Annals of Human Biology, March–April 2010; 37(2): 137–149

informa
healthcare

RESEARCH PAPER

Population differences in allele frequencies at the *OLR1* locus may suggest geographic disparities in cardiovascular risk events

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(Received 22 May 2009; accepted 29 September 2009)

Abstract

Background: Several studies have demonstrated a link between cardiovascular disease (CVD) susceptibility and the genetic background of populations.

Endothelial activation and dysfunction induced by oxidized low-density lipoprotein (ox-LDL) is one of the key steps in the initiation of atherosclerosis. The oxidized low density lipoprotein (lectin-like) receptor 1 (*OLR1*) gene is the main receptor of ox-LDL. We have previously characterized two polymorphisms (rs3736235 and rs11053646) associated with the risk for coronary artery disease (CAD) and acute myocardial infarction (AMI).

Aim: Given their clinical significance, it is of interest to know the distribution of these variants in populations from different continents.

Subjects and methods: A total of 1229 individuals from 17 different African, Asian and European populations was genotyped for the two considered markers.

Results: The high frequencies of ancestral alleles in South-Saharan populations is concordant with the African origin of our species. The results highlight that African populations are closer to Asians, and clearly separated from the Europeans.

Conclusion: The results confirm significant genetic structuring among populations and suggest a possible basis for varying susceptibility to CVD among groups correlated with the geographical location of populations linked with the migrations out of Africa, or with different lifestyle.

Keywords: *Cardiovascular disease, single nucleotide polymorphisms, OLR1*

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ISSN 0301-4460 print/ISSN 1464-5033 online © 2010 Informa UK Ltd.
DOI: 10.3109/03014460903393857

Introduction

The identification of genetic variants predisposing to complex diseases and the observed differences in the incidence of such variants among human populations are an open question in medical and population genetics (Rish et al. 2002; Ioannidis et al. 2004). In fact, substantial differences in susceptibility to common diseases such as cardiovascular disease (CVD) are present between self-identified ancestral/ethnic groups (Keys 1970; Levy and Kannel 2000), although evolutionary and clinical approaches must often be considered as different levels of analysis (Jackson 2008).

The basis for ethnic differences in CVD susceptibility is not fully understood although several studies have reported differences in the degree of cardiovascular risk in relation to the geographical origin of the populations (Kullo and Ding 2007; Romeo et al. 2007; Allison et al. 2008; Shiffman et al. 2008). The main obstacle for these studies lies in the variability of cardiovascular risk factors in different ethnic groups. In fact, complex diseases are the result of the interaction of different environmental factors with a genetic background, which is itself a gene–environment outcome that has been shaped through generations by sociocultural, abiotic and biotic environmental filters (Jackson 2008).

Traditional risk factors associated with atherogenesis include hypercholesterolaemia, smoking, male gender, hypertension, diabetes, and age. However, newly defined nontraditional risk factors are emerging as being equally important. Among these are the elevated plasma and tissue levels of oxidized low-density lipoprotein (oxLDL), that show significant positive correlation with the severity of acute coronary syndromes such as myocardial infarction and unstable angina, and the more severe lesions contain a significantly higher percentage of oxLDL-positive macrophages. Most of the effects of oxLDL are mediated by scavenger receptors (Dhaliwal et al. 1999). The best known endothelial scavenger receptor is the lectin-like oxidized low density lipoprotein receptor 1 receptor (LOX-1), a type-II membrane protein belonging to the C-type lectin family. LOX-1 consists of four domains: A short N-terminal cytoplasmic, a transmembrane, a connecting neck, and a lectin-like domain at the C terminus which binds oxLDL (Sawamura et al. 1997) and that is also known to be present in several natural killer receptors involved in innate immune response. As for many scavenger receptors, LOX-1 has the ability to bind with high affinity a broad spectrum of structurally unrelated exogenous (Gram+ and Gram– bacteria) and endogenous (ox-LDL, apoptotic cells, advanced glycation end products, C-reactive protein, HSP70) ligands (Chen and Du 2007). Importantly, the integrity of the lectin-like domain is critically required for its binding activity and is highly conserved among species (Park et al. 2005; Dunn et al. 2008).

The lectin-like oxidized LDL receptor gene (*OLR1*) encoding for LOX-1 receptor is mapped on chromosome 12p13.2-p12.3 (Yamanaka et al. 1998; Aoyama et al. 1999). It is constituted by six exons (Figure 1) and is expressed in endothelial cells, macrophages, vascular smooth muscle cells, platelets and cardiomyocytes. Different studies have demonstrated an involvement of the *OLR1* gene and its product in susceptibility to coronary artery disease (CAD) and acute myocardial infarction (AMI) (Chen et al. 2003; Mango et al. 2003; Tatsuguchi et al. 2003; Ohmori et al. 2004; Hattori et al. 2006; Novelli et al. 2006a,b), although discordant results were also reported (Sentinelli et al. 2006; Knowles et al. 2008). In particular, the functional role of two variants has been clearly demonstrated (Mango et al. 2005; Biocca et al. 2008, 2009).

Given the clinical and functional importance of the selected variants, the aim of the present study is to investigate the frequency and distribution of these two SNPs in various African, Asian and European populations, to check for genetic structuring and obtain

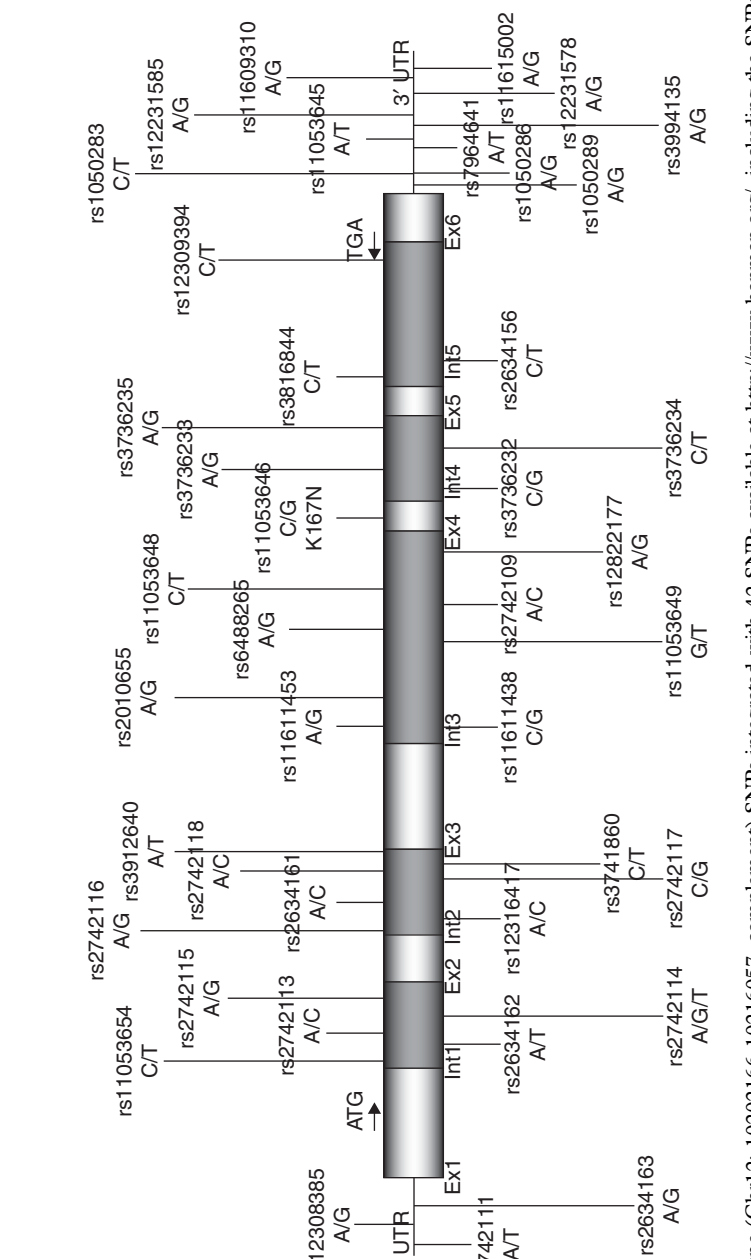

Figure 1. *OLR1* gene (Chr12: 10202166-10216057, complement) SNPs integrated with 42 SNPs available at http://www.hapmap.org/, including the SNPs described by Mango et al. (2003). The positions of the SNPs rs11053646 and rs3736235 are 10204715 and 10204342 respectively. Information about *OLR1* gene and SNPs positions are from the NCBI website.

population data regarding *OLR1* gene and its relationship with CAD susceptibility in different ethnic groups.

Materials and methods

Samples

We genotyped the SNPs IVS4-14A>G (rs3736235, chr12: 10204342) and K167N (rs11053646, chr12: 10204715) on a sample of 1229 individuals belonging to various African [Egypt, Libya (Tuareg), Benin (Bariba, Berba, Dendi, Fon)], Asian (Mongolia and Siberia), European (Italy, Spain, Bulgaria, Serbia, Turkey) and United States populations both of European and African ancestry. Each sample comprises autochthonous, unrelated and apparently healthy donors of both sexes, who gave their informed consent. Each donor was asked to supply their name, birthplace, language and ethnicity for three generations in order to exclude recent admixture. Further information on these samples can be found in several references (Biondi et al. 1996; Giambra et al. 2006; MartÍnez-Labarga et al. 2007; Giardina et al. 2008).

The Italian sample included a group of 174 clinically defined AMI cases, a group of 91 patients suffering from CAD and a group of 67 healthy subjects (ctrl). The patients were of both sexes, between 50 and 70 years of age, admitted to the hospital with suspected myocardial infarction and therefore underwent coronary angiography in order to assess the disease status. The assignment to one or another category was based on angiography data: Control subjects were defined individuals having a total absence of atherosclerotic lesions and clinically ascertained as having different problems than CAD. None of these samples had previous history of myocardial infarction.

Genomic DNA was isolated from whole blood by salting out method (Miller et al. 1988), or from mouthswab following the procedure reported in Budowle et al. (2000).

Furthermore, genotypic data for Chinese (CHB, 45 unrelated Han Chinese from Beijing, China), Japanese (JPT, 45 unrelated Japanese from Tokyo, Japan), Nigerian (YRI, 30 Yoruba mother–father–child trios from Ibadan, Nigeria) and European (CEU, 30 mother–father–child trios from the CEPH collection Utah residents with ancestry from northern and western Europe) samples were downloaded from the HapMap site (http://www.hapmap.org/genotypes/?N=D) for the two considered SNPs (The International HapMap Consortium 2005). In the CEU and YRI population the offspring's genotypes were removed from the sample in order to analyse unrelated subjects.

SNP genotyping

Genotyping was performed by TaqMan assays (Applied Biosystems, Foster City, CA, USA). Reactions were run in an AB7000 (Applied Biosystems) and interpreted using Sequence Detection System (SDS) 2.1 software. Each plate contained three positive controls (samples previously confirmed by direct sequencing as heterozygous and both homozygous) and two negative controls. Genotype assessment for each SNP was confirmed by post-genotyping direct re-sequencing of random samples.

Determination of ancestral alleles

Since *OLR1* sequence has a high level of similarity in *Homo sapiens*, *Pan troglodytes* and *Macaca mulatta*, ancestral alleles of the two SNPs considered were determined by the alignment of the sequences surrounding the variation in *Homo sapiens* with the sequences submitted in the *Pan*

troglodytes and *Macaca mulatta* databases through the Basic Local Alignment Search Tool available at http://blast.ncbi.nlm.nih.gov/Blast.cgi (Altschul et al. 1990).

The results obtained were compared with the data reported in the HapMap database available at http://www.hapmap.org/ (The International HapMap Consortium 2005).

Statistical analysis

Allele and haplotype frequencies were computed by a Bayesian approach implemented in the Phase v2.0 program (Stephens et al. 2001; Stephens and Donnelly 2003; Stephens and Scheet 2005) and by maximum-likelihood method using Arlequin package software (Excoffier and Slatkin 2005).

Linkage disequilibrium between each pair of loci was assessed in terms of *D*' through the Ldplotter software available at https://www.pharmgat.org/Tools/pbtoldplotform. In order to check whether some populations could be grouped in one, Bayesian 95% credible regions (CRs) for allele and haplotype frequencies were calculated with the computer program Sampling (kindly provided by V. Macaulay, Department of Statistics, University of Glasgow, UK).

The apportionment of genetic variation between and within populations was estimated by analysis of molecular variance (AMOVA) (Excoffier et al. 1992) either by comparing haplotype frequencies or by taking molecular differences into account using Arlequin 2.0. The statistical significance of *F* values was estimated by permutation analysis using 10 000 random permutations.

Distance matrices between populations were generated using two methods: Reynolds' distance (Reynolds et al. 1983) and Slatkin's linearized FST (Schneider et al. 2000). Multidimensional scaling (MDS, Kruskal and Wish 1978) based on the above reported distance matrices and correspondence analysis (CA) based on haplotype frequencies were performed with STATISTICA 5.5 package software (StatSoft Inc. 1995).

Results

Genotype and allele frequencies of the two polymorphisms are reported in Table I. No deviation from the Hardy–Weinberg equilibrium was detected.

The G allele of the rs3736235 was detected at low frequencies in Western African, Mongolian and Japanese populations, and in the Han Chinese from Beijing, while this SNP is very frequent in European populations. The highest frequency of the G allele was found in the Italian AMI subjects (60%, almost three-fold higher than the average value of the African samples, that was about 20%) and in the Italian CAD subjects (57%). Populations from north Africa show highly different values: The Libyan Tuaregs display a frequency (17.7%), which falls perfectly in the range of those observed in south Saharan Africa; the Egyptian sample, on the other hand, reaches a value higher than 50% typical of the European populations. The same stands for the Turkish who reach a value of 53%.

The exonic variation rs11053646 was revealed to be less frequent than the intronic variation in all populations studied, however also in this case a clear separation between European, populations of European Ancestry and non-European groups is evident. The only exceptions were the Utah sample and the Italian controls, characterized by frequencies significantly higher than those typical of European populations which range from 2 to 10%. Also for the rs11053646 polymorphism the Egyptians and the Turkish turned out to be more similar to the European populations, while the Tuareg show a value typical of those recorded in the African/Asian group.

Table I. Genotypic and allelic absolute (and relative) frequencies for the two polymorphisms in all populations.

		IVS4-14A>G (rs3736235)				K167N (G501C or rs11053646)			
Population	Chr. no.	AA freq.	AG freq.	GG freq.	G allele freq.	GG freq.	GC freq.	CC freq.	C(N) allele freq.
Bariba (Benin)	98	30 (0.612)	18 (0.367)	1 (0.020)	0.204±0.041	28 (0.571)	20 (0.408)	1 (0.020)	0.224±0.036
Berba (Benin)	72	19 (0.528)	15 (0.417)	2 (0.056)	0.278±0.065	23 (0.639)	11 (0.306)	2 (0.056)	0.208±0.041
Dendi (Benin)	98	33 (0.673)	14 (0.359)	2 (0.051)	0.184±0.043	33 (0.673)	14 (0.286)	2 (0.041)	0.184±0.040
Fon (Benin)	78	35 (0.714)	10 (0.204)	4 (0.082)	0.184±0.032	35 (0.714)	13 (0.265)	1 (0.020)	0.153±0.035
AA USA	38	13 (0.684)	5 (0.263)	1 (0.053)	0.184±0.052	12 (0.632)	7 (0.368)	0 (0.000)	0.184±0.077
HapMap YRI*	122	42 (0.734)	13 (0.228)	2 (0.035)	0.123±0.031	38 (0.623)	20 (0.328)	3 (0.049)	0.123±0.031
Egypt	122	13 (0.213)	30 (0.491)	18 (0.295)	0.541±0.050	53 (0.869)	8 (0.131)	0 (0.000)	0.066±0.022
Libyan Tuareg	164	55 (0.671)	25 (0.305)	2 (0.024)	0.177±0.022	60 (0.732)	20 (0.244)	2 (0.024)	0.152±0.032
Siberia	112	36 (0.625)	16 (0.286)	5 (0.089)	0.241±0.052	33 (0.643)	20 (0.375)	0 (0.000)	0.177±0.028
Mongolia	170	63 (0.741)	20 (0.235)	2 (0.023)	0.147±0.027	68 (0.800)	15 (0.176)	2 (0.024)	0.112±0.026
HapMap JPT*	90	27 (0.628)	11 (0.256)	5 (0.116)	0.216±0.044	30 (0.698)	10 (0.232)	3 (0.070)	0.250±0.046
HapMap CHB*	90	29 (0.659)	11 (0.250)	4 (0.091)	0.256±0.048	25 (0.555)	19 (0.422)	1 (0.022)	0.171±0.042
EA USA	290	43 (0.296)	71 (0.490)	31 (0.214)	0.455±0.027	124 (0.855)	20 (0.138)	1 (0.007)	0.077±0.017
HapMap CEU*	120	10 (0.172)	34 (0.586)	14 (0.241)	0.509±0.047	44 (0.733)	16 (0.267)	0 (0.000)	0.125±0.032
Turkey	132	19 (0.288)	24 (0.364)	23 (0.348)	0.530±0.049	63 (0.954)	3 (0.046)	0 (0.000)	0.023±0.009
Spain	172	22 (0.256)	43 (0.500)	21 (0.244)	0.500±0.037	77 (0.895)	9 (0.105)	0 (0.000)	0.052±0.019
Bulgaria	144	22 (0.305)	41 (0.569)	9 (0.125)	0.403±0.034	64 (0.889)	7 (0.097)	1 (0.014)	0.062±0.017
Serbia	78	19 (0.500)	15 (0.395)	4 (0.105)	0.308±0.038	30 (0.790)	8 (0.210)	0 (0.000)	0.103±0.025
Italy (ctrl)	134	16 (0.239)	38 (0.567)	13 (0.194)	0.478±0.050	54 (0.806)	10 (0.149)	3 (0.045)	0.119±0.027
Italy (AMI)	348	25 (0.143)	88 (0.506)	61 (0.351)	0.603±0.031	154 (0.885)	20 (0.115)	0 (0.000)	0.057±0.011
Italy (CAD)	182	15 (0.164)	48 (0.527)	28 (0.308)	0.571±0.040	75 (0.824)	14 (0.154)	2 (0.022)	0.099±0.023

*These populations were downloaded from http://www.hapmap.org/genotypes/?N=D.

AA, African ancestry; AMI, acute myocardial infarction patients; CAD, coronary artery disease patients; CEU, HapMap's population from the CEPH collection Utah residents with ancestry from northern and western Europe; CHB, HapMap's Han Chinese population from Beijing, China; EA, European Ancestry; JPT, HapMap's Japanese population from Tokyo, Japan; YRI, HapMap's population from Ibadan, Nigeria.

Chr. no., number of chromosomes screened; freq., frequency.

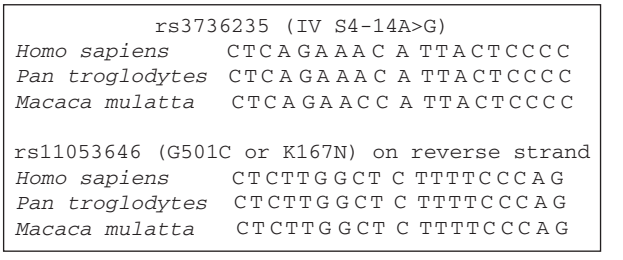

Figure 2. Determination of ancestral allele of the two SNPs by the alignment of the sequences surrounding the SNPs in *Homo sapiens* (>chromosome:NCBI36:12:10201571:10216657:-1), *Pan troglodytes* (>chromosome: CHIMP2.1:12:10455497:10470399:-1) and *Macaca mulatta* (>chromosome:MMUL_1:11:10350336:10365070:-1). It must be noted that the alleles reported in this work are reversed if compared to those submitted in the NCBI and Ensembl database and found in the alignment.

The high frequencies of the A allele of the rs3736235 SNP and the G allele (C on reverse strand, Lysine at the aminoacidic level) of the rs11053646 SNP in South-Saharan populations is concordant with the African origin of our species since, based on the alignment of the sequences in *Homo sapiens*, *Pan troglodytes* and *Macaca mulatta* (HapMap in the SNP database), these two alleles turned out to be the ancestral alleles (Figure 2).

Table II reports the haplotype frequencies observed and the linkage disequilibrium values.

Haplotype frequency estimations revealed a prevalence of the AG haplotype in most of the African and Asian samples and a high frequency of the GG haplotype in the Egyptian and in most of the European samples.

High *D*' values in most of the analysed populations reveal linkage disequilibrium between the two loci. We unified the Bariba, Berba, Dendi and Fon samples in a Benin population; Spain, Bulgaria, Serbia and Italian controls in a South European population; and Italian AMI and CAD samples in a Italian atherosclerosis population in order to improve the sample sizes from which to evaluate linkage disequilibrium values. As described in the Materials and methods section, to justify this union, we calculated Bayesian credible regions for the allele frequencies in the groups to check whether the frequencies were comparable.

D' values and statistical significances were similar to those obtained before. *D*' levels are high and statistically significant in almost all populations, and therefore we can conclude that the two polymorphisms are truly associated.

In order to detect possible genetic structure among populations, AMOVA analysis was performed either by comparing haplotype frequencies or by taking molecular differences into account. The AMOVA was firstly applied to the populations subdivided according to the geographical criterion, i.e. their geographical location. Particularly, five groups have been identified: European and European–American, Near Eastern, South Saharan, North African and Eastern Asian. The percentages of variations obtained using molecular differences are reported in Table III; similar results were achieved by considering haplotype frequencies (data not shown). As reported for other genetic markers, also in this case most of the variance observed (~92%) was due to intra-population variability even though high and significant differences were found among geographic groups (9%; $p < 0.0001$) and to a lesser extent within groups (2.5%; $p < 0.0001$).

The second and third grouping were performed, respectively, on the basis of the dynamics of the expansion of our specie out of the African cradle, and on the different lifestyle of the populations considered. Although and not surprisingly, by far the largest fraction of genetic

Table II. Haplotype frequencies and linkage disequilibrium expressed in terms of *D*' and *p*-values.

Population	AG haplotype	AC haplotype	GG haplotype	GC haplotype	*D*'	*p*-value
Bariba (Benin)	0.586±0.019	0.204±0.019	0.192±0.020	0.018±0.020	1.000	0.005
Berba (Benin)	0.531±0.018	0.214±0.018	0.248±0.011	0.007±0.011	1.000	0.005
Dendi (Benin)	0.633±0.000	0.184±0.000	0.184±0.000	0.000±0.000	1.000	0.025
Fon (Benin)	0.673±0.012	0.143±0.012	0.174±0.012	0.010±0.012	1.000	0.001
Benin (pooled)	0.607±0.049	0.187±0.049	0.197±0.043	0.009±0.043	1.000	0.000
AA USA	0.658±0.023	0.166±0.023	0.155±0.019	0.020±0.019	1.000	0.100
HapMap YRI*	0.663±0.010	0.214±0.006	0.120±0.010	0.002±0.006	1.000	0.000
Egypt	0.389±0.016	0.054±0.012	0.547±0.016	0.009±0.012	1.000	0.000
Libyan Tuareg	0.683±0.010	0.143±0.006	0.174±0.008	0.001±0.002	1.000	0.001
Siberia	0.586±0.014	0.178±0.014	0.235±0.002	0.000±0.002	1.000	0.001
Mongolia	0.732±0.008	0.112±0.008	0.155±0.003	0.001±0.003	1.000	0.005
HapMap JPT*	0.540±0.008	0.243±0.004	0.215±0.009	0.001±0.004	1.000	0.005
HapMap CHB*	0.574±0.012	0.186±0.012	0.238±0.009	0.002±0.006	1.000	0.010
EA USA EA (EA)	0.459±0.006	0.073±0.006	0.466±0.006	0.002±0.006	1.000	0.000
HapMap CEU* (EA)	0.392±0.024	0.105±0.021	0.488±0.022	0.015±0.019	1.000	0.000
European Ancestry (pooled)	0.439±0.030	0.082±0.027	0.458±0.028	0.006±0.025	1.000	0.000
Turkey (SE)	0.471±0.007	0.018±0.007	0.506±0.006	0.005±0.006	1.000	0.000
Spain (SE)	0.429±0.008	0.045±0.008	0.520±0.009	0.005±0.009	1.000	0.000
Bulgaria (SE)	0.534±0.009	0.057±0.009	0.404±0.008	0.004±0.008	1.000	0.001
Serbia (SE)	0.601±0.026	0.078±0.014	0.307±0.024	0.014±0.007	0.587	0.000
Italy (ctrl)	0.415±0.006	0.107±0.006	0.465±0.006	0.012±0.006	0.784	0.005
Southern Europe (pooled)	0.478±0.062	0.059±0.044	0.456±0.053	0.007±0.036	0.802	0.000
Italy (AMI)	0.356±0.011	0.040±0.011	0.586±0.011	0.017±0.011	0.817	0.000
Italy (CAD)	0.378±0.009	0.051±0.009	0.523±0.009	0.048±0.009	0.308	0.001
Italian CAD patients (pooled)	0.363±0.020	0.044±0.020	0.564±0.020	0.028±0.020	0.548	0.000

*These populations were downloaded from http://www.hapmap.org/genotypes/?N=D.
AA, African ancestry; AMI, acute myocardial infarction patients; CAD, coronary artery disease patients. CEU, HapMap's population from the CEPH collection Utah residents with ancestry from northern and western Europe; CHB, HapMap's Han Chinese population from Beijing, China; EA, European ancestry; JPT, HapMap's Japanese population from Tokyo, Japan; YRI, HapMap's population from Ibadan, Nigeria; SE, Southern Europe.

variation was found within populations, still in all groupings the proportion of genetic variation among groups was clearly higher and statistically significant than that within groups (see Table III).

All these results clearly demonstrate the presence of both phylogeographic and social structure of the investigated polymorphisms variation in the populations analysed.

The degree of biological relatedness among the populations was tested applying correspondence analysis and different distance methods. Figure 3 reports the two-dimensional plot of the CA. Similar results have been achieved using distance methods and MDS representation. The first dimension clearly separates the populations into two groups with South-Saharan African, African derived and eastern Asian populations at one pole, and the Europeans and Middle Eastern populations at the other pole. In this cluster the Egyptian samples and the AMI and CAD groups from Italy are comprised.

Discussion

The CA reveals that most of the inertia of the haplotype considered is explained by only one dimension, in fact most of the variability is explained by the IVS4-14A>G (rs3736235)

Table III. Analysis of molecular variance (AMOVA) results based on molecular differences.

		Among groups % variation	Within groups % variation	Within populations % variation	Fixation indices	Significance tests (*p*-value)
Different continents (Turkish within Asian group)						
Africa	Bariba, Berba, Dendi, Fon, AA USA	6.14	2.15	91.71	FSC: 0.023	0.000
Northern Africa	Egypt, Libya (Tuareg)				FST: 0.083	0.000
Asia	Siberia, Mongolia, Turkey				FCT: 0.061	0.002
Europe	Spain, Bulgaria, Serbia, EA USA, Italy (AMI), Italy (CAD), Italy (ctrl)					
Different continents (Turkish within European group)						
Africa	Bariba, Berba, Dendi, Fon, AA USA	3.23	4.18	92.59	FSC: 0.043	0.000
Northern Africa	Egypt, Libyan Tuaregs				FST: 0.074	0.000
Asia	Siberia, Mongolia				FCT: 0.032	0.033
Europe	Spain, Bulgaria, Serbia, Turkey, EA USA, Italy (AMI), Italy (CAD), Italy (ctrl)					
Out-of-Africa expansion						
Europe	Italy (AMI), Italy (CAD), Italy (ctrl), Spain, Serbia, Bulgaria	15.17	1.57	83.26	FSC: 0.018	0.000
					FST: 0.167	0.000
Out of Europe	Dendi, Fon, Bariba, Berba, Siberia, Mongolia, HapMap CHB*, HapMap YRI*				FCT: 0.152	0.000
Different lifestyles						
Occidental lifestyle	Italy (AMI), Italy (CAD), Italy (ctrl), Spain, EA USA	16.62	0.72	82.66	FSC: 0.009	0.000
					FST: 0.173	0.004
African lifestyle	Bariba, Berba, Dendi, Fon, HapMap YRI*				FCT: 0.166	0.004

*These populations were downloaded from http://www.hapmap.org/genotypes/?N=D.
AA, African ancestry; AMI, acute myocardial infarction patients; CAD, coronary artery disease patients; CHB, HapMap's Han Chinese population from Beijing, China; EA, European Ancestry; YRI, HapMap's population from Ibadan, Nigeria.

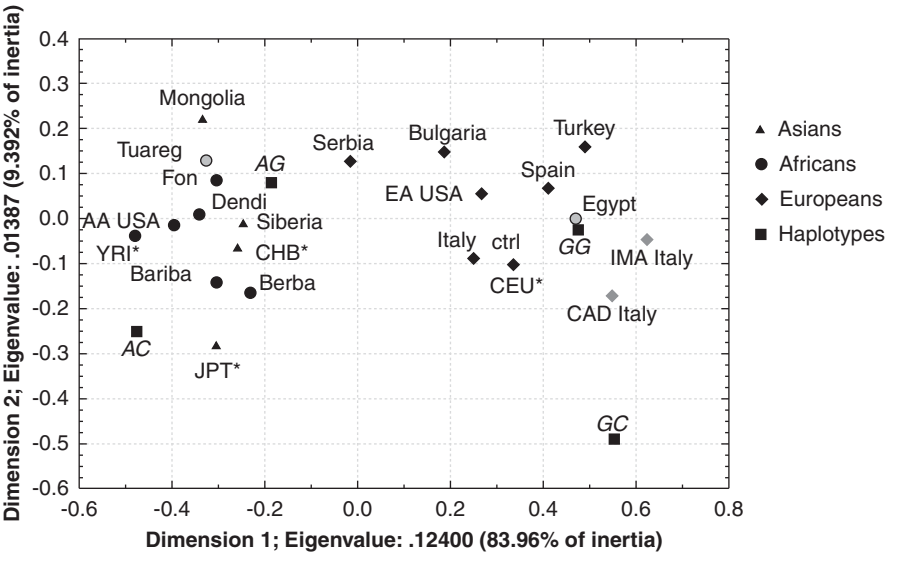

Figure 3. Two-dimensional plot of correspondence analysis based on *OLR1* haplotype frequencies in the considered populations. AA, African Ancestry; EA, European Ancestry; YRI, HapMap's population from Ibadan, Nigeria; CEU, HapMap's population from the CEPH collection Utah residents with ancestry from northern and western Europe; CHB, HapMap's Han Chinese population from Beijing, China; JPT, HapMap's Japanese population from Tokyo, Japan; AMI, acute myocardial infarction patients; CAD, coronary artery disease patients. The four observed haplotypes are reported as *AC*, *GC*, *AG* and *GG*. * These populations were downloaded from http://www.hapmap.org/genotypes/?N=D.

variant, whose ancestral allele is much more frequent in African, Asian and Tuareg populations. The G allele becomes very frequent in Egyptians and in European populations, and seems to be the risk allele since it has its highest frequency in the Italian AMI sample. This result is in accordance with the data presented by Lohmueller and collaborators, who observed, through simulations, the presence, in general, of more deleterious variations in European populations compared to populations from other continents. As the authors suggest, this could be a consequence of a bottleneck that our species experienced at the time of migration out of Africa towards Western Eurasia (Lohmueller et al. 2008).

Similar observations can be made from the frequency distribution of the K167N (G501C or rs11053646) variant, in which the C (asparagine, N) has a higher frequency in African populations and functionally appears to have a slightly protective role against myocardial infarction. Furthermore, the AMOVA, the CA and the MDS representation of genetic distance matrix performed between the studied population samples highlight that the African populations are close to the Asian populations and clearly separated from the Europeans and the populations of European ancestry. Further work will nevertheless be necessary to investigate the totality of the effects of *OLR1* gene variants on different phenotypes and populations. Due to its potential pleiotropy, in fact, the effects could be different in different populations and in different environments.

In summary, our results demonstrate a pattern of population differentiation as did many functional and nonfunctional variants localized on genes involved in the aetiological pathway of CVD previously studied by Kullo and co-workers (Kullo and Ding 2007), who demonstrated that the highest levels of FST among different populations were obtained comparing genes involved in apoptosis, lipoprotein metabolism, and immune response, all processes in which LOX-1 receptor is potentially or demonstrated to be involved.

We found significantly higher frequency of deleterious alleles in European populations as a probable effect of the bottleneck that our species underwent in the Out-of-Africa expansion. However, it must be considered that there are examples in which functionally deleterious alleles lying on CVD associated genes (*GJA4*, *SERPINE1* and *MMP3*) have also been observed at higher frequencies in African than in populations of European ancestry (Lanfear et al. 2004). This suggests that population differentiation could depend on many factors such as the variant's impact in the population (on which its persistence through the generations depends), on its age (i.e. how many generations ago it appeared) and on the interaction between each variant in each different environment. In fact, previous studies have identified population differentiations in genes implicated in common metabolic disorders in response to climate (Hancock et al. 2008), in several genes involved in nutrition (Muoio and Newgard 2008) and in pathogen infection (Verra et al. 2009). These genes and their relations with environment are still subject to numerous researches.

Although no neutrality indexes were computed because of the loss of information that would implicate the analysis of only two variants, the sequences surrounding the considered SNPs demonstrate a very high similarity between different organisms, suggesting that this domain could be subject to selective pressure and therefore highly conserved. In fact, sequence alignment between different species shows high similarity, suggesting high conservation of *OLR1* gene across catarrhine primates and of the C-type lectin like domain across mammals. We were not able to find a record in the *Pan troglodytes* and *Macaca mulatta* SNP database by performing a SNP blast of the two considered variants, neither checking for them in the MamuSNP database available at http://mamusnp.ucdavis.edu nor in the chimp SNP database available at http://www.broad.mit.edu/mammals/chimp, but the absence of this result does not prove the absence of the polymorphism. Is therefore suggestive to deepen the analysis in the consideration of the whole sequence of the gene in different species.

In conclusion, we propose a role for *OLR1*, in cooperation with environmental and other genetic risk factors, in determining susceptibility to CVD among groups and suggest a possible basis for the research of traces of evolutive pressures on *OLR1* as consequence of the Out-of-Africa expansion.

Acknowledgements

The authors declare no conflicts of interest. This study was supported in part by funds from the Italian Ministry of Health and Italian Ministry of University and Research. We thank Professor Guido Modiano, Professor Gianpaolo Scalia Tomba and Professor Alessandra Nardi for the helpful discussions.

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