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— SHORT COMMUNICATION —

Oxidized LDL receptor 1 (OLR1) SNPs and CAD: a case-control association study in a North Indian population

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The human lectin-like oxidized low-density lipoprotein receptor 1 (OLR1/LOX-1) is the major receptor for oxidized LDL on the endothelium. Single nucleotide polymorphisms (SNPs) in OLR1 may play an important role in pathogenesis of atherosclerosis. We investigated G501C, IVS4-73C>T, 3′UTR 188C>T SNPs of OLR1 gene in 329 patients with coronary artery disease (CAD) and 331 age and sex-matched healthy controls by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. A significant association was observed for C allele (OLR1 G501C) with coronary artery disease (C allele frequency; CAD 0.178, control 0.098, $p = 0.00$, odds ratio (OR) 1.99, 95% CI 1.42-2.78). However, no association was found for the IVS4-73C>T (T allele frequency; CAD 0.40, control 0.388, $p = 0.66$, OR 1.05, 95% CI 0.84-1.32) and 3′UTR 188C>T (T allele frequency; CAD 0.33, control 0.307, OR 1.13, 95% CI 0.89-1.43) polymorphisms of OLR1 gene. Coronary artery disease is significantly associated with G501C polymorphism but is not associated with the IVS4-73C>T and 3′UTR 188C>T polymorphisms of OLR1 gene in North India.

Key words: OLD receptor, coronary artery disease, SNPs, polymorphism, PCR-RFLP.

INTRODUCTION

The human lectin-like oxidized low-density lipoprotein receptor 1 (OLR1/LOX-1) is expressed on the endothelial cells, macrophages, and vascular smooth muscle cells. It internalizes Ox-LDL leading to multiple effects on endothelial dysfunctions and atherosclerosis (Knowles *et al*., 2008). The expression of OLR1 gene has been observed *in vivo* (placenta, lungs, brain, and liver) and *in vitro* (aortic endothelial cell) (Sawamura *et al*., 1997). Furthermore, Ox-LDL and inflammatory cytokines can up-regulate the expression of leukocyte adhesion molecules which are involved in atherosclerosis (Kume & Gimbrone, 1994).

The association of OLR1 gene polymorphisms with acute myocardial infarction (Chen *et al*., 2003; Mango *et al*., 2003; Tatsuguchi *et al*., 2003; Ohmori *et al*., 2004; Trabetti *et al*., 2006), ischemic cerebrovascular disease (Hattori *et al*., 2006), and essential hypertension (Hou *et al*., 2008) has been reported in different populations. In the present paper we investigated for the first time the association of OLR1 gene polymorphisms (G501C, IVS4-73C>T, and 3′UTR 188C>T) with coronary artery disease in an Indian population.

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MATERIALS AND METHODS

Study subjects

The patients were randomly recruited from the outpatient and inpatient services of the Department of Cardiology, Sanjay Gandhi Postgraduate Institute of Medical Sciences from February 2006 to November 2008. Patients with evidence of more than 50% stenosis in coronary arteries were included in the study.

The controls were matched by age and sex, they had no history of coronary artery disease, and their treadmill stress test results were negative. Histories of diabetes, hypertension, and smoking habit were recorded in patient and control groups on the basis of a standard clinical examination (Fauci *et al*., 2008). The family history of coronary artery disease was noted on the basis of the presence of disease in first-degree relatives. Body mass index was calculated in accordance with Fauci *et al.* (2008). The exclusion criteria were cardiomyopathy, febrile condition, rheumatic heart disease, congenital heart disease, and systemic disorders. Prior written informed consent was obtained from all patients and controls. The study was approved by our institutional ethical committee.

DNA extraction and genotyping

Genomic DNA was extracted using the standard phenol/chloroform method from peripheral blood samples anticoagulated with EDTA (Poncz *et al*., 1982). Oligonucleotides (Genetix, UK) were synthesized according to Trabetti *et al.* (2006). The primer sequences and PCR conditions are given in Table 1. The amplified PCR products of 239 bp were digested with the *Nla*IV (NEB, UK) restriction enzyme. Two bands, 217 and 22 bp, were obtained for G allele and a single band of 239 bp for C allele of OLR1 G501C SNP. The PCR product of 268 bp, on digestion with *Bam*H1 (NEB, UK), produced 220 bp and 47 bp bands for C allele while an undigested 268 bp band was obtained for T allele of IVS4-73C>T SNP. A 207 bp PCR product of 3′UTR 188C>T SNP, on digestion with *Rsa*I (NEB, UK), produced 184 bp and 23 bp bands for C allele (wild type) while the restriction site was abolished in the presence of T allele. The digested products were electrophoresed on 3% agarose gels.

Statistical analysis

Genotype and allele frequencies in patient and control groups were compared by the chi-square test. All analyses were performed using SPSS for Windows, version 11.5 (SPSS, Chicago, IL). Statistical significance was defined as a p value < 0.05.

TABLE 1. Primer sequences and PCR conditions of genotyped SNPs

SNPs	Primer sequences	PCR conditions
501G>C	(F) 5′ GGCTCATTTAACTGGGAAAG 3′ (R) 5′ CCGTCCAAGGTCATACACAA 3′	94°C-3 min, 94C°-1 min, 54°C-30 sec, 72°C-1 min, 72C°-5 min, 35 cycles
IVS4-73C>T	(F) 5′ CAGTCAAGGGGATGTCAAAGA 3′ (R) 5′ GAGGCATCAAAAAGAATGGG 3′	94°C-3 min, 94C°-1 min, 56°C-30 sec, 72°C-1 min, 72C°-5 min, 35 cycles
3′UTR 188C>T	(F) 5′ TGTCAACATTTTTGATTCTAGGTA 3′ (R) 5′ GTTCTCCATGTTCTGTCTTTCA 3′	94°C-3 min, 94C°-1 min, 58°C-30 sec, 72°C-1 min, 72C°-5 min, 35 cycles

TABLE 2. Clinical characteristics of controls and patients

Characteristics	CAD patient (n = 329)	Control (n = 331)	*p* value
Age	57.09 ± 9.66*	53.18 ± 9.18*	0.217
Sex (M/F)	260/69	245/86	0.756
BMI	25.54 ± 2.97*	25.12 ± 3.12*	0.077
Diabetic/Non-diabetic	142/187	82/249	0.000
Hypertensive/Normotensive	156/173	76/255	0.000
Smoker/Non-smoker	66/263	33/298	0.000
Triglyceride (45-150 mg/dl)†	165.04 ± 81.33*	95.75 ± 27.95*	0.000
Total cholesterol (125-250 mg/dl)†	170.29 ± 48.56*	154.70 ± 39.73*	0.000
HDL-cholesterol (23-60 mg/dl)†	36.51 ± 7.01*	38.34 ± 7.79*	0.002
LDL-cholesterol (92-148 mg/dl)†	103.99 ± 43.67*	97.82 ± 31.72*	0.039
VLDL-cholesterol (92-148 mg/dl)†	29.80 ± 13.90*	19.59 ± 6.65*	0.000

* mean ± s.d., † laboratory reference values

TABLE 3. Distribution of genotypes and allele frequencies between cases and controls

Genotype/Allele	CAD patients (n = 329)	Controls (n = 331)	*p* value, OR (95% CI)
501GG	233 (0.708)	284 (0.858)	
501GC	75 (0.228)	29 (0.088)	0.000
501CC	21 (0.064)	18 (0.054)	
501G allele	541 (0.822)	597 (0.902)	
501C allele	117 (0.178)	65 (0.098)	0.000, 1.99 (1.42-2.78)
IVS4-73 CC	74 (0.225)	78 (0.236)	
IVS4-73 CT	247 (0.751)	249 (0.752)	0.722
IVS4-73 TT	8 (0.024)	4 (0.012)	
IVS4-73 C allele	395 (0.60)	405 (0.612)	
IVS4-73 T allele	263 (0.40)	257 (0.388)	0.66, 1.05 (0.84-1.32)
3′UTR 188CC	118 (0.359)	131 (0.396)	
3′UTR 188CT	203 (0.617)	197 (0.595)	0.240
3′UTR 188TT	8 (0.024)	3 (0.009)	
3′UTR 188C allele	439 (0.667)	459 (0.693)	
3′UTR 188T allele	219 (0.333)	203 (0.307)	0.30, 1.13 (0.89-1.43)

RESULTS

The clinical and demographic characteristics of controls and patients are indicated in Table 2. Table 3 shows the genotype distributions and the allele frequencies of OLR1 SNPs between cases and controls. Genotype distributions in patients and controls were compatible with the Hardy-Weinberg equilibrium. A significant association was observed for G501C SNP with CAD while IVS4-73C>T and 3′UTR 188C>T SNPs showed no association. Furthermore, we observed no association for the three SNPs with the severity of CAD, measured as the number of stenosed vessels.

DISCUSSION

The present study examined the relationship between CAD and G501C, IVS4-73C>T, and 3′UTR 188C>T SNPs in exon 4, IVS4, and the 3′ untranslated region of OLR1 gene, respectively. A significant association was observed between G501C SNP with CAD while no association was obtained for the other two SNPs. Our results are similar to Tatsuguchi *et al.* (2003) who also reported a significant association, however, other workers found no association of OLR1 G501C polymorphism with myocardial infarction (Ohmori *et al*., 2004; Trabetti *et al*., 2006), ischemic cerebrovascular disease (Hattori *et al*., 2006), and essential hypertension (Hou *et al.,* 2008). We observed no association of IVS4-73C>T SNP with CAD which is quite similar to Trabetti *et al.* (2006). The 3′UTR 188C>T SNP we have tested in our population also showed no association with CAD whereas Chen *et al.* (2003) and Mango *et al.* (2003) reported significant association of this polymorphism with acute myocardial infarction. Furthermore, on comparison of genotypes with the severity of the disease in relation to one, two, and three obstructed vessels, we observed no association. A stepwise decrease in the percentage of patients with the GC+CC genotype depending on the severity of CAD has been reported (Ohmori *et al*., 2004), although other groups have showed a significant association between the GG genotype and the severity of CAD (Trabetti *et al*., 2006). The above discrepancies might be due to genetic variation in different populations.

In conclusion, the G to C substitution in exon 4 showed an association with CAD whereas SNPs in IVS4 and the 3′ UTR region are not associated with CAD in a North Indian population.

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