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Influence of cholesteryl ester transfer protein, peroxisome proliferator–activated receptor α, apolipoprotein E, and apolipoprotein A-I polymorphisms on high-density lipoprotein cholesterol, apolipoprotein A-I, lipoprotein A-I, and lipoprotein A-I:A-II concentrations: the Prospective Epidemiological Study of Myocardial Infarction study

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Abstract

The plasma level of high-density lipoprotein cholesterol (HDL-C) is known to be inversely associated with cardiovascular risk. However, besides lifestyle, gene polymorphism may influence the HDL-C concentration. The aim of this study was to investigate the possibility of interactions between CETP, PPARA, APOE, and APOAI polymorphisms and HDL-C, apolipoprotein (apo) A-I, lipoprotein (Lp) A-I, and Lp A-I:A-II in a sample selected from the Prospective Epidemiological Study of Myocardial Infarction (PRIME) study population who remained free of cardiovascular events over 5 years of follow-up. Healthy individuals (857) were randomly selected for genotyping the PRIME study subjects. The population was selected so as to provide 25% of subjects in the lowest tertile of HDL-C (≤28 mg/dL) in the whole PRIME study sample, 25% of subjects in the highest tertile of HDL-C (≥73 mg/dL), and 50% of subjects in

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the medium tertile of HDL-C (28-73 mg/dL). Genotyping was performed by using a polymerase chain reaction system with predeveloped TaqMan allelic discrimination assay. The CETP A373P rare allele *c* was less frequent in the group of subjects with high HDL-C, apo A-I, Lp A-I, and Lp A-I:A-II concentrations. Apolipoprotein A-I and Lp A-I were also found to be higher in the presence of the ε2 allele coding for APOE. The effect of the CETP A373P rare allele c on HDL-C was independent of all tested parameters except triglycerides. The respective effect of these polymorphisms and triglycerides on cardiovascular risk should be evaluated prospectively.

1. Introduction

The plasma level of high-density lipoprotein cholesterol (HDL-C) is known to be inversely associated with cardiovascular risk [1]. Apolipoproteins (apos) A-I and A-II are the major apolipoproteins of HDL. Increased concentrations of lipoprotein (Lp) A-I, the subfraction containing apo A-I but free of apo A-II, were reported to be associated with higher HDL-C levels. However, elevated concentrations of Lp A-I:A-II, the subfraction containing both apo A-I and apo A-II, showed contrasting associations with coronary heart disease (CHD) risk [2].

Many factors influencing the HDL-C level are known, including dietary habits [3], physical exercise [4], alcohol consumption [5], family history [6], and genetic factors [7].

High-density lipoprotein cholesterol depends on the levels of apolipoproteins building the HDL particle, other apolipoproteins, and the transfer proteins participating in the lipid exchange between triglyceride-rich lipoproteins and HDL and the transcription factors of the genes coding for those proteins.

Apolipoprotein E acts as a receptor ligand in the lipoprotein catabolism process. It mediates the catabolism of chylomicron and very low-density lipoprotein remnants via remnant and low-density lipoprotein (LDL) receptors [8]. There are 3 isoforms of apo E: E2, E3, and E4. APO E4 allele is reported to be associated with early-onset CHD [9].

The peroxisome proliferator–activated receptors (PPARs) are members of the nuclear receptor superfamily. The genes regulated by PPARα participate in the regulation of key proteins involved in extracellular lipid metabolism, fatty acid oxidation, homeostasis, and inflammation [10]. As such, PPARA is a candidate gene whose expression or activity may influence CHD risk through multiple pathways including alterations in lipid concentrations, obesity, insulin resistance, or the inflammatory response. Several polymorphisms of the human PPARA gene have been described. Of these, a C→G transversion at position 484 in exon 5 leads to a substitution of valine for leucine at codon 162. In the Framingham Offspring Study, the rare *g* allele was associated with higher concentrations of total and LDL cholesterol (LDL-C), apo B, and apo C-III [10]. Another polymorphism of PPARA is Val227Ala. In a Japanese population, the carriers of Ala227 have lower levels of serum total cholesterol and triglycerides [11].

Apolipoprotein A-I is the major apolipoprotein of HDL. It plays an important role in reverse cholesterol transport and can be a protective factor in atherosclerosis. The −75G/A polymorphism in the promoter of gene APOAI was associated with changes in blood pressure, variability in the HDL-C response to statins, HDL subfractions, and dietary fat intake [4].

Cholesteryl ester transfer protein (CETP) mediates the transfer of cholesterol ester from HDL to LDL, very low-density lipoprotein, and triglyceride in the reverse direction. This represents a key role in the reverse cholesterol transport. D442G polymorphism, a substitution of A by G in exon 15 of CETP gene, was found to be associated with CHD in a Chinese population [12]. In whites, the A373P polymorphism, a substitution of G by C in exon 12, has effects on plasma lipid concentrations. The *g* allele carriers have higher levels of HDL-C [13].

All these polymorphisms have been sometimes studied individually. The hypothesis is how they can interact together. However, the availability of a large population may be helpful to determine if they interact together and with lifestyles for the level of HDL-C and associated lipoprotein particles.

The aim of this analysis was to investigate the possibility of interactions between the CETP (A373P, D442G), PPARA (L162V, Val227Ala), APOE, and APOAI (−75G/A) with lifestyles on HDL-C, apo A-I, Lp A-I, and Lp A-I:A-II levels in healthy individuals selected in the cohort Prospective Epidemiological Study of Myocardial Infarction (PRIME) study.

2. Methods

2.1. Subjects

Results of the PRIME study have been described in great detail elsewhere [14]. Briefly, it is a prospective cohort study that was set up to investigate risk factors for ischemic heart disease. From 1991 to 1993, 10 600 men aged 50 to 59 years living in France and in Northern Ireland were recruited to broadly match the social class structure of the background population. On entry, questionnaires relating to medical history and tobacco consumption were obtained; and physical measurements were taken. Additional data were collected on all participants every year over 5 years of follow-up. Only subjects without any history of CHD on entry were included in this study. At the fifth year of follow up, 289 cases of CHD were found, leaving 8784 subjects free of any disease. Among

those, we randomly selected a pool of 857 individuals to participate in our research. This sample was calculated to provide 25% of subjects in the lowest tertile of HDL-C (≤28 mg/dL) in the whole PRIME study sample, 25% of subjects in the highest tertile of HDL-C (≥73 mg/dL), and 50% of subjects in the medium tertile of HDL-C (28-73 mg/dL). A final set of 792 samples with DNA available was used in this analysis.

2.2. DNA analysis

Genomic DNA was isolated from peripheral blood leukocytes by standard methods [15]. Genotyping was carried out on an Applied Biosystems (Cortaboeuf, France) 7900 sequence detection system with TaqMan probes for allelic discrimination. The method has been described by the supplier. Polymerase chain reaction amplification was carried out in the presence of 2 probes with different reporter dyes attached to their 5′ ends (in this case, 6-carbon fluorescein and VIC) and a fluorescent quencher (6-carboxy-tetramethylrhodamine) at the 3′ ends. One probe was complementary to the wild-type DNA strand and the other to the DNA strand with the mutation. Polymerase chain reaction was performed in 5-μL reaction mixtures containing 0.1 μL of assay mix, 2 μL of TaqMan universal master mix from Applied Biosystems, and 20 ng DNA. After 1 step at 95°C for 10 minutes, 45 cycles of 92°C for 15 seconds and 60°C for 1 minute were performed in the ABI Prism 7900 sequence detection system (Applied Biosystems), in which the fluorescence intensity in each well of the plate was read. Fluorescence data files from each plate were analyzed using automated software (SDS 2.1, Applied Biosystems). The primers and probes sequences in the assay mix are listed in Table 2. Polymorphism determination was possible for 730 subjects for apo E, 714 subjects for PPARα, 774 subjects for apo A-I, and 764 subjects for CETP.

2.3. Serum measurements

A blood sample was drawn at inclusion after a 12-hour fast into tubes containing EDTA. Plasma was separated by centrifugation at 4°C within 15 minutes at each clinic and kept at 4°C. All samples were analyzed at the Pasteur Institute in Lille in exactly the same way. Cholesterol and triglyceride levels were determined by enzymatic procedures (Boehringer, Meylan, France). Cholesterol was measured in HDL-containing supernatant after phosphotungstate/magnesium chloride precipitation of apo B–containing lipoproteins (Boehringer). Apolipoprotein A-I was quantified by immunonephelometry (Behringwerke, Marburg, Germany). Lipoprotein A-I was measured using differential immunoelectrophoresis [16]. The Lp A-I:A-II level was calculated as its apo A-I content by subtracting Lp A-I from total apo A-I. Low-density lipoprotein cholesterol was calculated according to the Friedewald formula [17]. Therefore, this parameter was not determined in subjects with triglycerides greater than 4 g/L (n = 51).

2.4. Statistical analysis

Statistical analysis was conducted using SAS (SAS Institute, Cary, NC) software. The relationships between PPARA, APOE, CETP, and APOAI alleles and lipid or apolipoprotein measures were evaluated by using *t* test; and χ^2 test was used to examine differences in allele frequencies between HDL-C level classes defined as low, medium, or high as described above (≤28 mg/dL, between 28 and 73 mg/dL, or ≥73 mg/dL). Interactions between alleles and other variables well known to be linked to HDL-C (physical activity, body mass index [BMI], tobacco consumption, alcohol consumption, and major diet intake such as total fat, cheese, eggs, fruits, and vegetables) were tested using a cumulative logistic regression model with HDL-C level in 3 classes (as above) being the explained ordinal variable. The model contained all variables linked to the HDL-C level in univariate analysis with a *P* value less than .25. In this model, alcohol consumption was categorized as less than or greater than the median (< or ≥29.83 mL/d); and hypertriglyceridemia was considered when triglycerides were at least 1.50 g/L.

3. Results

The main characteristics of the selected PRIME sample population and the various polymorphisms studied are shown in Table 1. The HDL-C level of this population reflects the criteria used for the selection of the sample. The frequencies of the polymorphisms given in Table 1 were determined using the probes described in Table 2. The polymorphisms of PPARA V227A and CETP D442G were not found in any of the 792 individuals studied. The frequency distribution of the different alleles, according to the level of HDL-C, is shown in Table 3. A statistically significant different distribution was found only for the CETP A373P rare allele *c*, which was shown to be less frequent in the group of subjects with a high HDL-C concentration. Accordingly, as shown in Table 4, the CETP rare allele was associated with a lower concentration of HDL-C (42.6 ± 23.2 vs 51.9 ± 24.1 mg/dL, *P* = .0006, presence vs absence of the rare allele), apo A-I (140 ± 37 vs 154 ± 38 mg/dL, *P* = .0007, presence vs absence of the rare allele), Lp A-I (42.3 ± 13.7 vs 48.4 ± 15.6 mg/dL, *P* = .0004, presence vs absence of the rare allele), and Lp A-I:A-II (98 ± 27 vs 106 ± 27 mg/dL, *P* = .007, presence vs absence of the rare allele). The plasma concentrations of apo A-I (159 ± 42 vs 151 ± 37 mg/dL, *P* = .046, presence vs absence of the rare allele) and Lp A-I (51.3 ± 17.0 vs 46.9 ± 15.2 mg/dL, *P* = .007, presence vs absence of the rare allele) were also found to be significantly higher in the presence of the ε2 allele coding for APOE.

To study the independent influence of each allele on plasma concentrations of HDL-C, a multiple logistic regression model was run with all the rare alleles included and variables known to influence the HDL-C level: BMI

Table 1
Main characteristics of the studied population

Variables		Means and (5th-95th percentiles)
Cholesterol (g/L)		2.21 (1.53-2.85)
Triglycerides (g/L)		1.80 (0.58-4.59)
HDL-C (g/L)		0.51 (0.21-0.91)
LDL-C (g/L)[a]		1.37 (0.81-1.95)
BMI (kg/m^2)		26.5 (21.1-33.1)
Alcohol consumption (mL/d)		39.12 (0.00-122.57)
Variables		**n (%)**
Physical activity	No	117 (14.77%)
	Light once a week	476 (60.10%)
	Intense once or twice a week	150 (18.94%)
	Intense ≥3 times a week	49 (6.19%)
Tobacco consumption	Never	229 (28.91%)
	Actual	253 (31.94%)
	Past	310 (39.14%)
APOE genotype	*e2*/*e2*	4 (0.55%)
	e2/*e3*	93 (12.72%)
	e2/*e4*	14 (1.92%)
	e3/*e3*	441 (60.33%)
	e3/*e4*	153 (20.93%)
	e4/*e4*	26 (3.56%)
PPARA genotype	*c*/*c*	618 (86.55%)
	c/*g*	92 (12.89%)
	g/*g*	4 (0.56%)
APOAI genotype	*a*/*a*	21 (2.71%)
	g/*a*	196 (25.32%)
	g/*g*	557 (71.96%)
CETP genotype	*c*/*c*	3 (0.39%)
	c/*g*	88 (11.52%)
	g/*g*	673 (88.09%)

Results are means (5th-95th percentiles) or numbers (percentage).
[a] n = 741.

(in kilograms per square meter), physical activity, tobacco consumption, alcohol consumption, and major food intake. Physical activity was grossly estimated as no activity, light activity once a week, intense activity once or twice a week, or intense activity 3 times or more a week; tobacco consumption was classified as never, actual, or past consumption; alcohol consumption was determined in milliliters per day; total fat intake was divided into 7 categories from the lowest to the highest; cheese and egg consumptions were divided into 4 separate categories from the lowest to the highest (number of portions and frequencies); and fruit and vegetable consumption was grouped into 10 categories according to the number of units taken and frequencies. As shown in Table 5, the CETP rare *c* allele remained independently and negatively associated with the HDL-C (odds ratio, 0.58 [0.36-0.92]; $P = .0197$), even when physical activity, tobacco consumption, alcohol consumption, and major food intake were added to the model. However, in addition to CETP, the presence of the rare ε2 allele was slightly associated with HDL-C level ($P = .0773$). In this model, besides these genotypes, only BMI (odds ratio, 0.87 [0.83-0.91]; $P < .0001$), tobacco consumption (odds ratio, 0.98 [0.97-1.00]; $P = .05$), fruit and vegetable consumption (odds ratio, 1.08 [1.00-1.16]; $P = .04$), and alcohol consumption (odds ratio, 2.74 [2.03-3.70]; $P < .0001$)

Table 3
Frequency distribution (percentage) of each allele according to the level of HDL-C

	HDL-C ≤28 mg/dL	28 < HDL-C < 73 mg/dL	HDL-C ≥73 mg/dL	*P*
PPARA L162V Allele *g*	17.9	11.7	14.8	.2203
PPARA L162V Allele *c*	98.9	99.5	99.5	.7877
CETP A373P Allele *c*	13.9	14.7	5.7	.0027
CETP A373P Allele *g*	100.0	99.5	99.6	.7945
APOAI −75G/A Allele *a*	26.9	28.4	27.8	.9516
APOAI −75G/A Allele *g*	96.1	97.9	96.5	.4150
APOE Allele ε*3*	93.9	93.8	94.4	.9599
APOE Allele ε*2*	13.1	13.6	19.2	.1445
APOE Allele ε*4*	28.3	24.8	28.6	.5306

Table 2
Sequences of primers and probes used for the polymorphism determination

SNP	Primers	Probes	NCBI SNP reference
APOAI −75G/A			rs670
PPARA	CAGAAACAAATGCCAGTATTGTCGAT	VIC-ACAAGTGCCTTTCTG	rs1800206
L162V	CCTTACCTACCGTTGTGTGACATC	FAM-CAAGTGCGTTTCTG	
CETP A373P	TCTCCCCAGGATATCGTGACTAC	VIC-TCTTAGAATAGGAGGCCTGGA	rs5880
	GCAGCACATACTGGAAATCCAAGA	FAM-TTAGAATAGGAGGGCTGGA	
CETP D442G	GCCCTCATGAACAGCAAAGG	VIC-CCTCTTCGACATCATC	rs2303790
	GCTTTGTACTCACATCTCGAGTGAT	FAM-TCTTCGGCATCATC	
PPARA Val227Ala	CTACGAGGCCTACTTGAAGAACTTC	VIC-CTGAGAGGATGGCCCG	rs1800234
	GCCGCAAACACCTACTGGAT	FAM-CTGAGAGGATGACCCG	
APOE T388C	GCACGGCTGTCCAAGGA	VIC-CATGGAGGACGTGTGC	rs429358
	GCTTGCGCAGGTGGGA	FAM-ATGGAGGACGTGCGC	
APOE C526T			rs7412

Table 4
Effect of the presence of each rare allele on the concentration of HDL- and apo A-I–containing lipoproteins

	PPARA L162V		CETP A373P		APOAI −75G/A		APOE		APOE	
	g (n =99)	no *g* (n =618)	*c* (n =91)	no *c* (n =673)	*a* (n =217)	no *a* (n =557)	*ε2* (n =111)	no *ε2* (n =620)	*ε4* (n =193)	no *ε4* (n =538)
HDL-C (mg/dL)	49.9 (25.5)	50.8 (23.9)	42.6 (23.2)	51.9 (24.1)	50.9 (23.4)	50.8 (24.4)	53.4 (23.8)	50.1 (24.1)	51.5 (23.7)	50.3 (24.2)
	P = .737		*P* = .0006		*P* = .978		*P* = .186		*P* = .545	
Apo A-I (mg/dL)	151 (38)	152 (38)	140 (37)	154 (38)	154 (38)	152 (38)	159 (42)	151 (37)	153 (36)	152 (39)
	P = .764		*P* = .0007		*P* = .468		*P* = .046		*P* = .676	
Lp A-I (mg/dL)	48.3 (15.6)	47.7 (15.5)	42.3 (13.7)	48.4 (15.6)	47.5 (14.4)	47.7 (15.9)	51.3 (17.0)	46.9 (15.2)	47.7 (16.6)	47.6 (15.2)
	P = .708		*P* = .0004		*P* = .856		*P* = .007		*P* = .934	
Lp A-I:A-II (mg/dL)	103 (26)	105 (27)	98 (27)	106 (27)	107 (28)	104 (27)	108 (30)	104 (27)	106 (25)	104 (28)
	P = .527		*P* = .007		*P* = .267		*P* = .215		*P* = .592	

Results are presented as means (SD).

had an independent effect on the level of HDL-C (data not shown).

Because triglyceride concentration is known to be inversely correlated with HDL and its related components, a second multiple logistic regression model including all the tested rare alleles and triglycerides was run to test for the independent association of these parameters with HDL-C, after adjustment for BMI, tobacco, alcohol, and various food intakes. As shown in Table 5, hypertriglyceridemia remained independently and negatively associated with the concentrations of HDL. However, the presence of hypertriglyceridemia in the model led to a lack of significant association between CETP *c* allele and the concentrations of HDL.

4. Discussion

This study was run on men who were free of cardiovascular disease, at an age when most of the people start to develop atherosclerosis. High-density lipoprotein and associated lipoprotein particles may help predict CHD risk; this is why we have studied associations between the polymorphisms and these parameters. However, it is clear that this study cannot link these polymorphisms to CHD risk. This is a real limitation of our study.

The samples obtained for our ancillary study of the PRIME study were selected among the subjects who remained free of any cardiovascular disease at the 5-year follow-up analysis [14]. The main result is that the CETP A373P polymorphism modulates HDL-C, apo A-I, Lp A-I, and Lp A-I:A-II levels.

The frequency of this single nucleotide polymorphism (SNP) in our population in the second tertile of HDL-C (14.68%) almost matches that of the white population (10.3%) [13]. Our data are in agreement with the Copenhagen City Heart Study [13] and with the Atorvastatin Comparative Cholesterol Efficacy and Safety Study showing that carriers of *c* allele have lower level of HDL-C than homozygotes for the *g* allele [13,18]. We show that this rare allele is also associated with a lower concentration of Lp A-I and Lp A-I:A-II. A biologically plausible explanation for such association could be that the mutation at position 373 induces higher CETP mass or activity. We were not able to test these hypotheses; however, in a previous study, this

Table 5
Effect of each rare allele on the HDL-C concentration defined in class level, after adjustment for physical activity, BMI, food intake, and tobacco and alcohol consumption

	Univariate model	Multivariate model without triglycerides	Multivariate model with triglycerides
	Odds ratio (5th-95th percentile)	Odds ratio (5th-95th percentile)	Odds ratio (5th-95th percentile)
PPARA L162V	0.98 (0.64-1.49)	–	–
Allele *g*	*P* = .9153		
CETP A373P	0.54 (0.35-0.83)	0.58 (0.36-0.92)	0.67 (0.42-1.07)
Allele *c*	*P* = .0048	*P* = .0197	*P* = .0911
APOAI −75G/A	1.01 (0.74-1.37)	–	–
Allele *a*	*P* = .9508		
APOE	1.44 (0.97-2.14)	1.45 (0.96-2.20)	1.53 (1.00-2.34)
Allele *ε2*	*P* = .0682	*P* = .0773	*P* = .0517
APOE	1.08 (0.78-1.49)	–	–
Allele *ε4*	*P* = .6421		
Triglycerides (≥ vs <1.50 g/L)	0.14 (0.10-0.20)	–	0.20 (014-0.29)
	P < .0001		*P* < .0001

The logistic multiple regression model contains all variables linked to the HDL-C level (≤28 mg/dL, between 28 and 73 mg/dL, or ≥73 mg/dL) in the univariate model with a *P* value less than .25 as well as BMI, physical activity, tobacco, alcohol, and various food intakes (total fat, cheese, eggs, fruits, and vegetables).

variant was associated with higher CETP mass [18]. Although our results do not argue in favor of any of these hypotheses, they indicate that the effect of this CETP rare allele is independent of any effect of the genes studied because its influence remained significant in our multiple logistic regression model, entering all rare alleles studied, as well as variables classically linked to HDL-C, such as physical activity, BMI, food intake, and tobacco and alcohol consumption, clearly indicating that the effect of CETP rare allele is independent of these variables. However, when the presence of hypertriglyceridemia (≥1.50 g/L) was included in the model, the association between this CETP rare allele and the HDL level was much weaker, indicating that the effect of this polymorphism may be modulated by the triglyceride concentration.

CETP D442G was not detected in the PRIME population. This can be explained by its absence in European and North American populations as previously suggested. This mutation was observed in the Japanese and Chinese populations [12,19]. Besides these 2 polymorphisms, other functional variants of CETP gene influence the concentration of HDL-C concentrations, for example, C-629A. The A allele is associated with reduced CHD risk probably mediated by elevated HDL concentration [20].

The allele frequencies of APOAI −75G/A in our population are similar to those reported by others [4,21]. However, we did not find any association with plasma lipids or lipoprotein concentrations. An early study [22] showed that men with the *a* allele had higher HDL-C and apo A-I concentrations than *g* homozygotes. Pagani et al [23] reported that the frequency of the *a* allele increased from the lowest to highest decile of HDL-C concentration in women only. Ruaño et al [4] found no effect of the APOAI −75G/A SNP on the concentration of HDL-C. In the Framingham Study, a significant gene-diet interaction was found for this SNP [21]. Indeed, higher polyunsaturated fatty acid intakes were associated with higher HDL-C in female carriers of allele, whereas the opposite effect was observed in *g* homozygotes. Therefore, the effects of this SNP could depend on environmental and sex factors that differ between populations.

The frequencies of APOE alleles are in agreement with those found in previous research in white populations [8] but not in Greek and Turkish populations [24,25]. In addition, in our multiple logistic regression models, the ε2 allele was not clearly linked to the HDL-C level, although it was independent of the presence of hypertriglyceridemia. By contrast, we found that apo A-I and Lp A-I were significantly higher in the presence of this rare allele when compared with subjects who lacked this allele. However, because APOE gene has been associated with other serum lipid concentrations [8,24,25], this effect could reflect an indirect effect.

PPARA V227A polymorphism was not found in our population, although it was detected in the Japanese population [11] and associated with changes in plasma cholesterol and triglyceride concentrations. The frequencies of PPARA L162V alleles are in agreement with those published earlier by other researchers [10,26,27]. Our data did not show any significant increase in the levels of HDL-C, Lp A-I, and Lp A-I:A-II in the presence of the PPARA *g* allele. However (data not shown), the levels of triglycerides were decreased in the presence of this rare allele. The same trend has been reported in other studies [21,28] in which the *g* allele appeared to be a protective factor against CHD. This allele was associated with high levels of apo B and LDL-C [27] and reduced levels of HDL-C [29]. These discrepancies could be explained by differences in study populations and environmental factors.

In conclusion, our results indicate a major effect of the CETP A373P polymorphism on HDL-C, Lp A-I, and Lp A-I: A-II and a minor effect of the APOE2 polymorphism on apo A-I and Lp A-I. These effects should contribute to the risk of cardiovascular disease. This should be further evaluated in prospective studies.

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