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Impact of *APOE* gene polymorphisms on the lipid profile in an Algerian population

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Abstract

Background: The importance of apolipoprotein E (APOE) in lipid and lipoprotein metabolism is well established. However, the impact of *APOE* polymorphisms has never been investigated in an Algerian population. This study assessed, for the fist time, the relationships between three *APOE* polymorphisms (epsilon, rs439401, rs4420638) and plasma lipid concentrations in a general population sample from Algeria.

Methods: The association analysis was performed in the ISOR study, a representative sample of the population living in Oran (787 subjects aged between 30 and 64). Polymorphisms were considered both individually and as haplotypes.

Results: In the ISOR sample, *APOE* ε4 allele carriers had higher plasma triglyceride (*p*=0.0002), total cholesterol (*p*=0.009) and LDL-cholesterol (*p*=0.003) levels than ε3 allele carriers. No significant associations were detected for the rs4420638 and rs439401 SNPs. Linkage disequilibrium and haplotype analyses confirmed the respectively deleterious and protective impacts of the ε4 and ε2 alleles on LDL-cholesterol levels and showed that the G allele of the rs4420638 polymorphism may exert a protective effect on LDL-cholesterol levels in subjects bearing the *APOE* epsilon 4 allele.

Conclusion: Our results showed that (i) the *APOE* epsilon polymorphism has the expected impact on the plasma lipid profile and (ii) the rs4420638 G allele may counterbalance the deleterious effect of the ε4 allele on LDL-cholesterol levels in an Algerian population.

Keywords: APOE, Polymorphism, Algerian population, Lipid parameters, Cardiovascular risk, General population sample, North Africa

Background

Dyslipidemia (defined by elevated levels of fasting and post-prandial plasma triglyceride-rich lipoproteins, abnormally low high-density lipoprotein (HDL) levels and elevated low-density lipoprotein (LDL) concentrations) is associated with atherosclerosis and coronary heart disease (CHD) [1,2]. Coronary artery disease is a major cause of morbidity and mortality in both industrialized countries and developing countries, such as Algeria [3,4]. It has been estimated that there were 7.3 million deaths worldwide from ischemic heart disease in 2008 (12.4% of all mortality). Furthermore, it is predicted that CHD will still be the leading cause of death in 2020 [5,6].

Commonly studied intermediate traits associated with CHD include plasma levels of cholesterol (total cholesterol, LDL-cholesterol and HDL-cholesterol), body mass index (BMI) and blood pressure. These traits are influenced by a combination of genetic [7] and environmental factors (such as diet, alcohol and physical activity) [8-10]. The results of several meta-analyses have firmly established that the apolipoprotein E (*APOE*) epsilon polymorphism (defined by the rs7412 and rs429358 single nucleotide polymorphisms (SNPs)) is a genetic risk factor for CHD [11-14]. Indeed, APOE has an important role in the metabolism of lipoproteins and is a ligand for LDL-cholesterol and APOE receptors [15]. The epsilon

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polymorphism in the *APOE* gene leads to the generation of APOE2, APOE3 and APOE4 isoforms, which are coded by three codominant alleles (designated as ε2, ε3 and ε4). The three isoforms differ by an amino acid substitution at position 112 or position 158 in the 299-amino-acid peptide chain [16]. The isoforms interact differently with specific lipoprotein receptors and thus influence plasma cholesterol concentrations [17]. The *APOE* ε4 allele is associated with higher total and LDL-cholesterol levels and a higher risk of CHD, whereas the *APOE* ε2 allele is associated with the opposite (i.e. protective) effects in Caucasian populations [18-21].

Furthermore, two other *APOE* polymorphisms have been found to display associations with various metabolic traits. Firstly, the rs439401 SNP was associated with higher plasma triglyceride and lower plasma HDL-cholesterol concentrations in a meta-analysis of genome-wide association studies (GWAS) in 16 European cohorts [22]. Secondly, the rs4420638 SNP is reportedly associated with lower plasma HDL-cholesterol levels, higher total cholesterol and LDL-cholesterol levels and higher total cholesterol/HDL-cholesterol and LDL-cholesterol/HDL-cholesterol ratios [14,23-25].

Very few studies have investigated putative associations between the *APOE* epsilon polymorphism and plasma lipid levels in North African populations. Indeed, only two studies (in Moroccan and Tunisian populations) reported that the *APOE* ε4 allele is associated with higher plasma concentrations of total cholesterol and LDL-cholesterol, whereas the *APOE* ε2 allele shows the opposite association [26-28]. However, no data for the rs439401 and rs4420638 polymorphisms in these populations are available.

To the best of our knowledge, the relationship between *APOE* polymorphisms and plasma lipid and lipoprotein concentrations in an Algerian population has never previously been studied. We therefore decided to assess the relationships between *APOE* epsilon, rs439401 and rs4420638 polymorphisms and plasma lipid concentrations in a population sample from the city of Oran in north-west Algeria, the ISOR study.

Results
Genotype and allele distributions
The allele and genotype distributions of the *APOE* polymorphisms are presented in Table 1. There was no evidence of significant deviation from Hardy-Weinberg equilibrium in any distributions.

Linkage disequilibrium
We evaluated the linkage disequilibrium (LD) between the *APOE* epsilon (rs429358 and rs7412), rs439401 and rs4420638 polymorphisms (Figure 1). The rs439401 and rs4420638 SNPs were not in LD with the *APOE* epsilon

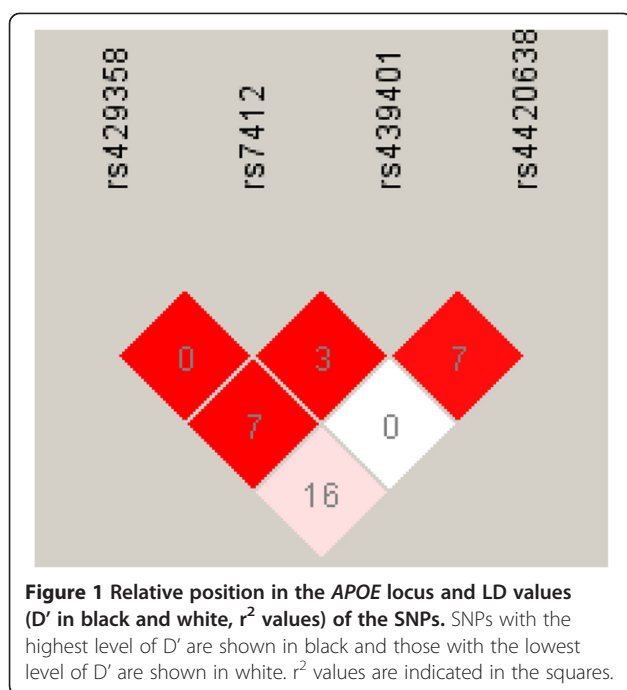**Table 1 Genotype distributions of the *APOE* polymorphisms in the ISOR study**

Polymorphism		n	(%)
Epsilon	Genotype		
	ε2/ε2	3	(0.4)
	ε2/ε3	64	(8.7)
	ε2/ε4	3	(0.4)
	ε3/ε3	525	(71.8)
	ε3/ε4	124	(16.9)
	ε4/ε4	13	(1.8)
	Total	732	
	pH-W	0.07	
	Allele		
	ε2	73	(5.0)
	ε3	1238	(84.6)
	ε4	153	(10.4)
rs439401	Genotype		
	CC	288	(39.0)
	CT	335	(45.4)
	TT	115	(15.6)
	Total	738	
	pH-W	0.28	
	Allele		
	C	911	(61.7)
	T	565	(38.3)
rs4420638	Genotype		
	AA	596	(78.9)
	AG	149	(19.8)
	GG	10	(1.3)
	Total	755	
	pH-W	0.84	
	Allele		
	A	1341	(88.8)
	G	169	(11.2)

H-W: Hardy-Weinberg equilibrium.

polymorphism ($r^2<0.16$). So the analysis of all polymorphisms was investigated.

Association studies
The various associations between the three *APOE* polymorphisms and the anthropometric phenotypes (weight, waist and hip circumferences and BMI), biochemical phenotypes (glucose, insulin, fasting plasma triglyceride, total cholesterol, HDL-cholesterol and LDL-cholesterol levels) and clinical phenotypes (SBP and DBP) were assessed (Tables 2–3).

Figure 1 Relative position in the *APOE* locus and LD values (D' in black and white, r² values) of the SNPs. SNPs with the highest level of D' are shown in black and those with the lowest level of D' are shown in white. r² values are indicated in the squares.

The APOE epsilon polymorphism

No significant association could be detected when comparing ε2 allele carriers with ε3 allele carriers (Table 2). In contrast, ε4 allele carriers had significantly higher mean plasma triglyceride (p=0.0002), mean plasma total cholesterol (p=0.009), mean LDL-cholesterol (p=0.003) levels and LDL-cholesterol/HDL-cholesterol ratio (p=0.001) than ε3 allele carriers did (Table 2).

The rs4420638 and rs439401 polymorphisms

No significant associations were detected for the rs4420638 SNP in the ISOR study (Table 3). In contrast, the T allele of rs439401 was significantly associated with lower plasma triglyceride levels (p=0.005) (Table 3). This association disappeared after further adjustment for the *APOE* epsilon polymorphism (p=0.25).

Gene-environment analyses

As gender, menopausal status in women and lipid-lowering drugs use may significantly impact plasma lipid levels, we took into account these confounders and investigated associations between the *APOE* polymorphisms and plasma lipids, in men and women separately and in the non-menopausal women group. All previously described associations were replicated in each group, meaning that gender

Table 2 Association between the *APOE* epsilon polymorphism and anthropometric, biochemical and clinical parameters in the ISOR study

	ε2ε2+ε2ε3	ε3ε3	ε3ε4+ε4ε4	p^a	p^b
n	67	522	137		
Weight (kg)	74.6 ± 17.9	71.0 ± 14.2	70.7 ± 13.6	0.05	0.6
Waist (cm)	89.4 ± 13.3	87.6 ± 12.5	87.4 ± 11.6	0.33	0.81
Hip (cm)	103.0 ± 10.6	101.9 ± 9.7	101.0 ± 9.3	0.16	0.6
BMI (kg/m²)	27.3 ± 6.2	26.1 ± 5.1	25.5 ± 4.5	0.02	0.42
Waist-to-hip ratio	0.87 ± 0.08	0.86 ± 0.09	0.87 ± 0.08	0.93	0.71
Fasting glucose (mmol/L)*	4.98 ± 1.28	5.04 ± 1.30	5.20 ± 1.53	0.23	0.25
Fasting insulin (µIU/mL)*	9.18 ± 6.65	7.95 ± 5.48	8.65 ± 8.31	0.76	0.35
Triglycerides (mmol/L)†	1.34 ± 0.78	1.09 ± 0.44	1.24 ± 0.48	0.10	**0.0002**
Total cholesterol (mmol/L)†	4.25 ± 0.95	4.41 ± 0.86	4.63 ± 1.11	0.10	**0.009**
HDL-cholesterol (mmol/L)†	1.27 ± 0.34	1.26 ± 0.30	1.22 ± 0.32	0.40	0.06
LDL-cholesterol (mmol/L)†	2.47 ± 0.85	2.64 ± 0.82	2.88 ± 1.05	0.06	**0.003**
LDL-cholesterol/HDL-cholesterol ratio†	2.14 ± 1.07	2.25 ± 1.00	2.55 ± 1.23	0.18	**0.001**
SBP (mmHg)††	122.6 ± 16.3	120.6 ± 14.8	122.7 ± 17.8	0.95	0.13
DBP (mmHg)††	75.5 ± 8.8	76.6 ± 9.8	76.3 ± 11.1	0.83	0.38

Data are expressed as the mean ± SD. SBP: systolic blood pressure; DBP: diastolic blood pressure.
p values were adjusted for age, gender, smoking status and physical activity for anthropometric variables.
p values were adjusted for age, gender, BMI, smoking status and physical activity for biological and biochemical variables.
p^a: p values obtained when comparing ε2ε2+ε2ε3 subjects with ε3ε3 subjects.
p^b: p values obtained when comparing ε3ε4+ε4ε4 subjects with ε3ε3 subjects.
*subjects treated for diabetes were excluded (n=54).
†subjects treated with lipid-lowering drugs were excluded (n=36).
††subjects treated for hypertension were excluded (n=89).

Table 3 Association between the *APOE* rs439401 and rs4420638 SNPs and anthropometric, biochemical and clinical parameters in the ISOR study

	rs439401					rs4420638				
	CC	CT	TT	p^1	p^2	AA	AG	GG	p^1	p^2
n	288	335	115			596	149	10		
Weight (kg)	71.6 ± 14.5	71.5 ± 15.0	70.8 ± 13.6	0.87	0.68	71.7 ± 14.9	69.4 ± 13.4	67.7 ± 5.9	0.04	0.04
Waist (cm)	88.1 ± 12.6	87.7 ± 12.2	87.4 ± 12.4	0.83	0.66	88.0 ± 12.5	86.7 ± 11.9	83.5 ± 11.2	0.11	0.07
Hip (cm)	102.2 ± 10.0	102.0 ± 10.00	101.3 ± 9.1	0.56	0.45	102.2 ± 9.9	100.8 ± 9.3	97.0 ± 6.2	0.04	0.04
BMI (kg/m^2)	26.0 ± 5.2	26.3 ± 5.1	26.0 ± 4.9	0.65	0.79	26.2 ± 5.2	25.4 ± 4.6	24.5 ± 2.9	0.05	0.08
Waist-to-hip ratio	0.86 ± 0.08	0.86 ± 0.10	0.86 ± 0.08	0.83	0.92	0.86 ± 0.08	0.86 ± 0.11	0.86 ± 0.11	0.99	0.64
Fasting glucose (mmol/L)*	5.04 ± 1.20	5.10 ± 1.52	5.06 ± 1.14	0.69	0.48	5.03 ± 1.26	5.20 ± 1.60	5.03 ± 0.70	0.18	0.34
Fasting insulin (μIU/mL)*	8.02 ± 6.09	8.57 ± 6.53	7.58 ± 5.62	0.72	0.7	8.07 ± 5.62	8.75 ± 8.28	6.61 ± 2.89	0.31	0.58
Triglycerides (mmol/L)†	1.20 ± 0.51	1.14 ± 0.50	1.07 ± 0.47	**0.005**	0.25	1.14 ± 0.49	1.19 ± 0.55	1.21 ± 0.41	0.06	0.89
Total cholesterol (mmol/L)†	4.50 ± 1.02	4.42 ± 0.86	4.35 ± 0.82	0.14	0.42	4.44 ± 0.90	4.43 ± 0.98	4.51 ±1.02	0.76	0.42
HDL-cholesterol (mmol/L)†	1.24 ± 0.31	1.26 ± 0.33	1.27 ± 0.27	0.29	0.41	1.25 ± 0.30	1.27 ± 0.34	1.17 ± 0.24	0.82	0.55
LDL-cholesterol (mmol/L)†	2.74 ± 0.97	2.66 ± 0.82	2.58 ± 0.82	0.11	0.31	2.69 ± 0.87	2.64 ± 0.88	2.78 ± 0.88	0.94	0.20
LDL-cholesterol/HDL-cholesterol ratio†	2.39 ± 1.15	2.38 ± 2.12	2.17 ± 0.88	0.31	0.19	2.37 ± 1.76	2.25 ± 1.02	2.36 ± 0.52	0.65	0.09
SBP (mmHg)††	121.4 ± 16.4	120.9 ± 14.8	121.1 ± 15.1	0.66	0.44	120.9 ± 14.8	121.0 ± 17.6	128.1 ± 18.9	0.36	0.51
DBP (mmHg)††	75.4 ± 10.1	75.9 ± 8.7	76.4 ± 8.6	0.14	0.09	75.7 ± 9.0	75.7 ± 10.1	78.1 ± 12.2	0.56	0.58

Data are expressed as the mean ± SD. SBP: systolic blood pressure, DBP: diastolic blood pressure.
p^1: p values were adjusted for age, gender, smoking status and physical activity for anthropometric variables. p values were adjusted for age, gender, BMI, smoking status and physical activity for biological and biochemical variables.
p^2: as above and additionally for *APOE* epsilon status.
*subjects treated for diabetes were excluded (n=54).
†subjects treated with lipid-lowering drugs were excluded (n=36).
††subjects treated for hypertension were excluded (n=89).

and menopausal status had no notable influence on the present associations (data not shown).

Haplotype analysis

We explored the haplotype effects of the *APOE* epsilon (rs429358 and rs7412) and the rs439401 and rs4420638 SNPs on plasma LDL-cholesterol levels. First, we selected the most informative haplotype configuration. The best haplotype model included the *APOE* epsilon and the rs4420638 polymorphisms (*p*=0.002, with 4 d.f.). We then performed haplotype analysis (using Thesias [29]) for the *APOE* epsilon and rs4420638. Five haplotypes were inferred (ε3A, ε3G, ε4A, ε4G and ε2A) (Table 4). The test for an overall haplotype effect was significant (*p*=0.002).

When compared with the most frequent (reference) ε3A haplotype, the ε4A haplotype was associated with higher LDL-cholesterol levels (Δ [95% CI]=+0.35 [0.18 — 0.51] mmol/L, *p*=0.0001), whereas the ε2A haplotype was associated with lower LDL-cholesterol levels (Δ [95% CI]=-0.20 [-0.41 — -0.01] mmol/L, *p*=0.05), confirming the respectively deleterious and protective impacts of the ε4 and ε2 alleles. The ε3G haplotype was associated with similar LDL-cholesterol levels (Δ [95% confidence interval (CI)]=0.02 [-0.17 — +0.21] mmol/L, *p*=0.74) compared with carriers of the ε3A haplotype; this result suggests that the rs4420638 G allele does not have an effect in an ε3 background. Interestingly and contrarily to what we observed for the ε4A haplotype, the ε4G haplotype was not associated with higher LDL-cholesterol levels, relative to

Table 4 Effect of the *APOE* haplotypes on plasma LDL-cholesterol levels in the ISOR study

Haplotypes				
rs429358/rs7412/rs4420638	ε/rs4420638	Frequency	Haplotype effect [95% CI] (mmol/L)	*p*
TCA	ε3A	0.784	reference	-
TCG	ε3G	0.061	0.02 [-0.17 — 0.21]	0.74
CCA	ε4A	0.054	0.35 [0.18 — 0.51]	**0.0001**
CCG	ε4G	0.051	−0.05 [-0.27 — 0.17]	0.81
TTA	ε2A	0.050	−0.20 [-0.41 — -0.01]	**0.05**

Polymorphisms are ordered according to their position in the genomic sequence.
Values are the difference in means [95% CI] when compared with the ε3A reference haplotype (mean [95% CI]=1.33 [1.29 — 1.37] mmol/L) using Thesias software.

the reference ε3A haplotype (Δ [95% CI]=-0.05 [-0.27 — -0.17] mmol/L, *p*=0.81). Hence, the G allele of the rs4420638 SNP may have counterbalanced the deleterious effect of the ε4 allele.

Similar haplotype results were obtained for plasma total cholesterol levels (data not shown).

Discussion

The importance of APOE in lipid and lipoprotein metabolism is well established. However the impact of *APOE* polymorphisms has never been investigated in an Algerian population. So in this study, we assessed the relationship between three *APOE* polymorphisms (epsilon, rs439401 and rs4420638) and metabolic trait variations in a population from Oran, Algeria, the ISOR study. To the best of our knowledge, this was the first study to characterize associations of the three above-mentioned *APOE* gene polymorphisms with anthropometric, biochemical and clinical parameters in an Algerian population.

The frequency of the ε4 allele in Europe parallels the incidence of CHD and other diseases [11,30,31]. It follows a north-to-south gradient and ranges from more than 0.22 in Finland and Greenland [32-34] to less than 0.07 in Greece and Italy [35-37]. In our study, the ε4 allele frequency (0.10) was similar to values observed in other North African populations (around 0.10 in Moroccan populations [26,38] and around 0.08 in Tunisian populations [27,28]).

The minor allele frequency of the rs439401 SNP (located within the *APOE/APOC1* cluster) was 0.38 in the ISOR study. Similar frequencies have been reported in the literature and range from 0.33 to 0.36 [14,39].

For the rs4420638 SNP (situated lying 14 kb downstream of the *APOE* locus in the adjacent *APOC1* gene), the minor allele frequency (0.11) was markedly lower than the value observed in European samples [14,24,40].

In the ISOR study, the ε2 allele was not significantly associated with plasma lipid variations (0.06≤*p*≤0.18). This is probably due to too few of ε2 carriers (n=67). Only the ε4 allele showed an association with higher levels of total cholesterol and LDL-cholesterol. Furthermore, the ε4 allele was associated with higher concentrations of triglycerides, as previously observed by Kofler *et al.* in a study performed in the United Kingdom [41]. The APOE ε4 isoform (which binds preferentially to VLDLs) can interfere with plasma lipase activity or with the triglyceride removal system; this results in delayed lipolysis or clearance of plasma triglycerides in subjects bearing the ε4/ε3 phenotype [18]. Therefore, our results are consistent with previous studies [42-45]. In contrast with previous studies in multi-ethnic populations [41,46-48] but consistently with other studies in Tunisian subjects [27,28], we did not detect significant associations between the *APOE* epsilon polymorphism and HDL-cholesterol

levels in the ISOR sample. Other studies conducted in Arab population samples are therefore needed to conclude.

Despite the deleterious effect of the ε4 allele on lipid parameters observed in the ISOR study, the lipid concentrations did not reach abnormal threshold values. A case–control study on the risk of myocardial infarction conducted in Oran in 2001 also showed that plasma concentrations of all lipid parameters were systematically lower in Algerian subjects than in Irish and French individuals [49].

We found significant association between the rs439401 SNP and triglyceride concentrations. This association has previously been observed in European GWAS of blood lipid levels [14,22]. However, this association disappeared after further adjustment for the *APOE* epsilon polymorphism, suggesting that the effect of rs439401 was overshadowed by the epsilon polymorphism.

In the ISOR study, the rs4420638 SNP was not significantly associated with any metabolic traits, contrary to what has been previously described [50]. These discrepancies could be explained by differences in the LD structure between rs4420638 and the *APOE* epsilon polymorphism in the Algerian and European populations (D'=0 and +16 with rs7412 and rs429358, respectively in the ISOR study versus D'=+66 and –69 with rs7412 and rs429358, respectively in the EUR panel of the 1000 Genomes database (December 2012 release, http://browser.1000genomes.org)). Therefore the effect of the rs4420638 SNP could be mainly due to the epsilon polymorphism in European samples but be independent in the ISOR study. This result should be investigated in other North-African populations.

Haplotype analysis was performed to better understand the information provided by the individual SNP analysis. This analysis showed that regarding plasma LDL-cholesterol (or total cholesterol) levels, the rs4420638 polymorphism could counterbalance the deleterious effect of the epsilon 4 allele.

Conclusion

We showed that the impact of the *APOE* ε4 allele on the plasma lipid profile is similar in an Algerian population to what is known in other countries. It is noteworthy that the rs4420638 G allele could counterbalance the deleterious effect of the *APOE* ε4 allele on LDL-cholesterol levels in a North African population. Replications in larger studies are required.

Methods
Subjects
The ISOR (InSulino-résistance à ORan) study
The ISOR study was performed between 2007 and 2009. The study's objectives and procedures were approved by the independent ethics committee at the Algerian National

Agency for the Development of Health Research. The ISOR study was a population-based, cross-sectional study of a representative sample of 787 subjects (378 men and 409 women, aged between 30 and 64) recruited within the city of Oran. Subjects were selected at random from social security rolls. All subjects consented freely to participation in the study. A questionnaire on lifestyle (physical activity, tobacco use and alcohol intake), personal and family medical histories, current medication and socio-economic and educational levels was completed during a face-to-face interview. Anthropometric data were also recorded.

The level of physical activity was defined in quartiles as "none", "low", "medium" and "high" after summing exercise scores for sporting activities, walking, housework and physical activity at work.

In terms of tobacco use, participants were categorized as either smokers (i.e. individuals reporting at least one cigarette per day) or non-smokers. In the study questionnaire, subjects were asked to report their weekly consumption of wine, beer, and spirits. As there were very few drinkers (n=25, 3.2%), this variable was not taken into account in the final analysis.

The anthropometric measurements included height, body weight, waist circumference and hip circumference. Height and weight were measured while the subject was barefoot and lightly dressed. The BMI was calculated according to the Quetelet equation. Systolic and diastolic blood pressure values (SBP and DBP, respectively) were measured on the right arm with the subject in the sitting position, using a standard mercury sphygmomanometer. Measurements were made before and after completion of the questionnaire, with an interval of at least 10 minutes. The mean value of the blood pressure readings was considered in the final analysis.

After a 12 h overnight fast, two 15 ml blood samples were collected for each subject (in a disodium EDTA tube for subsequent DNA analysis and in a heparin tube for clinical chemistry tests).

A multichannel analyzer and dedicated kits (Humastar®, HUMAN Diagnostics, Wiesbaden, Germany) were used for the colorimetric, enzymatic measurement of cholesterol (kit: monotest cholesterol with cholesterol esterase, cholesterol oxidase and peroxidase), triglycerides (kit: peridochrom triglyceride with glycerol phosphate oxidase and peroxidase) and glucose (kit: glucose, glucose oxidase and peroxidase). Plasma LDL-cholesterol levels were calculated according to the Friedwald equation. High-density lipoprotein cholesterol levels were measured after sodium phosphotungstate/magnesium chloride precipitation of chylomicrons and VLDL and LDL-cholesterol and then centrifugation. Plasma insulin levels were measured in a microparticle enzyme immune assay running on an AxSYM analyzer (Abbott Laboratories, Abbott Park, Illinois, USA).

Genomic DNA was extracted from white blood cells by using the Stratagene® kit (Agilent Technologies, Les Ulis, France), according to the manufacturer's protocol.

Genotyping

The *APOE* SNPs (rs429358, rs7412, rs439401 and rs4420638) were genotyped using KASPar technology (KBioscience, Hoddesdon, UK) with the following probes: rs429358: [GACATGGAGGACGTG[C/T]GCGGCCGCC TGGTGC], rs7412: [GATGACCTGCAGAAG[C/T]GCC TGGCAGTGTACC], rs439401: [GCCGGCACTCTCTTC [C/T]CCTCCCACCCCTCA], rs4420638: [TGCTACAC TTTTCCT[A/G]GTGTGGTCTACCCGA]. The genotyping success rates ranged from 93% to 96%.

Statistical analyses

Statistical analyses were performed with SAS 9.1 software (SAS Institute Inc., Cary, NC, USA). The Hardy-Weinberg equilibrium was tested using a χ^2 test with one degree of freedom (d.f.).

Intergroup comparisons of means were performed with (i) a general linear model comparing ε2 carriers (ε2ε2 and ε2ε3 subjects) or ε4 carriers (ε4ε4 and ε4ε3 subjects) with ε3 carriers (ε3ε3 homozygotes) for the *APOE* epsilon polymorphism and (ii) an additive model for the rs439401 and rs4420638 polymorphisms. Subjects with the ε2ε4 genotype (n=3) were excluded from the analyses because of the possible opposing biological effects of the ε2 and ε4 alleles.

Data on triglycerides, glucose and insulin levels were log-transformed to obtain normal distributions. Estimated means were subsequently back–transformed for presentation in the tables.

For anthropometric variables, the confounding variables were age, gender, smoking status and physical activity. For biological and biochemical variables, the confounding variables were age, gender, BMI, smoking status and physical activity. For the rs439401 and rs4420638 polymorphisms, data were further adjusted for the *APOE* epsilon polymorphism coded in three genotypes as follows: ε2 carriers (ε2ε2 and ε2ε3 subjects); ε4 carriers (ε4ε4 and ε4ε3 subjects) and ε3 carriers (ε3ε3 subjects).

After Bonferroni correction, only associations with an uncorrected p value below 0.017 were considered to be statistically significant (i.e. 0.05 divided by the number of polymorphisms considered).

General linear models were used to investigate potential interactions by adding an interaction ((gender or BMI) x polymorphism) term.

Linkage disequilibrium figure and values were calculated with Haploview 4.2 (http://www.broadinstitute. org/scientific-community/science/programs/medical-and-population-genetics/haploview/haploview).

Haplotype frequencies derived from all studied polymorphisms were estimated independently of phenotype. A two-step haplotype analysis was performed. Firstly, in order to reduce the haplotype dimension and select the most informative, parsimonious haplotype configuration for the prediction of phenotypic variability, we applied the maximum likelihood model to all the possible 1 to *k*-loci combinations of polymorphisms that could be derived from the 4 *APOE* SNPs with the GridHaplo software [51]. Akaike's information criterion (AIC) was calculated for each model (including a model with no polymorphisms) [52]. All AIC values were rescaled by subtracting the smallest AIC value obtained for the whole set of models. According to a rule derived by extensive Monte Carlo simulation, all models with a rescaled AIC ≤2 can be considered to be "equivalent" to the model with the lowest min *AIC*. The most parsimonious of the latter (corresponding to the minimal haplotype configuration) was selected. Secondly, haplotype analyses were performed using the Thesias software package (http://ecgene.net/genecanvas) [29]. Haplotype analyses were adjusted for age, gender, BMI, smoking status and physical activity level.

Abbreviations
AIC: Akaike's information criterion; APOE: Apolipoprotein E; BMI: Body mass index; CHD: Coronary heart disease; DBP: Diastolic blood pressure; DNA: Deoxyribonucleic acid; d.f.: Degree of freedom; GWAS: Genome-wide association studies; HDL: High-density lipoprotein; ISOR: InSulino-résistance à ORan; LD: Linkage disequilibrium; LDL: Low-density lipoprotein; SBP: Systolic blood pressure; SNP: Single nucleotide polymorphism; VLDL: Very low-density lipoprotein.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
SMB, LH, NSM, PA, AM and LG designed research; SMB, LH, IMH, AM and LG conducted research; HOD, SLH, IMH, SMB and LH participated in the recruitment of subjects; LG built the database with the support of BGB; XH, DNM and HB performed the DNA extraction under the supervision of LG; HB and LG performed the statistical analyses; HB, SMB, AM and LG interpreted the results. IMH assayed biochemical parameters; HB wrote the paper under the supervision of SMB, AM and LG; HB, SMB, AM and LG had primary responsibility for final content. All authors read and approved the final manuscript.

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References
1. Davignon J, Gregg RE, Sing CF: **Apolipoprotein E polymorphism and atherosclerosis.** *Arteriosclerosis* 1988, **8**:1–21.
2. Eichner JE, Dunn ST, Perveen G, Thompson DM, Stewart KE, Stroehla BC: **Apolipoprotein E polymorphism and cardiovascular disease: a HuGE review.** *Am J Epidemiol* 2002, **155**:487–495.
3. Institut National de Santé Publique: *Transition épidémiologique et système de santé. Synthèse de l'enquête morbidité.* Alger: INSP Ed: Projet TAHINA; 2002.
4. Mackay JMG: *The Atlas of Heart Disease and Stroke.* Geneva: World Health Organization; 2004.
5. World Health Organization (WHO): *The World Health Report 2008.* 2013. Available from: URL: http://www.who.int/whr/2008/whr08_en.pdf.
6. Murray CJ, Lopez AD: **Alternative projections of mortality and disability by cause 1990–2020: Global Burden of Disease Study.** *Lancet* 1997, **349**:1498–1504.
7. Watkins H, Farrall M: **Genetic susceptibility to coronary artery disease: from promise to progress.** *Nat Rev Genet* 2006, **7**:163–173.
8. Ginsberg HN, Kris-Etherton P, Dennis B, Elmer PJ, Ershow A, Lefevre M, Pearson T, Roheim P, Ramakrishnan R, Reed R, *et al*: **Effects of reducing dietary saturated fatty acids on plasma lipids and lipoproteins in healthy subjects: the DELTA Study, protocol 1.** *Arterioscler Thromb Vasc Biol* 1998, **18**:441–449.
9. Savolainen MJ, Kesaniemi YA: **Effects of alcohol on lipoproteins in relation to coronary heart disease.** *Curr Opin Lipidol* 1995, **6**:243–250.
10. Gielen S, Schuler G, Hambrecht R: **Exercise training in coronary artery disease and coronary vasomotion.** *Circulation* 2001, **103**:E1–E6.
11. Wilson PW, Schaefer EJ, Larson MG, Ordovas JM: **Apolipoprotein E alleles and risk of coronary disease. A meta-analysis.** *Arterioscler Thromb Vasc Biol* 1996, **16**:1250–1255.
12. Song Y, Stampfer MJ, Liu S: **Meta-analysis: apolipoprotein E genotypes and risk for coronary heart disease.** *Ann Intern Med* 2004, **141**:137–147.
13. Bennet AM, Di Angelantonio E, Ye Z, Wensley F, Dahlin A, Ahlbom A, Keavney B, Collins R, Wiman B, de Faire U, *et al*: **Association of apolipoprotein E genotypes with lipid levels and coronary risk.** *JAMA* 2007, **298**:1300–1311.
14. Teslovich TM, Musunuru K, Smith AV, Edmondson AC, Stylianou IM, Koseki M, Pirruccello JP, Ripatti S, Chasman DI, Willer CJ, *et al*: **Biological, clinical and population relevance of 95 loci for blood lipids.** *Nature* 2010, **466**:707–713.
15. Anoop S, Misra A, Meena K, Luthra K: **Apolipoprotein E polymorphism in cerebrovascular & coronary heart diseases.** *Indian J Med Res* 2010, **132**:363–378.
16. Das HK, McPherson J, Bruns GA, Karathanasis SK, Breslow JL: **Isolation, characterization, and mapping to chromosome 19 of the human apolipoprotein E gene.** *J Biol Chem* 1985, **260**:6240–6247.
17. Siest G, Pillot T, Regis-Bailly A, Leininger-Muller B, Steinmetz J, Galteau MM, Visvikis S: **Apolipoprotein E: an important gene and protein to follow in laboratory medicine.** *Clin Chem* 1995, **41**:1068–1086.
18. Dallongeville J, Lussier-Cacan S, Davignon J: **Modulation of plasma triglyceride levels by apoE phenotype: a meta-analysis.** *J Lipid Res* 1992, **33**:447–454.
19. Wilson PW, Myers RH, Larson MG, Ordovas JM, Wolf PA, Schaefer EJ: **Apolipoprotein E alleles, dyslipidemia, and coronary heart disease. The Framingham Offspring Study.** *JAMA* 1994, **272**:1666–1671.
20. Costanza MC, Beer-Borst S, James RW, Gaspoz JM, Morabia A: **Consistency between cross-sectional and longitudinal SNP: blood lipid associations.** *Eur J Epidemiol* 2012, **27**:131–138.
21. Petkeviciene J, Smalinskiene A, Luksiene DI, Jureniene K, Ramazauskiene V, Klumbiene J, Lesauskaite V: **Associations between apolipoprotein E genotype, diet, body mass index, and serum lipids in Lithuanian adult population.** *PLoS One* 2012, **7**:e41525.

22. Aulchenko YS, Ripatti S, Lindqvist I, Boomsma D, Heid IM, Pramstaller PP, Penninx BW, Janssens AC, Wilson JF, Spector T, et al: **Loci influencing lipid levels and coronary heart disease risk in 16 European population cohorts.** *Nat Genet* 2009, **41**:47–55.

23. Kathiresan S, Melander O, Guiducci C, Surti A, Burtt NP, Rieder MJ, Cooper GM, Roos C, Voight BF, Havulinna AS, et al: **Six new loci associated with blood low-density lipoprotein cholesterol, high-density lipoprotein cholesterol or triglycerides in humans.** *Nat Genet* 2008, **40**:189–197.

24. Willer CJ, Speliotes EK, Loos RJ, Li S, Lindgren CM, Heid IM, Berndt SI, Elliott AL, Jackson AU, Lamina C, et al: **Six new loci associated with body mass index highlight a neuronal influence on body weight regulation.** *Nat Genet* 2009, **41**:25–34.

25. Liu Y, Zhou D, Zhang Z, Song Y, Zhang D, Zhao T, Chen Z, Sun Y, Zhang D, Yang Y, et al: **Effects of genetic variants on lipid parameters and dyslipidemia in a Chinese population.** *J Lipid Res* 2011, **52**:354–360.

26. Lahlali-Kacemi N, Bamou Y, Guedira A, Hassani M, Visvikis S, Siest G, Alami N, Kabbaj O, Lahrichi M: **Apolipoprotein E polymorphism in a Moroccan population: allele frequency and relation to plasma lipid concentrations.** *Ann Biol Clin (Paris)* 2002, **60**:73–78.

27. Jemaa R, Elasmi M, Naouali C, Feki M, Kallel A, Souissi M, Sanhaji H, Hadj TS, Souheil O, Kaabachi N: **Apolipoprotein E polymorphism in the Tunisian population: frequency and effect on lipid parameters.** *Clin Biochem* 2006, **39**:816–820.

28. Bahri R, Esteban E, Moral P, Hassine M, Ben Hamda K, Chaabani H: **Apolipoprotein gene polymorphisms and plasma levels in healthy Tunisians and patients with coronary artery disease.** *Lipids Health Dis* 2008, **7**:46.

29. Tregouet DA, Garelle V: **A new JAVA interface implementation of THESIAS: testing haplotype effects in association studies.** *Bioinformatics* 2007, **23**:1038–1039.

30. Schiele F, De Bacquer D, Vincent-Viry M, Beisiegel U, Ehnholm C, Evans A, Kafatos A, Martins MC, Sans S, Sass C, et al: **Apolipoprotein E serum concentration and polymorphism in six European countries: the ApoEurope Project.** *Atherosclerosis* 2000, **152**:475–488.

31. Kosunen O, Talasniemi S, Lehtovirta M, Heinonen O, Helisalmi S, Mannermaa A, Paljarvi L, Ryynanen M, Riekkinen PJ Sr, Soininen H: **Relation of coronary atherosclerosis and apolipoprotein E genotypes in Alzheimer patients.** *Stroke* 1995, **26**:743–748.

32. Gerdes LU: **The common polymorphism of apolipoprotein E: geographical aspects and new pathophysiological relations.** *Clin Chem Lab Med* 2003, **41**:628–631.

33. Ehnholm C, Lukka M, Kuusi T, Nikkila E, Utermann G: **Apolipoprotein E polymorphism in the Finnish population: gene frequencies and relation to lipoprotein concentrations.** *J Lipid Res* 1986, **27**:227–235.

34. de Knijff P, Johansen LG, Rosseneu M, Frants RR, Jespersen J, Havekes LM: **Lipoprotein profile of a Greenland Inuit population. Influence of anthropometric variables, Apo E and A4 polymorphism, and lifestyle.** *Arterioscler Thromb* 1992, **12**:1371–1379.

35. Sklavounou E, Economou-Petersen E, Karadima G, Panas M, Avramopoulos D, Varsou A, Vassilopoulos D, Petersen MB: **Apolipoprotein E polymorphism in the Greek population.** *Clin Genet* 1997, **52**:216–218.

36. Deiana L, Pes GM, Carru C, Errigo A, Pettinato S, Carcassi C, Baggio G, Contu L: **Lack of influence of apolipoprotein E4 on lipoprotein levels in the island population of Sardinia.** *Eur J Clin Invest* 1998, **28**:290–294.

37. Barbagallo CM, Polizzi F, Severino M, Rizzo M, Vivona N, Onorato F, Caldarella R, Cefalu AB, Noto D, Notarbartolo A, et al: **ApoE polymorphism in a small Mediterranean island: relationships with plasma lipids, lipoproteins and LDL particle size.** *Eur J Epidemiol* 2001, **17**:707–713.

38. Valveny N, Esteban E, Kandil M, Moral P: **APO E polymorphism in Spanish and Moroccan populations.** *Clin Genet* 1997, **51**:354–356.

39. Kring SI, Brummett BH, Barefoot J, Garrett ME, Ashley-Koch AE, Boyle SH, Siegler IC, Sorensen TI, Williams RB: **Impact of psychological stress on the associations between apolipoprotein E variants and metabolic traits: findings in an American sample of caregivers and controls.** *Psychosom Med* 2010, **72**:427–433.

40. Barber MJ, Mangravite LM, Hyde CL, Chasman DI, Smith JD, McCarty CA, Li X, Wilke RA, Rieder MJ, Williams PT, et al: **Genome-wide association of lipid-lowering response to statins in combined study populations.** *PLoS One* 2010, **5**:e9763.

41. Kofler BM, Miles EA, Curtis P, Armah CK, Tricon S, Grew J, Napper FL, Farrell L, Lietz G, Packard CJ, et al: **Apolipoprotein E genotype and the cardiovascular disease risk phenotype: impact of sex and adiposity (the FINGEN study).** *Atherosclerosis* 2012, **221**:467–470.

42. Hallman DM, Boerwinkle E, Saha N, Sandholzer C, Menzel HJ, Csazar A, Utermann G: **The apolipoprotein E polymorphism: a comparison of allele frequencies and effects in nine populations.** *Am J Hum Genet* 1991, **49**:338–349.

43. Xhignesse M, Lussier-Cacan S, Sing CF, Kessling AM, Davignon J: **Influences of common variants of apolipoprotein E on measures of lipid metabolism in a sample selected for health.** *Arterioscler Thromb* 1991, **11**:1100–1110.

44. Howard BV, Gidding SS, Liu K: **Association of apolipoprotein E phenotype with plasma lipoproteins in African-American and white young adults. The CARDIA Study. Coronary Artery Risk Development in Young Adults.** *Am J Epidemiol* 1998, **148**:859–868.

45. Wilson HM, Patel JC, Russell D, Skinner ER: **Alterations in the concentration of an apolipoprotein E-containing subfraction of plasma high density lipoprotein in coronary heart disease.** *Clin Chim Acta* 1993, **220**:175–187.

46. Alvim RO, Freitas SR, Ferreira NE, Santos PC, Cunha RS, Mill JG, Krieger JE, Pereira AC: **APOE polymorphism is associated with lipid profile, but not with arterial stiffness in the general population.** *Lipids Health Dis* 2010, **9**:128.

47. Medina-Urrutia AX, Cardoso-Saldana GC, Zamora-Gonzalez J, Liria YK, Posadas-Romero C: **Apolipoprotein E polymorphism is related to plasma lipids and apolipoproteins in Mexican adolescents.** *Hum Biol* 2004, **76**:605–614.

48. Liang S, Steffen LM, Steffen BT, Guan W, Weir NL, Rich SS, Manichaikul A, Vargas JD, Tsai MY: **APOE genotype modifies the association between plasma omega-3 fatty acids and plasma lipids in the Multi-Ethnic Study of Atherosclerosis (MESA).** *Atherosclerosis* 2013, **228**:181–187.

49. Mediene-Benchekor S, Brousseau T, Richard F, Benhamamouch S, Amouyel P: **Blood lipid concentrations and risk of myocardial infarction.** *Lancet* 2001, **358**:1064–1065.

50. Pendergrass SA, Brown-Gentry K, Dudek S, Frase A, Torstenson ES, Goodloe R, Ambite JL, Avery CL, Buyske S, Buzkova P, et al: **Phenome-Wide Association Study (PheWAS) for Detection of Pleiotropy within the Population Architecture using Genomics and Epidemiology (PAGE) Network.** *PLoS Genet* 2013, **9**:e1003087.

51. Tregouet DA, Konig IR, Erdmann J, Munteanu A, Braund PS, Hall AS, Grosshennig A, Linsel-Nitschke P, Perret C, DeSuremain M, et al: **Genome-wide haplotype association study identifies the SLC22A3-LPAL2-LPA gene cluster as a risk locus for coronary artery disease.** *Nat Genet* 2009, **41**:283–285.

52. Akaike H: **A new look at the statistical model identification.** *IEEE Transactions on Automated Control AC* 1974, **19**:716–723.