European Journal of Human Genetics (2006) 14, 894–895

www.nature.com/ejhg

LETTER

OLR1 gene and coronary artery disease/acute myocardial infarction: replication in an independently collected sample

European Journal of Human Genetics (2006) **14,** 894–895.
doi:10.1038/sj.ejhg.5201636; published online 17 May 2006

In their recent publication, Trabetti *et al.*[1]reported new data on the association of the *OLR1* gene and acute myocardial infarction (AMI) or coronary artery disease (CAD), showing that they do not fully confirm the positive results of previous studies,[2–4] even though they observed an association of one OLR1 polymorphism with CAD severity. They also comment that these discrepancies could be owing to an ascertainment difference, or to a population difference, or to the sample size reported in some of the published studies. In 2003,[3] we identified and described a set of *OLR1* single-nucleotide polymorphisms (SNPs) in complete linkage disequilibrium that were found to be associated with AMI in a series of Italian patients and controls carefully selected. All subjects enrolled in the study underwent coronary angiography and left ventriculography. Control subjects were without any angiographically demonstrable coronary lesions and no evidence of active myocardial ischemia. On this basis, *ORL1* SNPs were considered an important risk factor in AMI.[3]

Difficulty in confirmation of the genetic association data has become a major impediment to progress in elucidating the basis of complex genetic disorders. This may relate to false-positive reports of association and uncertainty that originates from the unknown complexity of the overall genetic heterogeneity in the sampled populations. In addition, when genetic effects are modest in magnitude across the sample population with too many variables, such as race and ethnicity, replication may require very large sample size and appropriate selection of the control group.[5] Accuracy in the definition of the phenotype of patients and the clinical (and even the subclinical status) of controls is, therefore, crucial in these studies. Sometimes risk factors may be significantly different between the groups of controls and patients, and this can affect the association analysis.

In a recent study, we demonstrated that *ORL1* SNPs previously identified have a functional effect.[6] In fact, two different *OLR1* transcripts were isolated in the mRNA extracted from human monocyte-derived macrophages. One of these products corresponded to the full-length transcript, whereas the other lacked exon 5, and we termed it LOXIN. LOXIN was predicted to code a protein that lacks two-third of the lectin-like domain of LOX-1, a region that is important for oxLDL binding. Both isoforms were detected in several cell types, such as endothelial cells, fibroblasts, and smooth muscle cells, and tissues, including the heart, kidney, and brain.[6] We were able to demonstrate that the OLR1/LOXIN mRNA ratio was 33% higher in human monocyte-derived macrophages of subjects homozygous for *risk* allele compared with homozygous for the *nonrisk* allele.[6] This suggests that the SNPs may result in reduced expression of LOXIN. Correspondingly, a relative increase in the amount of LOXIN was found in cells derived from subjects homozygous for the *nonrisk* allele, suggesting that the expression level of LOXIN influence the incidence of AMI in humans and, at least in part, explain the risk resulting from polymorphisms in the human *OLR1* gene.

Altogether, these data provide a biological plausibility of the *OLR1* SNPs association studies and offer support for an important role for *OLR1* in CAD and the evolution of AMI in certain patients.[7] However, vagaries in patient presentation, background risk factors, and extent of atherosclerosis may all relate to the variable penetrance of a large set of alleles in different genes.

Acknowledgements
This work was supported by the Italian Ministry of Health and Italian Ministry of Education, University, and Research.

Giuseppe Novelli*[,1,2], Paola Borgiani[1], Ruggiero Mango[1], Francesco Romeo[2,3] and Jawahar L Mehta[2]
[1]*Dipartimeno di Biopatologia e Diagnostica per Immagini, Università di Roma Tor Vergata, Roma, Italy;*
[2]*Division of Cardiovascular Medicine, University of Arkansas for Medical Sciences, Little Rock, AR, USA;*
[3]*Dipartimento di Medicina Interna, Università di Roma Tor Vergata, Roma, Italy*

**Correspondence: Professor G Novelli, Dipartimento di Biopatologia e Diagnostica Per Immagini, Università di Roma Tor Vergata, Via Montpellier 1, Roma 00133, Italy.Tel: + 39 06 20900665; Fax: + 39 06 20900669; E-mail: novelli@med.uniroma2.it*

References
1 Trabetti E, Biscuola M, Cavallari U *et al*: On the association of the oxidised LDL receptor 1 (OLR1) gene in patients with acute myocardial infarction or coronary artery disease. *Eur J Hum Genet* 2006; **14**: 127–130.
2 Tatsuguchi M, Furutani M, Hinagata J *et al*: Oxidized LDL receptor gene (OLR1) is associated with the risk of myocardial infarction. *Biochem Biophys Res Commun* 2003; **28**: 247–250.
3 Mango R, Clementi F, Borgiani P *et al*: Association of single nucleotide polymorphisms in the oxidised LDL receptor 1 (OLR1) gene in patients with acute myocardial infarction. *J Med Genet* 2003; **40**: 933–936.
4 Chen Q, Reis SE, Kammerer C *et al*: Genetic variation in lectin-like oxidized low-density lipoprotein receptor 1 (LOX1) gene and the risk of coronary artery disease. *Circulation* 2003; **107**: 3146–3151.
5 Novelli G, Borgiani P, Giardina E *et al*: Role of genetics in prevention of coronary atherosclerosis. *Curr Opin Cardiol* 2003; **18**: 368–371.
6 Mango R, Biocca S, del Vecchio F *et al*: *In vivo* and *in vitro* studies support that a new splicing isoform of OLR1 gene is protective against acute myocardial infarction. *Circ Res* 2005; **97**: 152–158.
7 Mehta JL, Chen J, Hermonat PL, Romeo F, 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* 2006; **69**: 36–45.

Reply to Novelli

European Journal of Human Genetics (2006) **14,** 895. doi:10.1038/sj.ejhg.5201637; published online 17 May 2006

We would like to thank Giuseppe Novelli and colleagues for their comments on our paper:[1] we have appreciated the importance of their demonstration of functional effects of intronic SNPs in the OLR1 gene.[2]

In our publication, we did not find an association with acute myocardial infarction (AMI) in 677 subjects, but observed an association of the 501G>C polymorphism with CAD severity in a survey of 350 individuals.

We have now extended that study (first set) in a new replication sample of 637 angiographically documented CAD patients (second set). Of the 637 CAD patients, 397 were with and 240 without AMI. All these subjects were consecutively recruited from the same clinical center as the first set, according to the previously described enrollment criteria. The IVS4−73C>T SNP, which is associated with the expression of full-length transcript or the LOXIN detected transcript in their study,[2] as well as the exon 4 K167N (501G>C) SNP, were genotyped.

As indicated in Table 1, no association was found in the replica set with the IVS4−73C>T or the K167N SNP ($P=0.15$, $P=0.89$, respectively). The grouped sample (first and second set) confirmed no association with either polymorphism ($P=0.29$, $P=0.84$, respectively).

Table 1 OLR1 IVS4−73C>T (a) and K167N (501G>C) (b) genotype frequencies in the replication sample

	AMI (N=397)	*AMI free (N=240)*
(a) IVS4—73C>T		
C/C	0.207	0.212
C/T	0.456	0.521
T/T	0.337	0.267
(b) K167N (501G>C)		
G/G	0.864	0.877
G/C	0.126	0.115
C/C	0.010	0.008

The association we previously observed between the K167N GG genotype frequency and CAD severity was not confirmed in the second set that included 322 patients with three, 159 patients with two, and 116 patients with one stenosed vessels, respectively ($P=0.86$). In the grouped sample, no significant association was detected ($P=0.73$).

In conclusion, we confirm no association with AMI in an extended sample; moreover, the apparent association with CAD severity was not replicated. Further studies are needed to elucidate the role of the OLR1 gene in cardiovascular disease.

Elisabetta Trabetti*[,1], Michele Biscuola[1], Ugo Cavallari[1], Giovanni Malerba[1], Alessandra Pasquali[1], Domenico Girelli[2], Oliviero Olivieri[2], Nicola Martinelli[2], Roberto Corrocher[2] and Pier Franco Pignatti[1]
[1]*Department of Mother and Child and of Biology-Genetics, Section of Biology and Genetics, University of Verona, Verona, Italy;*
[2]*Department of Clinical and Experimental Medicine, University of Verona, Verona, Italy*

**Correspondence: Dr E Trabetti, Department of Mother and Child and of Biology-Genetics, Section of Biology and Genetics, University of Verona, Strada Le Grazie 8, Verona 37134, Italy*
E-mail: elisabetta.trabetti@univr.it

References
1 Trabetti E, Biscuola M, Cavallari U *et al*: On the association of the oxidised LDL receptor 1 (OLR1) gene in patients with acute myocardial infarction or coronary artery disease. *Eur J Hum Genet* 2006; **14**: 127–130.
2 Mango R, Biocca S, del Vecchio F *et al*: *In vivo* and *in vitro* studies support that a new splicing isoform of OLR1 gene is protective against acute myocardial infarction. *Circ Res* 2005; **97**: 152–158.