Abstract. Background: Human lectin-like oxidized low-density lipoprotein receptor 1 (LOX-1, OLR1) has been identified as a cell surface endocytosis receptor for oxidized low-density lipoprotein (oxLDL) on vascular endothelial cells. OxLDLs are avidly ingested by macrophages, resulting in foam cell formation. OxLDLs are also involved in inducing smooth muscle cell migration, proliferation and transformation. A single nucleotide polymorphism K167N (G501C) of the LOX-1 gene results in an amino acid dimorphism (Lys/Asn) at residue 167. Replacement of this Lys residue causes reduced binding and internalization of oxLDL. The purpose of this study was to investigate the effect of the LOX-1 K167N gene polymorphism in Turkish patients with coronary artery disease (CAD). Materials and Methods: K167N polymorphism were studied in 91 patients with CAD and 72 healthy controls by the PCR-RFLP method. Results: The frequencies of the KK genotype and the K allele were higher in the CAD group than the controls (p<0.05), while the frequency of the NN genotype was higher in the control group than in the CAD group (p<0.05). It was observed that the decreased CAD risk in patients who had the N allele was reversed by male sex (OR: 0.400–0.481) and smoking (OR: 0.400–0.949). Although male sex and smoking were lower than other cardiovascular risk factors in patients with the N allele they were higher than other cardiovascular risk factors in patients with the K allele. Conclusion: Male sex and smoking decrease the protective effects of the N allele. The adverse effects of the K allele on the CAD risk resulting from the K167N polymorphism appear to be independent of other cardiovascular risk factors.
exhibits a strong negative charge since the lipid peroxidation products are generated by and linked to its apolipoprotein B moiety. The C-terminal lectin-like domain alone is sufficient for the binding of oxLDL (4, 10).

Previous studies have identified 7 novel polymorphisms in the LOX-1 gene (15). A single nucleotide polymorphism G501C of the LOX-1 gene in exon 4 is a missense mutation that involves a G-to-C transition at nucleotide 501, resulting in a nonconservative amino acid dimorphism (Lys/Asn) in codon 167 (9, 16). The amino acid residue 167 is located in the lectin domain of LOX-1, which is the ligand-binding domain. Basic residues in the lectin domain are important for strengthening the ligand binding and substitution of these residues causes reduced binding and internalization of oxLDL (9).

Previous studies have shown that variation in the LOX-1 gene, including the K167N polymorphism, is associated with acute myocardial infarction (MI) and CAD (17-19). The aim of this study was to investigate the effect of the LOX-1 K167N gene polymorphism in patients with CAD and its association with cardiovascular risk factors in Turkish patients.

Materials and Methods

Subjects. The LOX-1 K167N gene polymorphism was studied in 91 patients with severe CAD (25 (27.5%) women, 66 (72.5%) men) 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 MI, 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. The height and weight of each were also recorded, thus permitting calculation of the body mass index (BMI): (weight [kg]/height² [m]) x100.

Healthy persons (33 (45.8%) women, 39 (54.2%) men) without any symptoms of CAD were selected for the control group. 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 had any history of a vascular event.

Determination of K167N genotype. Blood samples were collected in tubes containing EDTA; DNA was prepared from these specimens by the salting-out method described by Miller et al. (20). LOX-1 K167N genotyping was performed by modifying the method described by Trabetti et al. (2). For the K167N detection, PCR was used with forward primers in order to establish a BspLI (MBI Fermentas, Lithuania) restriction site. The primer pairs 167F: 5’-GGCTCATTAACTGGGAGAAG-3’, 167R: 5’-CCGTCCAAAGGT CATACACAA-3’ were used. One μl DNA template was added to 25 μl of the reaction mixture which consisted of 2.5 μl 10 x reaction buffer (MBI Fermentas), 0.75 μl (50 pmol) of each primer, 2 μl dNTP (MBI Fermentas) mixture (1.000 μM), 0.3 μl Thermus aquaticus (taq) DNA polymerase (MBI Fermentas) and 16.2 μl sterile deionized water. Amplifications were achieved by 37 cycles of denaturation (45 s at 94°C), annealing (45 s at 60°C) and an extension (1 min at 72°C) followed by 5 min at 72°C using a Techne thermal cycler (Applied Biosystems Gene Amp PCR System 9700, Singapore). The K167N PCR product was 239 bp. The amplified PCR products were directly digested with the BspLI restriction enzyme (10 units/μl). After BspLI restriction, two fragments were obtained: 217 and 22 bp for the K allele and a single fragment of 239 bp for the N allele. The digested DNAs were separated on 3% Nusieve (Cambrex Bioscience, Rockland, Inc.) agarose gel in 1×Tris borate EDTA buffer followed by staining with ethidium bromide solution. The K167N genotypes were identified by visualization under ultraviolet light and were photographed using a Polaroid camera.

Serum lipid levels were measured enzymatically (21, 22).

Statistical methods. Statistical analyses were performed using SPSS software package (version 13.0; SPSS Inc., Chicago, IL, USA). The genotypes and allele frequencies were compared by Chi-square test. Clinical, nonclinical parameters and alleles were compared by Kruskal–Wallis test, while genotypes, clinical and nonclinical parameters were compared by Student’s t-test. Values of p<0.05 were considered statistically significant.

Results

The demographic characteristics are summarized in Table I. There were significant differences in age between the patient and the control groups (p=0.03). There was a significant difference in gender (p=0.015), smoking (p=0.004), BMI values (p=0.045) and diastolic pressure (p=0.020), while there were no statistically significant differences in systolic pressure, consumption of alcohol, family history of CAD and concentrations of serum triglyceride, LDL-cholesterol, HDL-cholesterol and VLDL-cholesterol between patients with CAD and the controls (p>0.05).

The genotype distributions and allele frequencies in the CAD and controls are shown in Table II. When the K167N polymorphism was compared, it was found that there was a statistically significant difference (Fisher’s exact test, p=0.016). In the patient group, the frequency of the normal K167N KK genotype was significantly higher than in the control group (p=0.006). The frequency of the NN mutant genotype was statistically higher in the control group than in the patients (p=0.007).

In the controls when the effects of K167N polymorphism on serum lipoprotein levels, BMI, and blood pressure were investigated, it was found that there were no effects of the K167N genotype and alleles on these parameters (p>0.05). The serum triglyceride levels were higher in the controls who had the K allele than in those who had the N allele, but it was not statistically significant different (K allele: 159.00±103.49 mg/dl; N allele: 152.95±60.288 mg/dl; p>0.05).

In the patient group, no effects of the K167N polymorphism on serum lipoprotein levels, BMI or blood pressure were observed. Serum triglyceride levels in the patients who had the N allele were higher in comparison
with these with the K allele, but there was no statistically significant difference (K allele: 139.16±69.89 mg/dl; N allele: 176.06±156.79 mg/dl; p>0.05). In individuals with the KK genotype, the risk of development of CAD was increased (odds ratio: 2.502; CI 95%:1.290-4.851). When the distribution of the alleles was compared, the frequencies of the normal 167K allele and the mutant 167N allele were high in the patients and in the control subjects (respectively, p=0.007 and p=0.006). It was determined that the 167K allele increased by 2.5 fold the risk of CAD (odds ratio: 2.520; CI 95%:1.283-4.949) (Table II).

The odds ratios for the development of CAD were 1.986 for smoking and the 167K allele together and 0.949 for smoking and the 167N allele together, while it was 3.373 for smoking, 2.52 for the 167K allele and 0.400 for the 167 N allele (Table III). If an odds ratio is greater than 1, it has causal etiological value and if it is smaller than 1, it has protective value.
effect of the mutant 167N allele. In males, the odds ratio for the development CAD was 2.234 alone, 3.008 together with the K allele and 0.481 together with the N allele (Table III).

Discussion

In recent years, studies on interactions between oxLDL and cells in lesion region as a factor in formation of early and late stage atherosclerotic lesion have increased (8).

When Mango et al. examined the gene variants of the K167N polymorphism in MI patients and controls, they reported that the frequency of the K variant was lower in patients with MI than in controls (9% vs. 18%) and indicated that the N allele may show a protective effect (17). Trabetti et al. was unable to find any association between K167N polymorphism and acute MI (AMI) in an Italian population of 350 patients and 327 controls (G/C: OR=2.89; 95% CI: 1.51-5.53), although a significant association was observed with the K167N GG genotype frequency in the patients having three obstructed vessels compared with those having one or two (OR=0.469; 95% CI: 0.199-1.01; p=0.045) (2).

Ohmori and his colleagues examined the K167N polymorphism in 568 CAD patients and observed that the percentage of patients who had either C/C or C/G genotypes (LOX-1 gene variants) was lower in patients with significant stenosis than in those with normal/minimal stenosis (36 vs. 49%, p<0.01). The frequency of the C allele was also lower in the patients with significant than in those with normal/minimal stenosis (21 vs. 28%, p<0.025). The frequency of LOX-1 gene variants (CC/GC) decreased as the severity of CAD increased, and the lowest frequency was observed in patients with triple-vessel disease. However, the frequency of the variants did not differ between patients with and without MI. The LOX-1 G501C gene variants were found to be inversely associated with the severity of CAD suggesting that this polymorphism may be modifying the severity of CAD (18).

Hattori et al. reported no association between the K167N polymorphism and ischemic cerebrovascular disease in a Japanese population (16). Tatsuguchi and his colleagues found that the Japanese population with the K167N mutation of LOX-1 (501G/C 501C/C) was significantly larger in the MI group (38.2%) than in the control group (17.6%). The difference was statistically significant (p<0.002) (19).

In this study, it was observed that the frequency of the K (G) allele was high (75.82%) and the frequency of the mutant N (C) allele was low (24.17%) in the patient group (p<0.01). Although our results were consistent with the findings of Mango et al. (17) and Ohmori et al. (18), they contradicted those of Trabetti et al. (2), Hattori et al. (16) and Tatsuguchi et al. (19). In the present patient group, when the distribution of the K167N alleles was not considered, the ratio of smoking (69.8%) and male gender (72.5%) was statistically higher than in the healthy group (p<0.05) (Table I). Fewer patients who had N allele were smokers (p<0.05), while many patients who had the K allele were smokers (p=0.023). In male patients, a significantly low frequency of the N allele (p<0.01) and high frequency of the K allele (p<0.01) were observed. The ratios of smoking and male sex were high in the patients with the K allele and were low in the patients with the N allele. However, it appeared that the adverse effect on the CAD risk of the K allele resulting from the LOX-1 K167N polymorphism was independent of the other cardiovascular risk factors. When the LOX-1 K167N polymorphism in the present controls was compared with the results of different populations, the distribution of the K167N alleles was different (17, 19). This study was the first that investigated LOX-1 K167N gene variants in a Turkish population. The mechanism by which LOX-1 affects the risk of CAD, has not yet been clarified. No interaction between the K167N polymorphism and plasma lipid levels was observed, but the LOX-1 gene is a candidate for CAD risk due to being a receptor for oxLDL which has a direct effect of the mechanism of CAD.

In conclusion, the frequency of the K167N (G501C) K (G) allele was high (75.82%) and of the N allele was low (24.17%) in the CAD patients. The LOX-1 K167N polymorphism might be an independent risk factor among the other cardiovascular risk factors.

Acknowledgements

The present work was supported by the Research Fund of Istanbul University. Project No. T–966/06102006.

References

12 Chen M, Masaki T and Sawamura T: LOX-1, the receptor for oxidized low-density lipoprotein identified from endothelial cells: implications in endothelial dysfunction and atherosclerosis. Pharmacol, Therapeut 95: 89-100, 2002.

Received June 17, 2009
Revised October 8, 2009
Accepted October 14, 2009