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SHORT REPORT

On the association of the oxidised LDL receptor 1 (OLR1) gene in patients with acute myocardial infarction or coronary artery disease

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The human oxidised low-density lipoprotein receptor 1 (OLR1) gene is a functional candidate for atherosclerosis. An association of the OLR1 gene with acute myocardial infarction (AMI) or coronary artery disease (CAD) has recently been reported. In the present study a total of 677 Italian subjects, 327 CAD-free, 350 CAD, of which 190 with AMI and 160 AMI-free, was genotyped for the following four OLR1 single nucleotide polymorphisms: exon 4 K167N, IVS4 −73C>T, IVS4 −14A>G, and 3′UTR 188 C>T. No statistically significant difference was observed in allele or genotype distribution of the exon 4, intron 4, or 3′UTR SNPs in CAD patients compared to CAD-free subjects, or within CAD, in AMI patients compared to AMI-free patients. A correlation was found between the K167N G/G genotype and the increased number of obstructed vessels. Even if the OLR1 genotype frequency distribution data in CAD or AMI subjects here reported do not fully confirm the positive results of some other association studies, an association with a marker of CAD severity was observed.
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Introduction

Several studies have attempted to identify candidate genes that may be associated with acute myocardial infarction (AMI) or coronary artery disease (CAD).[1,2] One such gene is the oxidized low-density lipoprotein (LDL) receptor 1 (OLR1, or lectin-like oxidised LDL receptor 1, LOX-1) gene. OLR1 is induced by proatherogenic stimuli and by inflammatory cytokines,[3] and it is upregulated in ischaemia reperfusion injury in the rat.[4,5] An atherosclerosis susceptibility locus in mice has been identified and mapped in a syntenic region with human chromosome 12p13–p12, which contains the OLR1 gene.[6]

The association of the OLR1 K167N polymorphism, and of other OLR1 gene SNPs in linkage disequilibrium (LD), with AMI has been reported.[7,8] These polymorphisms have also been associated with CAD.[9,10]

The aim of the present study was to confirm the association of the OLR1 gene with AMI or CAD in a novel, well phenotyped, and homogenous, population.

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Materials and methods

Subjects

Subjects with angiographic documentation of their coronary artery obstruction ($N=677$) were enrolled in this study. Of these subjects, 350 subjects had angiographically documented severe coronary atherosclerosis (CAD group),

that is, lesions with greater than 50% luminal stenosis, and 327 had normal coronary arteries (CAD-free group). Among the CAD subjects, the AMI ($N=190$), and the AMI-free ($N=160$) subgroups were selected. A complete clinical history that included also the assessment of conventional cardiovascular risk factors was collected for all participants. After a multivariate analysis, as expected, CAD patients had more conventional risk factors as compared with CAD-free controls: higher BMI, total and LDL cholesterol, a higher prevalence of hypertension and smoking, and lower HDL cholesterol ($P \leq 0.0005$). Details on the enrollment criteria have been described elsewhere.[11,12]

All individuals gave their consent before entering the study, which was approved by the Ethical Committee of the Verona Hospital.

SNP genotyping

We genotyped all the 677 subjects for the following three SNPs of the OLR1 gene: K167N (501G>C) in exon 4, IVS4-73C>T, and IVS4-14A>G. Moreover, we genotyped 100 subjects for the 3′UTR 188C>T, in order to confirm the previously described LD block.[8]

For K167N and 3′UTR 188C>T detection, a new method was used with modified forward primers in order to establish a *Nla*IV and a *Rsa*I restriction site, respectively (New England Biolabs, Beverley, MA, USA). The primer pairs 167F: 5′-GGCTCATTTAACTGGGAAAG-3′, 167R: 5′-CCGTCCAAGGTCATACACAA-3′, and 188F: 5′-TGTCAACA TTTTTGATTCTAGGTA, 188R: 5′-GTTCTCCATGTTCTGTC TTTCA-3′ were used, each modified nucleotide is underlined. The K167N PCR product was 239 bp. After *Nla*IV 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 3′UTR 188C>T PCR product was 207 bp. After *Rsa*I restriction two fragments were obtained: 184 and 23 bp for the C allele, and a single fragment of 207 bp for the T allele.

For the IVS4-73C>T and −14A>G SNPs, a single PCR was performed using the primers F: 5′-CAGTCAAGGGGA TGTCAAAGA-3′ and R: 5′-GAGGCATCAAAAAGAATG GG-3′, as previously described.[8] The PCR product was 267 bp. After *Bam*HI (New England Biolabs) restriction two fragments were obtained: 220 and 47 bp for the −73C allele, and a single fragment of 267 bp for the −73T allele. After *Mae*III (New England Biolabs) restriction two fragments were obtained: 157 and 110 bp for the −14G allele, and a single fragment of 267 bp for the −14A allele.

Statistical analysis

Genotype and allele frequencies of the four groups of patients were compared by χ^2 analysis. Hardy–Weinberg equilibrium was tested. All the calculations were performed with SPSS 11.5 statistical package (SPSS Inc., Chicago, IL, USA).

Results

Study subjects were genotyped as described for the following OLR1 gene SNPs: K167N (501G>C), IVS4-73C>T, IVS4-14A>G, 3′UTR 188C>T.

Table 1 reports the allele and genotype frequencies observed for CAD and AMI patients. Data are given in Table 1 only for K167N and IVS4-73C>T SNPs, as IVS4-73C>T is in complete LD with both IVS4-14A>G and 3′UTR 188C>T (C-A-C, respectively). The distribution of genotypes within each group was in Hardy–Weinberg equilibrium. The K167N polymorphism has an observed LD with IVS4-73C>T: $D'=0.87$. K167N (501G>C) – IVS4-73C>T haplotype frequencies were estimated, and the following three haplotypes with frequency >0.01 were observed: G-T (53%), G-C (39%), C-C (7.5%), C-T (0.5%).

No statistically significant difference was observed in allele or genotype frequencies for each polymorphism in CAD patients compared to CAD-free controls, in AMI patients compared to AMI-free patients, or in AMI patients compared to CAD-free controls.

An association analysis was performed by grouping OLR1 genotypes as follows: K167N (501G>C) GG *vs* GC+CC,[7] and IVS4 −73C>T CC *vs* CT or TT.[8] The results are given in Table 2. No significant association was observed.

No association was detected for OLR1 SNPs genotypes when the conventional cardiovascular risk factors were included in the model.

No association of K167N and −73C>T SNPs with CAD was observed in women[9] (50 CAD *vs* 108 CAD-free; $P=0.17$, $P=0.38$, respectively).

The distribution of K167N or IVS4-73C>T genotypes in the CAD patients did not show any significant difference or trend according to the number of stenosed vessels (one, 41 patients; two, 69 patients; or three, 231 patients; for nine subjects data were not available; $P=0.35$, $P=0.41$, respectively, for the two polymorphisms) (data not shown).

Table 1 OLR1 K167N (501G>C) (a), and IVS4-73C>T (b) genotype and allele frequencies, respectively, in the studied subjects

	CAD (N=350)	*CAD-free (N=327)*	*AMI (N=190)*	*AMI-free (N=160)*
(a)				
G/G	0.851	0.850	0.847	0.869
G/C	0.143	0.147	0.147	0.125
C/C	0.006	0.003	0.006	0.006
G	0.92	0.92	0.92	0.93
C	0.08	0.08	0.08	0.07
(b)				
C/C	0.197	0.260	0.195	0.200
C/T	0.506	0.453	0.531	0.475
T/T	0.297	0.287	0.274	0.325
C	0.45	0.49	0.46	0.44
T	0.55	0.51	0.54	0.56

Table 2 Association analysis for OLR1 K167N or IVS4-73C>T SNPs in CAD or AMI subjects

	CAD vs CAD-free				*AMI vs AMI-free*			
	OR	*SE*	*CI (95%)*	*P-value*	*OR*	*SE*	*CI (95%)*	*P-value*
K167N GG *vs* GC+CC	1.02	1.005	0.651–1.605	0.98	0.965	1.32	0.55–1.68	0.88
IVS4-73C>T CT+TT *vs* CC	1.42	1.20	0.99–2.04	0.065	1.31	1.27	0.82–2.10	0.29

Table 3 Association results between OLR1 gene polymorphisms and acute myocardial infarction (AMI) or coronary artery disease (CAD) severity in the previous studies and the present study

	Sample size	*Risk genotype*	*OR (CI)*	*P-value*	*Associated phenotype*
Tatsuguchi *et al*[7]	204	501(G/C+C/C)	2.89 (1.51–5.53)	<0.002	AMI
Mango *et al*[8]	253	3′UTR 188 CT or TT	3.74 (1.73–8.18)	<0.0001	AMI
Chen *et al*[9]	563	3′UTR 188 (CT+TT)	0.61 (0.41–0.91)	0.014	CAD severity
Ohmori *et al*[10]	586	501(G/C+C/C)	0.61 (0.41–0.92)	<0.025	CAD severity
Present study	350	501G/G	0.47 (0.19–1.01)	0.045	CAD severity

A significant association was observed when comparing the K167N GG genotype frequency in patients having three obstructed vessels *vs* patients having one or two (OR: 0.469, CI: 0.199–1.01, $P=0.045$).

Discussion

In the present study, the exon 4 K167N, the IVS4-73C>T and −14A>G, and the 3′UTR 188C>T polymorphisms of the OLR1 gene, described to be associated with AMI[7,8] or CAD,[9–10] were analysed. Table 3 shows a summary of the results from the above reported association studies and the present study for comparative purposes. Three common haplotypes account for 85% of the observed OLR1 haplotypes in the CEPH individuals from the HapMap Project (10 SNPs). Three LD blocks were described.[13] The SNPs reported in this study, not genotyped by the HapMap project, map in the third LD block. Mango *et al*[8] screened all exons and intron/exon boundaries of the gene, finding seven SNPs, and indicated that AMI risk was associated with SNPs mapping from exon 4 to the 3′UTR (third LD block). As the sample set described in the present manuscript and in Mango *et al*[8] are both from the Italian population, it is unlikely that other risk susceptibility SNPs in the first two blocks of the OLR1 gene could be present.

We did not confirm the association of the OLR1 K167N polymorphism and AMI previously described in the Japanese population (GC+CC: OR=2.89; CI: 1.51–5.53).[7] We did not confirm the different frequency distribution of the OLR1 IVS4−73T/T homozygotes between AMI and AMI-free groups previously described in another Italian population sample: 3′UTR 188C>T (which is in complete LD with IVS4-73C>T) and AMI (OR=3.74; CI: 1.73–8.18).[8] These discrepancies could be due to an ascertainment difference, or to a population difference, or to the limited study numerosity (350 in the present study, 204 in the Japanese study, and 253 in the previous Italian study, respectively).

Two studies reported a 3′UTR or a K167N association with CAD severity in non-Hispanic white women in the US population (CT+TT: OR=0.61; CI: 0.41–0.91)[9] and in the Japanese population (CC+CG: OR=0.61; CI: 0.41–0.92),[10] respectively. All CAD patients here described had severe coronary atherosclerosis, that is, lesions with greater than 50% luminal stenosis. The distribution of genotypes in CAD patients in the present study did not show any significant difference with increasing number of stenosed coronary arteries, while an association was detected in the most severely affected patient class. In this study, the K167N GG genotype has the opposite effect on CAD severity with respect to what described in the Japanese population.[10]

We performed an association analysis under the model used in two previous studies.[7,8] The association of IVS4-73C>T with CAD was close to statistical significance (results given in Table 2).

The present results suggest that the effect of the gene, if associated with CAD and/or AMI, might be smaller than previously reported, rejecting association at an OR≥1.5.

Further studies in a larger set of individuals may help in detecting SNPs associated with a more modest risk. Our calculations indicate that the sample size should be at least 2400 or 2730 for IVS4-73C>T or 4650 or 4950 for K167N to detect a significant association in CAD or AMI, respectively (significance level=0.001, power=80%, OR=1.4).

In conclusion, in accordance with some previous evidence of the oxidised LDL receptor involvement in atherogenesis, the OLR1 genotype frequency distribution data in CAD or AMI subjects here reported provide some indication of a possible association.

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