	63 (53.56)	18 (21.06)	55 (61.38)	136	-	-
Total	89	35	102	226	6.964	0.03

	Genotypes					
	GG	CC	GC	Total	χ^2	P-value
Ser447Ter						
Diabetes						
Diabetic	0	34 (26.84)	114 (121.5)	148	-	-
Non-diabetic	0	7 (14.17)	71 (63.84)	78	-	-
Total	0	41	185	226	6.74	0.009

Table IV. continued.

	Genotypes					
	GG	CC	GC	Total	χ^2	P-value
Dyslipidemia						
Positive	0	26 (23.22)	102 (104.77)	128	-	-
Negative	0	15 (17.78)	83 (80.22)	98	-	-
Total	0	41	185	226	0.33	0.214
Hypertension						
Hypertensive	0	32 (30.66)	137 (138.34)	169	-	-
Normotensive	0	9 (10.34)	48 (46.65)	57	-	-
Total	0	41	185	226	0.59	0.38
Smoking						
Smoker	0	13 (16.32)	77 (73.67)	90	-	-
Non-smoker	0	28 (24.67)	108 (111.32)	136	-	-
Total	0	41	185	226	0.24	0.16

Discussion

CAD is a complex disease with well-documented genetic and environmental components. The findings of most genetic studies of CAD are controversial, likely because the genetic risk of CAD is not based on a single gene but is instead based on interactions among several pathophysiological pathways involving multiple genes and environmental risk factors. LPL is a potential target for the treatment of CAD because LPL gene variants are involved in a number of pathophysiological conditions associated with CAD. Several mutations of the LPL gene have been identified thus far and 20% of these mutations occur in noncoding regions (20-21). The LPL gene is one of the most appealing candidate genes that may be used to explain some of the lipid and lipoprotein abnormalities encountered in numerous cases of CAD. In this study, we presented results on polymorphisms and haplotypes of the LPL gene in a Saudi population and the possible association of these polymorphisms with CAD and lipid profiles in some detail.

CAD is a multifactorial disorder believed to result from an interaction between the genetic background and environmental factors such as diet, smoking and physical activity. CAD is usually associated with conventional risk factors including hypertension, diabetes mellitus and hypercholesterolemia (22). In the present study, diabetes mellitus, dyslipidemia, hypertension and smoking were identified as risk factors for CAD and a positive association was found between LPL *Hin*dIII genotypes and hypertension and smoking. Additionally, an association between *Pvu*II genotypes and smoking was observed. The association between Ser447Ter genotypes and diabetes was evident in the present study. In general, individuals with hypertension, diabetes mellitus and hypercholesterolemia were considered at high risk for CAD, while subjects that did not exhibit any of these factors were considered low-risk subjects. Age, smoking, hypertension and diabetes have been established as independent risk factors for ischemic cardiovascular disease (23). Hypertension and dyslipidemia were found to influence CAD (24). Genetic factors were statistically independent of age, smoking, hyperuricemia, hypertension, diabetes mellitus and hypercholesterolemia. Smoking was considered an important environmental factor for CAD in men at low risk, consistent with the hypothesis that the cessation of smoking is important in the prevention of CAD in these individuals (23). It has been documented that dyslipidemia, diabetes mellitus and obesity were more common among patients with a +/- genotype than among controls of the same genotype, while dyslipidemia and hypertension were identified as independent risk factors for CAD (24).

The frequencies of the *Hin*dIII (TT, 45.1; TG, 35.8 and GG, 19%) and *Pvu*II genotypes (TT, 39.4; TG, 42.7 and CC, 15.5%) in the present study were similar to those found in different ethnic groups, including Northern Europeans (7), Caucasians (25), Russians (26) and Tunisians (27). The allele frequencies of *Pvu*II (C) and *Hin*dIII (G) in this study were similar to those found in other Caucasian populations. Ahn *et al* (25) reported allele frequencies of 0.45 and 0.26 for *Pvu*II(-) and *Hin*dIII(-), respectively, in a control population (n=539). Peacock *et al* (28) also reported frequencies of 0.435 and 0.228 for *Pvu*II(-) and *Hin*dIII(-), respectively, among 92 healthy control subjects. A strong linkage disequilibrium was also identified between the two *Pvu*II and *Hin*dIII polymorphisms in several other studies (28-33).

It was of interest to compare the Ser447Ter gene polymorphism frequency of our population (81.9 CC and 18.1% GC) with other published data as there is considerable variation in the reported frequency of this polymorphism in different populations (7.4-20%) (12,34,35). Komurcu-Bayrak *et al* (36) reported a Ser447Ter allele frequency of 11% among randomly selected Turkish participants and obtained a similar minor allele frequency for Ser447Ter in the group without CAD and a lower minor allele frequency in the group with significant CAD.

The *Hin*dIII TT (P=0.03), *Pvu*II TC (P=0.006) and Ser447Ter CC genotypes (P=0.003) were associated with significantly lower HDL-C levels in the CAD group compared to the control group (Table V).

Table V. Lipid concentration in three genotypes of lipoprotein lipase (*Hin*dIII, *Pvu*II and Ser447Ter).

Parameters	Groups	*Hin*dIII genotypes			*Pvu*II genotypes			Ser447Ter genotypes	
		TT	TG	GG	TT	TC	CC	GC	CC
TG (mmol/l)	Control (n)	42	35	26	46	44	13	11	92
	Mean ± SD	1.11±0.03	1.13±0.27	1.09±0.25	1.08±0.26	1.17±0.32	1.05±0.17	1.11±0.31	1.11±0.28
	Range	0.53-1.94	0.59-1.72	0.63-1.58	0.53-1.72	0.59-1.94	0.81-1.38	0.73-1.64	0.53-1.94
	CAD (n)	102	81	43	89	102	35	41	185
	Mean ± SD	2.88±0.98	2.70±0.88	2.77±1.23	2.81±1.07	2.74±0.87	2.92±1.16	2.93±1.31	2.77±0.91
	Range	1.84-8.70	1.84-7.4	1.89-8.5	1.87-8.70	1.84-7.40	1.84-5.90	1.92-8.50	1.84-8.70
	P-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
TC (mmol/l)	Control (n)	42	35	26	46	44	13	11	92
	Mean ± SD	3.74±0.46	3.95±0.74	3.75±0.37	3.79±0.66	3.78±0.42	3.98±0.56	3.77±0.37	3.82±0.58
	Range	3.01-5.11	3.12-7.11	3.19-4.53	3.11-7.11	3.01-4.91	3.21-4.91	3.14-4.19	3.01-7.11
	CAD (n)	102	81	43	89	102	35	41	185
	Mean ± SD	5.07±0.83	4.93±0.73	5.17±1.01	5.14±0.94	4.99±0.78	4.93±0.67	4.97±0.92	5.05±0.82
	Range	3.10-7.70	3.70-7.42	3.90-8.30	3.70-8.30	3.10-7.70	4.0-6.30	3.90-8.30	3.10-7.70
	P-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
HDL-C (mmol/l)	Control (n)	42	35	26	46	44	13	11	92
	Mean ± SD	1.29±0.42	1.18±0.32	1.25±0.37	1.23±0.42	1.27±0.36	1.23±0.28	1.27±0.37	1.24±0.38
	Range	0.76-1.99	0.76-2.11	0.81-2.15	0.76-2.15	0.76-2.11	0.79-1.68	0.89-1.99	0.76-2.15
	CAD (n)	102	81	43	89	102	35	41	185
	Mean ± SD	1.13±0.39	1.07±0.31	1.17±0.47	1.11±0.39	1.09±0.36	1.16±0.42	1.17±0.46	1.10±0.36
	Range	0.53-2.80	0.59-2.15	0.73-3.12	0.60-8.30	0.53-2.49	0.76-2.80	0.73-3.12	0.53-2.80
	P-value	0.03	0.085	0.462	0.101	0.006	0.581	0.071	0.003
LDL-C (mmol/l)	Control (n)	42	35	26	46	44	13	11	92
	Mean ± SD	1.62±0.55	1.74±0.70	1.58±0.71	1.60±0.67	1.59±0.48	2.02±0.65	1.76±0.57	1.64±0.61
	Range	0.86-2.80	0.91-4.50	0.97-3.12	0.86-4.50	0.90-2.50	1.02-3.12	0.90-2.51	0.86-4.50
	CAD (n)	102	81	43	89	102	35	41	185
	Mean ± SD	2.71±0.84	2.61±0.75	2.94±0.91	2.73±0.91	2.75±0.77	2.57±0.74	2.75±0.90	2.70±0.81
	Range	1.12-5.89	1.16-5.41	1.45-4.81	1.12-5.89	1.34-4.98	1.15-4.31	1.57-5.41	1.12-5.89
	P-value	0.000	0.000	0.000	0.000	0.000	0.023	0.000	0.000

SD, standard deviation; TG, triglyceride; CAD, coronary artery disease; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

The present study clarified the associations among the *Hin*dIII, *Pvu*II and Ser447Ter genotypes of the LPL gene and the lipid parameters of patients with CAD. TG, TC and LDL-C levels were higher in the CAD group compared to the control group, irrespective of the LPL genotype, whereas compared to the control group, HDL-C levels were significantly reduced in patients with CAD who had the *Hin*dIII TT, *Pvu*II TC and Ser447Ter CC genotypes. Previous studies have investigated the effect of LPL gene activity on the plasma levels of TG, TC, HDL-C and apolipoproteins; however, the results have been inconsistent (19,37-40). Clee *et al* (38) demonstrated that decreased plasma LPL activity is associated with high TG and low HDL-C levels in patient samples, and the Ser447Ter mutation is associated with higher plasma LPL activity. Common variants of the LPL gene have differential effects on plasma lipid concentrations and the development of atherosclerosis. The association of *Pvu*II and *Hin*dIII polymorphisms with hypertriglyceridemia, low HDL-C levels and CAD were reported in several studies (6-8). These effects of the *Hin*dIII polymorphism on TG and HDL-C levels are consistent with the physiological role of LPL, which both hydrolyzes TG-rich lipoproteins and modulates plasma HDL-C levels. The *Hin*dIII polymorphism is located in intron 8 of the LPL gene, and therefore, should not be the cause of the observed effects (41).

The relationship between hypertriglyceridemia and the *Pvu*II RFLP polymorphism of the LPL gene was investigated in different populations. An association between the LPL *Pvu*II polymorphism and lipid disorders was recorded in a Japanese population and this association occurred due to significantly higher triglyceride concentrations in the *Pvu*II +/+ compared to the -/- genotype group (30). Similar results have been reported for Australian (42), French (43) and Japanese school children (44) and the Welsh (32) population. Authors of those studies also reported a significant decrease in HDL-C concen-

Table VI. Associations among the *Hin*dIII, *Pvu*II and Ser447Ter genotypes

		LPL *Hin*dIII genotypes					
		TT	GG	TG	Total	χ^2	P-value
LPL *Pvu*II genotypes	TT	43 (40.17)	12 (16.93)	34 (31.90)	89	-	-
	CC	16 (15.79)	8 (6.65)	11 (12.54)	35	-	-
	TC	43 (46.03)	23 (19.41)	36 (36.55)	102		
Total		102	43	81	226	3.11	0.54

		LPL *Hin*dIII genotypes					
		TT	GG	TG	Total	χ^2	P-value
LPL Ser447Ter genotypes	CC	90 (83.49)	36 (35.19)	59 (66.30)	185	-	-
	GC	12 (18.50)	7 (7.80)	22 (14.69)	41	-	-
Total		102	43	81	226	7.33	0.03

		LPL *Pvu*II genotypes					
		TT	CC	TC	Total	χ^2	P-value
LPL Ser447Ter genotypes	CC	76 (72.85)	23 (28.65)	86 (83.49)	185	-	-
	GC	13 (16.14)	12 (6.35)	16 (18.50)	41	-	-
Total		89	35	102	226	7.3	0.02

LPL, lipoprotein lipase.

Table VII. Haplotype frequencies of the LPL gene polymorphisms (*Hin*dIII, *Pvu*II and Ser447Ter) in the CAD and controls groups.

Haplotypes	CAD (n=226) n (%)	Controls (n=103) n (%)	OR	95% CI	P-value
TTTTCC	39 (17.26)	13 (12.62)	1.44	0.73-2.83	0.287
TTTCCC	38 (16.81)	21 (20.40)	0.789	0.44-1.42	0.433
TGTTCC	29 (12.83)	13 (12.62)	1.02	0.51-2.05	0.957
TGTCCC	28 (12.39)	10 (9.71)	1.32	0.61-2.82	0.481
GGTCCC	20 (8.85)	8 (7.77)	1.15	0.49-2.71	0.744
TTTCGC	13 (5.75)	3 (2.91)	2.03	0.57-7.30	0.275
GGTTCC	9 (3.98)	14 (13.59)	0.263	0.11-0.63	0.003
TGCCGC	8 (3.54)	0	8.05	0.46-140.8	0.153
TGTCGC	8 (3.54)	1 (0.97)	3.74	0.46-30.32	0.216
GGCCCC	7 (3.10)	2 (1.94)	1.61	0.33-7.90	0.554
TTTCGC	5 (2.21)	3 (2.91)	0.754	0.18-3.21	0.703
TGTTTC	5 (2.21)	3 (2.91)	0.754	0.18-3.21	0.703
TTTTGC	4 (1.77)	2 (1.94)	0.91	0.164-5.05	0.914
TGCCCC	3 (1.33)	8 (7.77)	0.159	0.041-0.62	0.007
GGTCGC	3 (1.33)	1 (0.97)	1.37	0.14-13.35	0.785
GGTTGC	3 (1.33)	1 (0.97)	1.37	0.14-13.35	0.785
TTCCGC	3 (1.33)	0	3.24	0.17-63.33	0.44
GGCCGC	1 (0.44)	0	1.38	0.06-34.09	0.845

LPL, lipoprotein lipase; CAD, coronary artery disease; OR, odds ratio; CI, confidence interval.

trations in the *Pvu*II -/- genotype group of CAD patients not receiving lipid-lowering drugs. In contrast to these findings, the *Pvu*II -/- genotype was observed to be associated with higher TG concentrations than the +/+ genotype in a Chinese population in the Beijing area (45). Although Jemaa *et al* (46) failed to demonstrate any significant relationship between lipid concentrations and *Pvu*II polymorphisms in a French population, their observations suggested an association between *Pvu*II polymorphisms and the severity of coronary lesions. Similar results were reported by Wang *et al* (42) in a study of 500 Australian cardiology patients. In addition to a significant relationship between *Pvu*II RFLP polymorphisms and hypertriglyceridemia, this study also identified correlations between this polymorphism and CAD and diabetes.

In several studies, the Ser447Ter allele was associated with a reduced risk of CAD (12,47-50). The beneficial effects of the Ser447Ter (S447X) polymorphism may be related to favorable effects on lipid levels. Previous studies in the Turkish population demonstrated that carriers of the X447 allele, compared with noncarriers, had lower plasma TG levels and higher levels of HDL-C and they were protected against metabolic syndrome (18). Metabolic syndrome and hyperlipidemia increase the severity of CAD (51). Thus, we hypothesized that there is a relationship between the Ser447Ter polymorphism of the LPL gene and the severity of CAD.

In our study, the GGTTCC and TGCCCC haplotypes were significantly more common among the controls. However, the TGCCGC, TTCCGC and GGCCGC haplotypes were observed only in the CAD group. Anderson *et al* (7) reported that in 483 patients with CAD, the estimated frequency of the *Hin*dIII(+)/*Pvu*II(-) (TC) haplotype was nominally greater than that in the control subjects, whereas for the other haplotypes (TT, GT), the estimated frequencies were lower than those of the control subjects. Additionally, the frequency of the GC genotype was similar between the CAD and control groups. After adjustment for potential confounders, the OR for CAD associated with the GGTTCC haplotype compared to the wild type was 0.263 (95% CI, 0.11-0.63, P=0.003) and that for TGCCCC was 0.159 (95% CI, 0.041-0.62, P=0.007) (Table VII). Consequently, the GGTTCC and TGCCCC haplotypes may have been protective against CAD in our studied population.

Goodarzi *et al* (52) found several differences in the allele and haplotype frequencies of the three LPL markers. Such differences may affect the results of association studies conducted in different populations. Rebhi *et al* (27) reported that the CTGTAA haplotype was significantly more common among patients with significant coronary stenosis than among controls. However, the CGGGAA haplotype occurred significantly more frequently in the control group compared to the coronary stenosis group. The TGGTAG, TTAGAA and CGGGAG haplotypes were observed only in the coronary stenosis group.

In conclusion, differences in relative genotype frequencies were noted between the patient and control groups. However, these differences did not reach statistical significance (Table III). There were significant differences in the plasma levels of TC, LDL-C, HDL-C and TG in association with the LPL genotypes (Table V), suggesting an association between these polymorphisms and the lipid profiles of patients with CAD. CAD may therefore be a complex disorder caused by a combination of genetic and environmental factors that may influence the onset of disease (Tables II and IV).

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