

Is *LOX-1* K167N Polymorphism Protective for Coronary Artery Disease?

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Abstract. *Background:* Human lectin-like oxidized low-density lipoprotein receptor 1 (*LOX-1*, *OLRI*) 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.

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Coronary artery disease (CAD) is a major health problem in our country as in Western countries. Atherosclerosis is a degenerative disease of the tunica intima that starts with the formation of a fatty streak in the subintimal tissues. It is accepted that the progression of fatty streaks due to occurs interaction between lipoproteins in the circulation, hemodynamic factors, endothelial cells, vascular smooth muscle cells and macrophages (1). Several studies have attempted to identify candidate genes that may be associated with CAD. One such gene is the oxidized low density lipoprotein (oxLDL) receptor 1 (*OLRI*, or lectin-like oxLDL receptor 1, *LOX-1*) gene (2). *LOX-1* was cloned in 1997 as a mammalian endothelial receptor for oxLDL particles (3, 4). This receptor is considered a critical molecule responsible for the binding, internalization and degradation of oxLDL in endothelial cells and does not interact with modified LDL (5). Other studies have shown that *LOX-1* is also expressed in macrophages, vascular smooth muscle cells, monocytes, platelets and fibroblasts (5-7). OxLDL is taken up much more rapidly by macrophages than native LDL and this may explain in part the formation of foam cells in the developing fatty streak lesion of atherosclerosis (8). *LOX-1* plays a major role in the development of atherosclerosis via the binding and uptake of oxLDL in endothelial cells, macrophages and smooth muscle cells (9).

LOX-1 is a 50 kDa type II membrane protein that structurally belongs to the C-type lectin family and comprises four domains: a short N-terminal cytoplasmic domain, a transmembrane domain, a connecting neck domain (stalk region (10)), and a long extracellular C-type lectin domain (5, 11-13). The N- and the C-terminals are hydrophilic and are separated by a hydrophobic domain of 26 amino acids (5, 11). The lectin domain, also called the carbohydrate recognition domain, is the functional domain for oxLDL binding and is highly conserved among species, especially at the position of the six cysteine residues that form three disulfide bonds (5, 11, 14). This is consistent with its function as a ligand-binding domain and an initiator of internalization and phagocytosis (4). Positively charged amino acids within the lectin-like domain cooperatively recognize oxLDL which

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: $\geq 50\%$ 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]) $\times 100$.

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'-GGCTCATTAACTGGGAAAG-3', 167R: 5'-CCGTCCAAGGT CATAACAA-3' were used. One μ l DNA template was added to 25 μ l of the reaction mixture which consisted of 2.5 μ l 10 x reaction buffer (MBI Fermentas), 0.75 μ l (50 pmol) of each primer, 2 μ l dNTP (MBI Fermentas) mixture (1,000 μ M), 0.3 μ l *Thermus aquaticus* (taq) DNA polymerase (MBI Fermentas) and 16.2 μ l 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/ μ l). 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 1xTris 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

Table I. Characteristics of patient and control groups.

	Controls	Patients	P-value
Age (years)	57.26±10.13 (n=72)	60.72±9.83 (n=90)	0.030
Gender (F/M)	33/39	25/66	0.015
BMI	25.11±3.35 (n=66)	26.19±3.08 (n=80)	0.045
Cholesterol (mg/dl)	200.59±55.87 (n=46)	206.12±56.45 (n=86)	0.574
Triglycerides (mg/dl)	155.65±87.77 (n=46)	146.20±94.98 (n=86)	0.577
LDL-cholesterol (mg/dl)	131.61±54.28 (n=46)	130.20±45.26 (n=85)	0.881
HDL-cholesterol (mg/dl)	39.50±14.71 (n=46)	41.81±12.66 (n=85)	0.371
Total cholesterol/ HDL cholesterol	5.67±2.47 (n=46)	5.22±1.58 (n=85)	0.270
VLDL-cholesterol (mg/dl)	30.20±17.81 (n=40)	28.81±21.49 (n=81)	0.725
Smoking	13 (40.6%) (n=32)	60 (69.8%) (n=86)	0.004
Systolic pressure mmHg	120.83±11.07 (n=66)	124.65±27.52 (n=86)	0.245
Diastolic pressure mmHg	72.72±8.46 (n=66)	77.61±16.65 (n=86)	0.020
Alcohol consaption	6 (18.2%) (n=33)	11 (36.7%) (n=30)	0.099
Family history	8 (34.8%) (n=23)	32 (34.5%) (n=81)	0.681

The values in the table are shown as mean±SD; n: number of individuals.

Table II. Distribution of genotypes and alleles of K167N polymorphism in patients with CAD and controls.

K167N Genotypes	Group		Chi-square	P-value	Odds ratio (95% CI)
	Controls (n=72)	Patients (n=91)			
NN	31 (43.1%)	21 (23.1%)	7.385	0.007	0.397 (0.202-0.779)
KK	39 (54.2%)	68 (74.7%)	7.533	0.006	2.502 (1.29-4.85)
NK	2 (2.8%)	2 (2.2%)			

Fisher's exact test, $p=0.016$

K167N Alleles					
	Controls (n=72)	Patients (n=91)	Chi-square	P-value	Odds ratio (95% CI)
N	64 (44.44%)	44 (24.17%)	7.533	0.006	0.400 (0.206-0.775)
K	80 (55.55%)	138 (75.82%)	7.385	0.007	2.520 (1.283-4.949)

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.

Table III. Effects of smoking, male sex and K167N alleles together and separately on CAD risk in patients.

	Controls patients (%)	OR	95% CI
Smoking	17.8/82.2	3.373	1.453-7.831
Male gender	37.1/62.9	2.234	1.162-4.293
K allele	56.9/76.9	2.520	1.283-4.949
N allele	45.8/25.3	0.400	0.206-0.775
Smoking and K allele	81.4/89.7	1.986	0.767-5.143
Smoking and N allele	83.0/82.2	0.949	0.373-2.412
Male gender and K allele	34.7/61.5	3.008	1.581-5.723
Male gender and N allele	22.2/12.1	0.481	0.208-1.115

The odds ratio for the development of CAD risk that was low in the individuals who had the N allele, increased with smoking (0.400→0.949). Smoking reduced the protective

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 N 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 (GC+CC: 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.

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References

- Jain KS, Kathiravan MK, Somani RS and Shishoo CJ: The biology and chemistry of hyperlipidemia. *Bioorg Med Chem* 15: 4674-4699, 2007.
- Trabetti E, Biscuola M, Cavallari U, Malerba G, Girelli D, Olivieri O, Martinelli N, Corrocher R and Pignatti PF: On the association of the oxidised LDL receptor 1 (*OLR1*) gene in patients with acute myocardial infarction or coronary artery disease. *E J Hum Genet* 14: 127-130, 2006
- Sawamura T, Kume N, Aoyama T, Moriwaki H, Hoshikawa H, Aiba Y *et al.*: An endothelial receptor for oxidized low-density lipoprotein. *Nature* 386: 73-77, 1997.
- Mehta JL, Chen J, Hermonat PL, Romeo F and Novelli G: Lectin-like oxidized low-density lipoprotein receptor-1 (*LOX-1*): a critical player in the development of atherosclerosis and related disorders. *Cardiovasc Res* 69: 36-45, 2006 .
- Vecchione L, Gargiu E, Borgiani P, Predazzi I, Mango R, Romeo F, Magnani M and Novelli G: Genotyping *OLR1* gene: a genomic biomarker for cardiovascular diseases. *Recent Pat Cardiovasc Drug Discovery* 2: 147-151, 2007.
- Chen M, Narumiya S, Masaki T and Sawamura T: Conserved C-terminal residues within the lectin-like domain of *LOX-1* are essential for oxidized low-density lipoprotein binding. *Biochem J* 355: 289-296, 2001.

- 7 Kataoka H, Kume N, Miyamoto S, Minami M, Morimoto M, Hayashida K, Hashimoto N and Kita T: Oxidized LDL modulates Bax/Bcl-2 through the lectin-like Ox-LDL receptor-1 in vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* 21: 955-960, 2001.
- 8 Gillote KL, Hörkö S, Witztum JL and Steinberg D: Oxidized phospholipids, linked to apolipoprotein B of oxidized LDL, are ligands for macrophage scavenger receptors. *J Lipid Res* 41: 824-833, 2000.
- 9 Ellis TJ, 2006, *LOX-1* genotype, dietary fat intake, and aerobic exercise training: influence on endothelial function, oxidative stress, lipoprotein-lipids, and soluble *LOX-1*. Ph.D. Thesis. University of Maryland, MD, USA.
- 10 Tate S: Oxidized low-density lipoprotein receptor, *LOX-1*, on the endothelial cell-the receptor structure and functions of *LOX-1* in atherogenesis. *J Biol Macromol* 7(2): 11-22, 2007.
- 11 Chen X and Du G: Lectin-like oxidized low-density lipoprotein receptor-1: protein, ligands, expression and pathophysiological significance. *Chin Med J* 120(5): 421-426, 2007.
- 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.
- 13 Chen M and Sawamura T: Essential role of cytoplasmic sequences for cell surface sorting of the lectin-like oxidized LDL receptor-1 (*LOX-1*). *J Mol Cell Cardiol* 39: 553-561, 2005.
- 14 Murphy JE, Tedbury PR, Homer-Vanniasinkam S, Walker JH and Ponnambalam S: Biochemistry and cell biology of mammalian scavenger receptors. *Atherosclerosis* 182: 1-15, 2005.
- 15 Sentinelli F, Filippi E, Fallarino M, Romeo S, Fanelli M, Buzzetti R, Berni A and Baroni MG: The 3'-UTR C>T polymorphism of the oxidized LDL receptor 1 (*OLR1*) gene does not associate with coronary artery disease in Italian CAD patients or with the severity of coronary disease. *Nut Metab Cardiovasc Dis* 16: 345-352, 2006.
- 16 Hattori H, Sonoda A, Sato H, Ito D, Tanahashi N, Murata M, Saito I, Watanabe K and Suzuki N: G501C polymorphism of oxidized LDL receptor gene (*OLR1*) and ischemic stroke. *Brain Res* 1121: 246-249, 2006.
- 17 Mango R, Clementi F, Borgiani P, Forleo G B, Federici M, Contino G, Giardina E, Garza L, Fahdi I E, Lauro R, Mehta J L, Novelli G and Romeo F: Association of single nucleotide polymorphisms in the oxidised LDL receptor 1 (*OLR1*) gene in patients with acute myocardial infarction. *J Med Genet* 40: 933-936, 2003.
- 18 Ohmori R, Momiyama Y, Nagano M, Taniguchi H, Egashira T, Yonemura A, Nakamura H, Kondo K and Ohsuzu F: An oxidized low-density lipoprotein receptor gene variant is inversely associated with the severity of coronary artery disease. *Clin Cardiol* 27(11): 641-644, 2004.
- 19 Tatsuguchi M, Furutani M, Hinagata J, Tanaka T, Furutani Y, Imamura S *et al*: Oxidized LDL receptor gene (*OLR1*) is associated with the risk of myocardial infarction. *Biochem Biophys Res Commun* 303: 247-250, 2003.
- 20 Miller SA, Dykes DD and Poleskey HS: Simple salting-out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 16: 1215, 1988.
- 21 Richmond W: Preparation and properties of a cholesterol oxidase from *Nocardia* sp. and its application to the enzymatic assay of total cholesterol in serum. *Clin Chem* 19(2): 1350-1356, 1973.
- 22 Mc Gowan MW, Artiss JD, Stranbergh DR and Zak B: A peroxidase-coupled method for the colorimetric determination of serum triglycerides. *Clin Chem* 29(3): 538-542, 1983.

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