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# Associations of *Lipoprotein Lipase* S447X and *Apolipoprotein E* Genotypes with Low-density Lipoprotein Subfractions in Turkish Patients with Coronary Artery Disease

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**Abstract.** *Background: This study investigated associations of specific lipoprotein lipase (LPL) S447X and apolipoprotein (Apo)E allelic patterns with low-density lipoprotein (LDL) size and subfraction profiles in patients with coronary artery disease (CAD) and healthy individuals. Patients and Methods: Forty-one cases with CAD and 23 controls were compared regarding the occurrence of the Ser→Stop codon of the LPL and ApoE polymorphism. Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) techniques were utilized to perform genotyping, and LDL size and subfractions were assessed by a high-resolution, nongradient polyacrylamide gel electrophoresis technique. Results: The lowest small dense (sd) LDL level was observed for the homozygous LPLX447 genotype (6.00±4.00 mg/dl) while the highest sdLDL level was observed for LPLX447(+)/ApoE4(+) carriers (14.33±20.55 mg/dl) in the patient group. No protective effect of LPLX447 allele on the atherogenic LDL profile was observed when it was together with the ApoE4 allele. Furthermore, the detrimental effect of LPLS447 on the atherogenic LDL profile increased when it was present together with the ApoE4 allele. Conclusion: The X447 allele of the LPL gene may protect from atherogenic LDL subfraction, although this effect is small. We suggest that the S447X polymorphism of the LPL gene may modify the risk of atherogenic sdLDL fraction in an ApoE-dependent fashion.*

Major coronary artery disease (CAD) risk factors include advancing age, male sex, hypertension, smoking, diabetes, elevated total serum low-density lipoprotein (LDL) cholesterol, and reduced high-density lipoprotein (HDL) cholesterol (1).

Serum lipoproteins are important determinants of CAD and their metabolism is dictated to a large degree by their protein moieties, the apolipoproteins. Apolipoprotein E (ApoE) is a protein constituent of both triglyceride-rich lipoproteins (TRL) as well as HDL, which plays an important role in liver uptake of TRL remnants. Three apoE isoforms with cysteine/arginine interchanges at positions 112 and 158 have been identified in human plasma (2, 3). While ApoE2 and ApoE4 respectively have cysteine and arginine residues at both of these positions, ApoE3 has a cysteine residue at position 112 and arginine at position 158. Many studies assessing the role of *ApoE* genotypes on plasma lipids have shown that the presence of the E4 allele is associated with elevations in LDL cholesterol, while the presence of E2 is associated with reduced levels of LDL cholesterol (LDL-C) (4-9).The *ApoE4* allele is not only associated with elevated plasma cholesterol but has also been associated with atherosclerotic lesions in the thoracic and abdominal aortas and increased risk of heart disease (4, 10-12).

LPL is involved in the transformation of dietary lipids into sources of energy for peripheral tissues and plays an important role in triglyceride metabolism, since it is crucial for the hydrolysis of triglycerides and chylomicrons. The *LPL* gene is located on chromosome 8p22, spans 30 kb containing 10 exons, and 100 naturally occurring mutations have been described in this gene (13). Several polymorphisms at the *LPL* locus have been described that are associated with variations in LPL activity, serum lipid concentrations and the risk of CHD (14). The Ser447X polymorphism results in the premature truncation of LPL (15). *In vitro*, this polymorphism is associated with increased levels of LPL secretion (16, 17). This translates to higher plasma postheparin LPL activity *in vivo* (17). Previous studies on the *LPL*S447X on exon 9 have demonstrated its effect not only on reducing the plasma triglyceride level and increasing HDL-C, but also on decreasing blood pressure and protecting against coronary artery disease (18). Only a

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few have included the LDL-C concentration, and these showed variable results. Some studies showed similar LDL-C concentrations in X447 carriers as compared with S447 homozygotes (19-21), whereas others showed higher LDL-C concentrations in carriers of the X447 allele (22).

Increased blood concentrations of LDL have a high positive correlation with the incidence of cardiovascular disease. LDL has been shown to be a heterogeneous population of particles with respect to size, density and lipid composition. Quantimetrix Lipoprint LDL System separates LDL-C into 7 subfractions, from LDL-1 to LDL-7, by its size (23). LDL-1 and LDL-2 are defined as large LDL and LDL-3 to LDL-7 are defined as small dense (sd) LDL. Recent studies suggests that the presence of sdLDL is independently associated with increased risk of developing coronary artery disease (24, 25).

Recently, two studies were designed to show the relationship between specific allelic patterns of *ApoE* (26) and *CETP* (27) with LDL size and subfraction profiles in patients with CAD and healthy controls. Akanji *et al*. (26) found that although the *ApoE* allelic pattern, especially *ApoE2*, could be related to LDL subfraction profiles in controls, such associations could not be demonstrated in those with CAD. On the other hand, Wilund *et al*. (27) suggested that *CETP* genotype may contribute to the interindividual differences in plasma HDL-C subfraction changes occurring with endurance exercise training in sedentary middle- to older-aged men and women.

The aim of this study was to determine the genotype frequencies of *LPL*S447X and *ApoE* gene polymorphisms and their association with LDL subfractions in a Turkish population living in Istanbul, and to examine the potential effect of these associations by interactions with cardiac complications.

## Patients and Methods

*Study participants*. *LPL*S447X and *ApoE* gene polymorphisms and LDL subfraction analysis were studied in 41 patients with CAD (19 (46.34%) women, 22 (53.65%) men). Twenty-two out of forty-one patients had an sdLDL fraction. The patients with severe CAD were documented by angiography. Angiographic inclusion criteria were ≥50% stenosis of at least one major coronary vessel because of atherosclerosis and a vascular event, defined as myocardial infarction, percutaneous transluminal coronary angioplasty, or coronary artery by-pass grafting. Patients were included irrespective of concomitant risk factors for atherosclerosis, such as smoking and arterial hypertension and diabetes mellitus, without any lipid-lowering medication. Participants were of both sexes and the heights and weights of all individuals were also recorded, thus permitting calculation of the body mass index (BMI: weight (kg)/height$^2$ (m) ×100). Healthy persons (11 (47.82%) women, 12 (52.17%) men) lacking any symptoms of CAD were selected for the control group. None of these individuals had an sdLDL fraction. Coronary angiography was not performed on these individuals, and therefore the presence of atherosclerotic coronary arteries could not be excluded. However, none of these individuals nor their parents had any history of vascular events.

*PCR-Based detection of the Ser$^{447}$-Stop mutation*. Blood specimens were collected in tubes containing EDTA. DNA samples were extracted from whole blood with a salting-out procedure (28). Oligonucleotides located in introns 8 and 9 of the *LPL* gene were used to amplify the region of interest (5' primer, 5'-TACACTAGCAAT GTCTAGGTGA-3'; 3' primer, 5'-TCAGCTTTA GCCCAGAATGC-3') (17). The *LPL* Ser$^{447}$-Stop mutation can be detected by cutting the PCR product with the restriction endonuclease *Mnl* I. The PCR product of 488 bp contains two *Mnl* I restriction sites, of which one is polymorphic, which reveals the Ser$^{447}$-Stop mutation. Digestion of the PCR product with 10 U *Mnl* I results in three fragments of 290, 250 and 200 bp, respectively. Analysis was performed on 2% agarose gels stained with ethidium bromide (17). *ApoE* genotypes were determined by HhaI restriction enzyme (MBI Fermentas, Lithuania) digestion of the amplified specific polymerase chain reaction product was carried out in a DNA thermal cycler (GeneAmp 9700 PCR System; Applied Biosystems, CA, USA) as described by Kontula *et al*. (29).

*Lipid measurement*. Blood samples were drawn into plain tubes after the participants had fasted overnight. The samples were centrifuged for 10 min at 1500×g at room temperature and serum was immediately removed and frozen at –20˚C. Serum total cholesterol levels were measured enzymically (30). Serum HDL-C was measured following precipitation of apolipoprotein B-containing lipoproteins with phosphotungstic acid and magnesium ions. Serum triglyceride levels were measured enzymatically (31).

*LDL subtyping*. Each serum sample was analyzed for LDL subfractions on a LIPOPRINT SYSTEM (Quantimetrix, CA, USA), which has been well validated. This system uses a high-resolution, nongradient polyacrylamide gel electrophoresis to separate lipoprotein particles into various fractions: very low-density lipoprotein, 3 midbands corresponding to intermediate-density lipoproteins (IDL) and 7 LDL subfractions: LDL-1 to -2 (large, buoyant, pattern A); LDL-3 to -7 (small, dense; pattern B), and HDL. The system also gives the mean LDL particle size (23).

*Statistical methods*. Statistical analyses were performed using the SPSS software package (revision 11.5 SPSS Inc., Chicago, IL, U.S.A.). Clinical laboratory data are expressed as means±SD. Mean values were compared between patients and controls by unpaired Student's *t*-test. Differences in the distribution of *LPL*S447X and *ApoE* genotypes or alleles between cases and controls were tested using the Chi-square statistic, respectively. Allele frequencies were estimated by gene counting methods. Values of $p<0.05$ were considered statistically significant.

## Results

Patients with CAD and controls had similar distributions of sex and age. The patient group had a significantly higher level of large LDL (22.24±14.02 *vs*. 15.47±8.64 mg/dl; $p=0.04$), total cholesterol (193.58±28.99 *vs*. 168.30±22.84 mg/dl; $p=0.001$), triglycerides (167.70±50.04 *vs*. 119.47±32.92 mg/dl; $p=0.001$), as well as BMI (27.70±6.28 *vs*. 24.58±1.66 kg/m$^2$; $p=0.027$) when compared to the healthy controls. Levels of LDL-1,

Table I. *Characteristics of patient and control groups.*

| Characteristic | Groups | | $p$-Value |
|---|---|---|---|
| | Patient (n=41) | Control (n=23) | |
| Age (years) | 57.63±8.47 | 53.95±10.67 | 0.146 |
| Gender (female/male)(n) | 19/22 | 11/12 | 0.909 |
| BMI (kg/m$^2$) | 27.70±6.28 | 24.58±1.66 | 0.027 |
| LDL-1 (mg/dl) | 12.95±7.01 | 10.0±4.08 | 0.08 |
| LDL-2 (mg/dl) | 9.36±7.67 | 6.17±4.63 | 0.11 |
| LDL-3 (mg/dl) | 4.22±1.99 | 3.50±3.50 | 0.480 |
| LDL-4 (mg/dl) | 3.18±1.94 | 3.00±1.41 | 0.903 |
| LDL-5 (mg/dl) | 4.00±2.82 | - | |
| LDL-6 (mg/dl) | 3.80±2.48 | - | |
| LDL-7 (mg/dl) | 6.25±5.56 | - | |
| large LDL (LDL-1 to -2) (mg/dl) | 22.24±14.02 | 15.47±8.64 | 0.04 |
| sdLDL (LDL-3 to -7) (mg/dl) | 9.09±9.54 | 3.77±4.84 | 0.125 |
| HDL-cholesterol (mg/dl) | 36.63±13.92 | 37.56±15.43 | 0.806 |
| Total cholesterol (mg/dl) | 193.58±28.99 | 168.30±22.84 | 0.001 |
| Triglycerides (mg/dl) | 167.70±50.04 | 119.47±32.92 | 0.001 |
| Smoking (%) | 63.4 | 43.5 | 0.123 |
| Hypertension(%) | 53.6 | - | |
| Obesity (%) | 29.0 | - | |
| Family history (%) | 56.1 | 39.1 | 0.193 |
| Diabetes mellitus (%) | 43.2 | - | |
| LVH (%) | 44.8 | - | |

n, Number of individuals; LVH, left ventricular hypertrophy The results are shown as means±SD.

Table II. *Prevalences of the LPLS447X and ApoE genotypes and alleles in patients and controls.*

| | Control (n=23) | Patient (n=41) | $p$-Value |
|---|---|---|---|
| *LPLS*447X Genotype | | | |
| S447/S447 | 17 (73.9%) | 27 (65.9%) | 0.504 |
| X447/X447 | 2 (8.7%) | 10 (24.4%) | 0.123 |
| S447/X447 | 4 (17.4%) | 4 (9.8%) | 0.376 |
| *LPLS*447X Allele | | | |
| S447 | 38 (82.60%) | 58 (70.73%) | 0.123 |
| X447 | 8 (17.39%) | 24 (29.26%) | 0.504 |
| *ApoE* Genotype | | | |
| E2E3 | 3 (13.0%) | 4 (9.8%) | 0.686 |
| E2E4 | 1 (4.3%) | 0 | 0.178 |
| E3E3 | 15 (65.2%) | 28 (68.3%) | 0.801 |
| E3E4 | 4 (17.4%) | 9 (22.0%) | 0.664 |
| *ApoE* Allele | | | |
| E2 | 4 (17.39%) | 4 (4.87%) | 0.376 |
| E3 | 37 (80.43%) | 69 (84.14%) | 0.178 |
| E4 | 5 (11.62%) | 9 (10.97%) | 0.984 |

Chi-square test was used to compare of *LPLS*447X and *ApoE* genotypes in the study groups.

LDL-3 and LDL-4 subfractions, HDL-C and cigarette consumption were similar for both groups. In this study, the large LDL (LDL-1 to -2) subfraction (patients: 22.24±14.02 *vs*. controls: 15.47±8.64 mg/dl; $p$=0.04) was found to be the most significant difference in LDL subfractions between the CAD and control groups (Table I).

As shown in Table II, the genotype distribution and allele frequencies for the *LPL* and *ApoE* genes were not significantly different between the patients with CAD and the controls. The *LPL* and *ApoE* polymorphisms had no major significant effect on plasma lipid levels in any of the groups ($p$>0.05) (data not shown). There was a statistically significant difference in the levels of large-LDL subfractions (LDL-1to -2) of the patients and control group ($p$=0.04). On the other hand, Kruskal-Wallis test did not show statistically significant differences in LDL subfractions serum levels with regard to genotypes, nor could any statistically significant difference be shown among the carriers of different genotypes, neither within the large-LDL nor within the sdLDL fractions in the patient group ($p$>0.05) (Table III).

The lowest sdLDL level was observed for X447X447/S447(–) (6.00±4.00 mg/dl) while the highest sdLDL level was observed for X447(+)/ApoE4(+) individuals (14.33±20.55 mg/dl) in the patient group (Figure 1). No protective effect of the *LPL*X447 allele on atherogenic LDL profile (lowest sdLDL level) was observed when it was present together with the *ApoE4* allele. Furthermore, the detrimental effect of *LPLS*447 on the atherogenic LDL profile (high sdLDL level) increased when it was present together with the *ApoE4* allele.

We found a higher prevalance of hypertension in the patients with CAD with left ventricular hypertrophy (LVH) than these (73.3% *vs*. 30.8%; $p$=0.024) (data not shown). Moreover, no significant influence of *LPLS*447X and *ApoE* genotypes or alleles on the blood pressure (data not shown) or presence of LVH in the patient group ($p$>0.05) (Table IV) were observed.

## Discussion

We investigated associations of *LPLS*447X and *ApoE* polymorphism with the risk of CAD, and serum LDL subfraction concentrations in a case-control study in a Turkish population. Our study is the first study to examine these associations.

The causal role of LDL particles in the pathogenesis of CAD is well established. LDL-C is used as a parameter to estimate LDL-associated CAD risk. More recently, assessment of the number of LDL particles has been put forward as a more reliable method reflecting atherogenicity of the LDL fraction (32). The presence of sdLDL is closely associated with an increased risk of developing CAD. Small

Table III. *Effects of LPLS447X and ApoE genotypes on serum LDL subfractions in the patient group.*

| | *LPL*S447X | | | | *ApoE* | | | |
|---|---|---|---|---|---|---|---|---|
| Serum LDL subfraction (mg/dl) | S447/ S447 | X447/ X447 | S447/ X447 | *p*-Value | E2E3 | E3E3 | E3E4 | *p*-Value |
| Large-LDL | 22.04 (n=27) | 19.35 (n=10) | 18.13 (n=4) | 0.732 | 25.63 (n=4) | 21.04 (n=28) | 18.83 (n=9) | 0.640 |
| sd-LDL | 12.42 (n=12) | 9.83 (n=6) | 11.25 (n=4) | 0.724 | 9.75 (n=2) | 11.64 (n=14) | 11.75 (n=6) | 0.922 |

Non-parametric Kruskal-Wallis test was used to show the effects of *LPL* S447X and *ApoE* genotypes on serum sdLDL levels. Data are given as mean rank.

dense LDL particles show greater susceptibility to oxidative modification than typical LDL particles *in vitro*. The oxidative LDL has been strongly associated with the progression of atherosclerosis (33).

Jemaa *et al.* (34) investigated associations of *LPL* polymorphisms (HindIII, PvuII, Ser447→Ter) with the risk of myocardial infarction (MI), severity of atherosclerosis, and fasting plasma lipoprotein concentrations in the ECTIM study (614 patients and 733 controls). They reported that the X447 allele had a lowering effect on triglyceride ($p<0.01$) and VLDL-C ($p<0.05$), and a raising effect on apoA-I levels ($p<0.05$). They suggested that S447X had the largest effects on lipid traits, but this polymorphism had no significant impact on the risk of MI.

Yang *et al.* (35) found that the frequency of the X447 allele was significantly lower in cases than in controls (6.2% *vs.* 7.6%; $p<0.01$). They suggested the X447 allele of the *LPL* gene may protect from MI risk, although this effect is small. In this study, we found no association between the *LPL*S447X genotype and the risk of CAD. The distribution of the *LPL*S447X polymorphism was similar between CAD patients and controls. However, Ak *et al.* reported *LPL*S447X to be associated with lower levels of interleukin-8 after coronary artery bypass grafting in a case control study in Turkish patients with CAD (36).

In another case-control study, a significant association was found between S447X and patients with persistent hypertension and elevated triglycerides ($p=0.02$). *LPL* variants were suggested to play a causal role in the development of hypertension in Taiwan Han Chinese (37). In the present study, no significant influence of either *LPL*S447X or *ApoE* genotype and alleles on the blood pressure and presence of LVH in the patient group were detected.

Akanji *et al.* (26) investigated LDL subfraction heterogeneity and phenotypes as well as possible contribution of *ApoE* polymorphism on these lipid profiles in patients with CAD. They found that *ApoE* genotype and allele frequencies were similar for CAD and control groups. In both groups, the median large LDL level was greater in controls (51.0% *vs.* 46.5%, $p<0.001$) and the sdLDL level was greater in CAD patients (9.0% *vs.* 3.0%, $p<0.001$).

*Table IV. Association of the LPLS447X polymorphism and left ventricular hypertrophy in the patient group.*

| | LVH (–) (n=22) | LVH (+) (n=19) | *p*-Value |
|---|---|---|---|
| *LPL*S447X Genotype | | | |
| S447/S447 | 15 (68.2%) | 12 (63.2%) | 0.73 |
| X447/X447 | 5 (22.7%) | 5 (26.3%) | 0.79 |
| S447/X447 | 2 (9.1%) | 2 (10.5%) | 0.87 |
| *LPL*S447X Allele | | | |
| S447 | 32 (72.7%) | 26 (68.4%) | 0.79 |
| X447 | 12 (27.3%) | 12 (31.6%) | 0.73 |
| *ApoE* Allele | | | |
| E2 | 4 (100.0%) | 0 | 0.11 |
| E3 | 33 (47.8%) | 36 (52.2%) | * |
| E4 | 7 (77.8%) | 2 (22.2%) | 0.100 |

LVH, left ventricular hypertrophy. Chi-square test was used to show the effects of *LPL* S447X and *ApoE* genotypes on LVH. *All patients have ApoE3 allele. Therefore, we could not analyse the effect of presence of E3 allele on presence of LVH in the patient group.

Moreover, the sdLDL of the CAD group was higher compared with controls in the present study but this was not statistically significant (9.09±9.54 *vs.* 3.77±4.84 mg/dl; $p=0.125$). Moreover, levels of large-LDL subfractions of the patient group were higher than those of the control group ($p=0.04$).

Akanji *et al.* (26) observed that the sdLDL level was significantly lower for *ApoE2* carriers than for non-*ApoE2* in the controls (4.0% *vs.* 0.0%, $p<0.05$); in patients with CAD, patients with *ApoE2* had smaller LDL particle size, and the sdLDL fraction was significantly lower with non-*ApoE2* than with *ApoE2* carriers (15.0 *vs.* 8.0, $p<0.05$). With respect to *ApoE4*, control non-*ApoE4* carriers had a smaller median sdLDL level than did *ApoE4* carriers; otherwise, there were no significant findings in relation to *ApoE* or LDL size and subfractions in either group. Although the *ApoE* allelic pattern, especially *ApoE2*, could be related to LDL subfraction profiles in controls, such associations could not be demonstrated in patients with CAD.

Sing and Davignon (38) reported that ApoE accounted for ~10% of the LDL phenotypic variance; our results are very

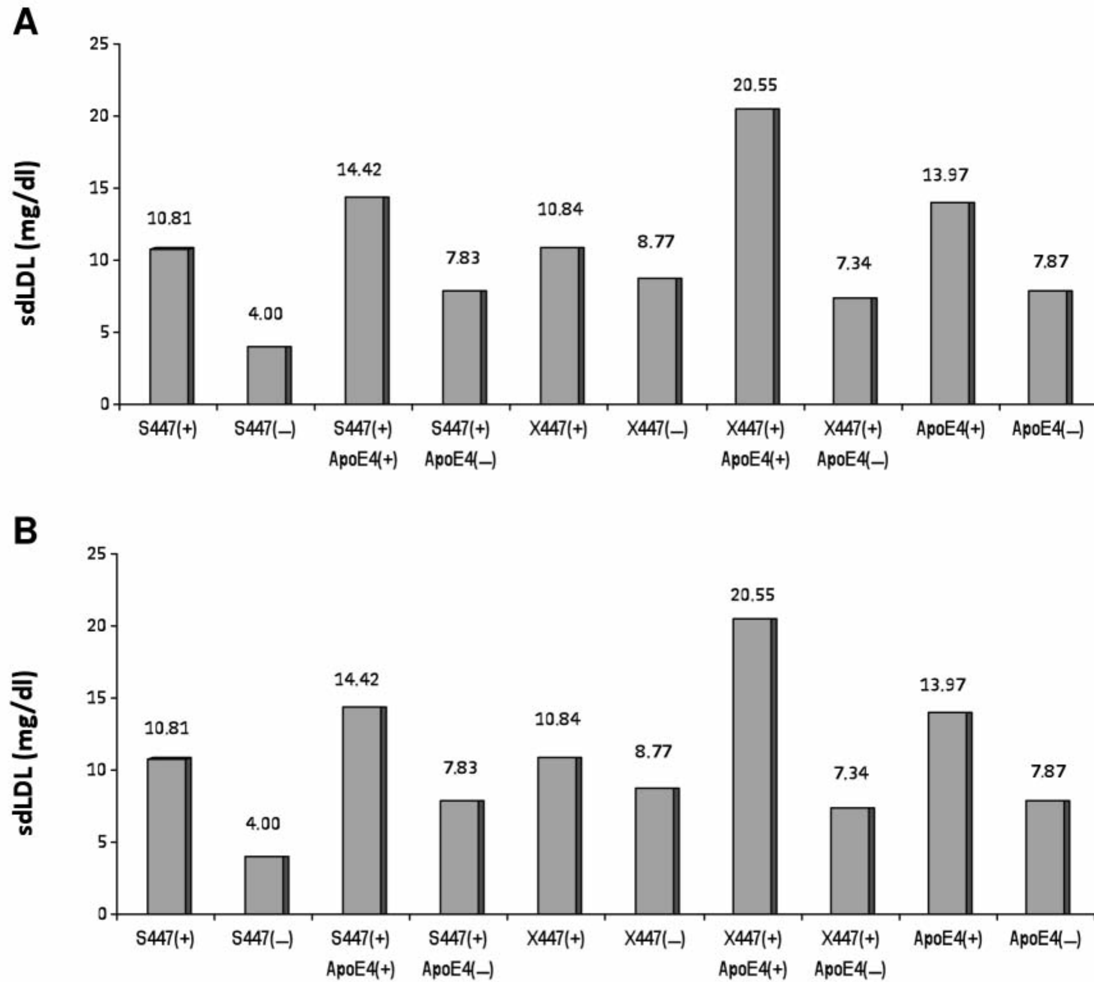


Figure 1. *Mean values for LDL subfractions. (A, sdLDL; B, large LDL) according to LPLS447X and ApoE4 alleles in the patient group. +, Carriers of allele; –, not carriers of allele. Values above bars indicate the standard deviation.*

much in accordance with this. The lowest sdLDL level was observed at for X447X447 (6.00±4.00 mg/dl) while the highest sdLDL level was observed for X447(+)/*ApoE4*(+) (14.33±20.55 mg/dl) (Figure 1). The protective effect of the X447 allele on the atherogenic LDL profile (lowest sdLDL level) was not observed when it was present together with the *ApoE4* allele. Furthermore, the detrimental effect of S447 on atherogenic LDL profile (high sdLDL level) increased when it was present together with the *ApoE4* allele. We suggest that the S447X polymorphism of the *LPL* gene may modify the risk of atherogenic sdLDL fraction in an *ApoE*-dependent fashion.

In conclusion, despite an effect of the *LPL*S447X and *ApoE* polymorphisms on atherogenic lipoprotein phenotype, we did not observe any associations between the these genotypes and a risk of CAD in the Turkish population. Our results do suggest that the *LPL* and *ApoE* genes are related to lipoprotein metabolism. We believe that a further study with a higher number of cases and controls than this and previous studies (26) may be necessary to conclude with greater certainty the association between *LPL* and *ApoE* genes and predisposition to CAD.

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