Combined effects of lipoprotein lipase and apolipoprotein E polymorphisms on lipid and lipoprotein levels in the Stanislas cohort

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Abstract We have genotyped 1101 supposedly healthy subjects from the Stanislas cohort for the lipoprotein lipase (LPL) gene Ser^{447}(C) → stop (G) polymorphism and/or for the apolipoprotein (apo)E common polymorphism. Genotypic effects of the two polymorphisms on fasting serum triglycerides (TG), total cholesterol (Tchol), high density lipoprotein-cholesterol (HDLc), low density lipoprotein-cholesterol (LDLc), apoB, apoA-I, and apoE levels were studied separately for each polymorphism and in conjunction. ε4 allele and high apoE levels were associated with high levels of LDLc, Tchol, apoB, and TG. The G allele of LPL was significantly associated with low TG levels. We found a clear interaction between the LPL/apoE polymorphisms and apoE levels on serum TG variation. Total variability of TG levels in women and men of 42.31% and 53.62% respectively, were mainly explained by apoE concentration and these two polymorphisms. ApoE and LPL genes simultaneously modulated TG levels.—**Salah, D., K. Bohnet, R. Gueguen, G. Siest, and S. Visvikis.** Combined effects of lipoprotein lipase and apolipoprotein E polymorphisms on lipid and lipoprotein levels in the Stanislas cohort. *J. Lipid Res.* 1997. **38:** 904–912.

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Apolipoprotein E (apoE) is a 34 kD protein synthesized mainly by the liver but also by other tissues including brain. ApoE has several functions in humans (1). Among them, it can mediate cellular uptake of lipoproteins via both apo B/E receptor (2), the low density lipoprotein receptor-related protein (LRP) (3), and the VLDL receptor (4, 5). Therefore, it plays an important role in the transport of cholesterol and the metabolism of lipoprotein particles.

Lipoprotein lipase (LPL) is a 57 kD glycoprotein (6) synthesized and secreted into the circulation by many tissues. It is bound in its active homodimeric form via glycosaminoglycans to the luminal surface of capillary endothelial cells (7). LPL plays a key role in lipoprotein metabolism, both as an enzyme that hydrolyzes triglycerides of very low density lipoproteins (VLDL) and chylomicrons (8), and as a mediator for lipoprotein interaction with cell surfaces and receptors (9–17).

ApoE and LPL genes are involved in the predisposition for coronary heart disease and apoE is involved in Alzheimer's disease. The apoE gene is located on chromosome 19, spans 3.6 kb, and includes 4 exons (18). It is polymorphic, with three common alleles (ε2, ε3, ε4) coding for three isoforms (E2, E3, E4), which differ by a single amino acid substitution at residues 112 and/or 158 (19). The LPL gene has been mapped to chromosome 8 (20), spanning about 35 kb and containing 10 exons (21, 22). Numerous mutations and several restriction fragment length polymorphisms have been reported at the LPL locus (23). One of the polymorphism of the LPL gene is the mutation Ser^{447} → Stop (C → G) located in exon 9, which gives rise to a truncated mature protein (LPL446) lacking two carboxyl-terminal amino acids (Ser–Gly) (24, 25). The isoform corresponding to this premature stop codon mutation in exon 9 is the only relatively common protein polymorphism of human lipoprotein lipase known today. LPL446 isoform is of great interest, because the carboxy-terminal dipeptide Ser–Gly is conserved in all known mammalian enzymes (human, mouse, bovine, and guinea pig) (26). This truncated form of LPL, which lacks the terminal glycine and serine residues, could give rise to altered

Abbreviations: BMI, body mass index; WHR, waist-to-hip ratio; OC, oral contraceptive; apo, apolipoprotein; LPL, lipoprotein lipase; LPL446, truncated form of LPL; (C/G)allele, gene LPL (absence/presence) of LPL Ser^{447} → stop mutation; Tchol, total cholesterol; [ApoE], apoE concentration; LDLc, low density lipoprotein cholesterol; HDLc, high density lipoprotein cholesterol; VLDL, very low density lipoprotein; TG, triglyceride; ε2 ε2, ε3 ε2, ε3 ε3, ε3 ε4, ε2 ε4, ε4 ε4, apoE genotypes; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; GGT, gamma-glutamyltransferase; PCR, polymerase chain reaction.

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transport, binding to endothelial cells, or distribution in the capillary endothelium. The regions coded for by exons 6 and 9 are rich in basic amino acids and probably contain the heparan sulfate glycosaminoglycan binding sites, by which the enzyme attaches to the capillary endothelium.

Since 1991, several recent studies have reported the interaction of both proteins, apoE and LPL, in the lipid metabolism as: *i*) apoE and LPL protein coordinately enhance binding and uptake of lipoproteins by cells (17, 27, 28); *ii*) apoE appears to reduce the lipase-mediated retention of LDL by the subendothelial matrix (12); and *iii*) also appears to modulate the LPL activity (29); and *iv*) finally, both LPL and apoE are expressed in atherosclerotic lesions and could function coordinately (30).

We were interested in studying such eventual interaction in a supposedly healthy population. We examined *1*) the impact of the apoE polymorphism and the (C/G)LPL^{447} polymorphism on lipid levels independently; *2*) the combined influence of the apoE common polymorphism and the (C/G)LPL^{447} polymorphism on lipid levels; and *3*) the interaction between these gene polymorphisms, apoE levels, and other factors (BMI, WHR, oral contraceptive (OC) use, gender, age, consumption of alcohol), concerning their influence on lipid traits.

MATERIAL AND METHODS

Study population

Men and women from the STANISLAS cohort (31), who underwent a routine health examination at the Center for Preventive Medicine in Nancy, France, were included in this study. These individuals (1101 total) were selected following the exclusion criteria which were the use of hypolipidemic medication and abnormal liver metabolism as defined by elevated activities of alanine aminotransferase (ALAT > 130 U/l), aspartate aminotransferase (ASAT > 90 U/l), or gamma-glutamyltransferase (GGT > 150 U/l). Subjects with TG levels higher than 6 mmol/l were also excluded from the study.

Cohort Stanislas

Population-Recruitment of families. Between January 1994 and August 1995, 1006 families consisting of two parents with at least two biological offspring of more than 8 years of age, were recruited at the rate of two families a day (in total around 4300 individuals). These families had to be of European origin and all the different individuals were free of chronic disease and/or free of medical treatment that could interfere with the study. The selection and the recruitment were carried out at the Center of Preventive Medicine in Vandoeuvre-lès-Nancy, from the population living in the Vosges and in the Meurthe et Moselle area.

The study was in accordance with the clauses of the French law Huriet, concerning the protection of persons in biomedical research. The participants in this study were volunteers and signed a statement of informed consent. All the subjects of the cohort voluntarily participated in the study and signed the patient consent form.

Main variables measured. i) Socio-professional environment and life-style; *ii*) physiological characteristics: (age, sex, puberty status, hormonal status); *iii*) problems of health; *iv*) personal and hereditary antecedents; *v*) clinical examination report; *vi*) medication taken; *vii*) corpulence (weight, size, waist and hips, perimeters of skin folds, bioelectric impedance); *viii*) cardiological examination (arterial pressure, electrocardiogram, cardiological echography); *ix*) biological variables (fasting serum); *x*) bases tests (albumin, TGO, TGP, GGT, glucose); *xi*) blood count and blood grouping; *xii*) lipid tests: (triglycerides, Tchol, HDLc, apolipoproteins A-I, B, and E, Lp[a]); *xiii*) genetic polymorphisms: (apolipoprotein E, LPL ((C/G)LPL^{447})) were determined.

Bank of biological material. A bank of plasma, serum, and DNA for each family members was maintained for analysis and to serve in different transverse and longitudinal studies. The plasma and serum samples were stored in liquid nitrogen at −196°C. After extraction of the DNA using the technique of Miller, Dykes, and Polesky (32), the samples were aliquoted in volumes of 10, 50, or 400 µl with an average of 400 µg/ml and stored at −80°C and +4°C.

Lipid parameters analysis

Blood was collected by venipuncture after an overnight fast, either in EDTA-containing Vacutainers or in Vacutainers containing a gel for serum separation (Beckton Dickinson, Rutherford, NJ). Blood was promptly centrifuged (1000 *g*, 10 min, room temperature) for plasma and serum separation and for the preparation of buffy coat. The buffy coat was frozen in liquid nitrogen until extraction of DNA. Total serum cholesterol, triglycerides, ALAT, ASAT, and GGT were measured using standard enzymatic methods (Merck, Germany), automated on AU5000 (Olympus Merck, Japan). Serum apoA-I and apoB were determined by immunonephelometry on a Behring Nephelometer analyzer, with Behring reagents (France); serum apoE was determined by turbidimetry using reagents from Daiichi (Japan) automated on COBAS MIRA, and HDLc was determined using reagents from Boehringer (Germany). LDLc was calculated according to the equation of Friedewald, Levy, and Fredrickson (33).

TABLE 1. Population mean (SD) values for age, BMI, serum lipid, and lipoproteins

Subjects	n	Age	BMI	Tchol	LDLc	HDLc	TG	ApoA-I	ApoB	ApoE
		yr	*kg/m²*			*mmol/l*			*g/l*	*mg/l*
Men	531	41.6	25.3	5.98	4.13	1.24	1.38	1.54	1.15	49.3
		(4.8)	(3.1)	(1.08)	(1.01)	(0.34)	(1.26)	(0.23)	(0.27)	(16.0)
Women	570	39.5	23.9	5.49	3.56	1.53	0.88	1.69	0.99	44.2
		(4.5)	(4.2)	(0.95)	(0.88)	(0.41)	(0.56)	(0.27)	(0.23)	(12.0)

Quality control

Different procedures enabled the intra- and interseries analytical variation to be controlled. Indeed, control serums were regularly taken in the series of dosages and the daily variability of the patient gave the short term and long term analytical variation. Quality assurance plan was specifically installed for the Stanislas Cohort study in order to obtain validated morphological and biological data. Pre-analytical variation was controlled by using a blood sampling standardization. Analytical variations for each parameter were described earlier (34) except for the apoE constituent. Within-day imprecision of apoE measurement ranged from 2.8% to 1.7% for mean values between 35.3 and 80.2 mg/l. The day-to-day reproducibility was up to 4.2% during the 6 months period of apoE measurements.

DNA analysis

DNA was extracted by the salting-out method (32). Polymerase chain reaction (PCR) amplification of LPL exon 9 was carried out using a modified 3′ primer that produces a 140 bp product containing a site for the restriction enzyme HinfI in the presence of the mutation C → G (Ser^{447} → Stop mutation) as previously described (35).

Primers were 5′ upstream 5′-CATCCATTTTCTTCCA CAGGG-3′; 3′ downstream 5′-TAGCCCAGAATGCTCA CCAGACT-3′. Thirty subsequent cycles of PCR were performed as follows: denaturation step at 94°C for 1 min (except for an initial denaturation for 5 min at 95°C), annealing step at 55°C for 1 min, and extension step at 72°C for 1 min. Amplified products were digested with HinfI (Boehringer Mannheim), and the resulting fragments were electrophoresed on 8% nondenaturing polyacrylamide gels in TBE buffer at 200 volts for 2 h. DNA was visualized by staining with ethidium bromide. The alleles were named G/C according to the presence/absence of the mutation stop 447. ApoE genotype was determined by PCR amplification and subsequent digestion with restriction enzyme HhaI as described by Hixson and Vernier (36). Two individuals were not genotyped for apoE and 28 individuals had not been analyzed for LPL polymorphism.

Statistical analysis

Analysis of variance and non-parametric methods (Kruskal-Wallis test) were used to test for differences among mean values across genotypes. Subjects with the less common genotypes (ε2ε2, CC/ε2ε4, CG/ε2ε4, CC/ε4ε4, CG/ε4ε4) were not included in all statistical analyses because of the small sample size.

Stepwise regression analysis was used to test and estimate the effect of covariates (polymorphisms of LPL and apoE, [apoE], BMI, WHR, OC use, alcohol consumption) and their interactions on lipid variables.

RESULTS

The main biological characteristics of the population studied here, such as age, body mass index (BMI) and concentration of Tchol, LDLc, apoB, TG, HDLc, apoA-I and apoE, are given in **Table 1.**

TABLE 2. Population mean (SD) of serum lipid and lipoprotein levels for each apoE genotype (1091 subjects)

	Genotype (n)					*P* Value	
	ε2ε4 (37)	ε3ε2 (153)	ε3ε3 (675)	ε3ε4 (203)	ε4ε4 (23)	F Test	K-W Test
Tchol, mmol/l	5.68 (0.94)	5.33 (0.98)	5.72 (1.00)	6.00 (1.07)	6.26 (1.04)	<0.001	<0.001
HDLc, mmol/l	1.44 (0.39)	1.45 (0.37)	1.38 (0.39)	1.37 (0.39)	1.44 (0.43)	NS	NS
LDLc, mmol/l	3.64 (0.95)	3.41 (0.93)	3.87 (0.93)	4.10 (1.01)	4.37 (0.88)	<0.001	<0.001
LDLc/HDLc	2.79 (1.17)	2.56 (1.14)	3.11 (1.30)	3.28 (1.38)	3.30 (1.15)	<0.001	<0.001
TG, mmol/l	1.30 (0.84)	1.04 (0.60)	1.09 (0.86)	1.23 (1.55)	0.94 (0.43)	NS	NS
ApoA-I, g/l	1.69 (0.26)	1.68 (0.24)	1.61 (0.27)	1.60 (0.25)	1.62 (0.25)	0.01	0.008
ApoB, g/l	1.00 (0.22)	0.93 (0.25)	1.07 (0.25)	1.15 (0.27)	1.19 (0.25)	<0.001	<0.001
ApoE, mg/l	57.1 (18.0)	54.8 (13.0)	44.8 (10.8)	43.6 (16.4)	39.7 (14.0)	<0.001	<0.001

NS, not significant; F test, Fisher-Snedecor test; K-W test, Kruskal-Wallis test.

TABLE 3. Population mean (SD) of serum lipid and lipoprotein levels for LPL genotypes (1073 subjects)

	Genotype (n)			*P* Value	
	CC (831)	CG (229)	GG (13)	F Test	K-W Test
Tchol, mmol/l	5.75 (1.05)	5.63 (1.01)	5.87 (1.17)	NS	NS
HDLc, mmol/l	1.37 (0.40)	1.43 (0.40)	1.38 (0.70)	NS	NS
LDLc, mmol/l	3.84 (0.98)	3.77 (0.95)	4.08 (1.19)	NS	NS
LDLc/HDLc	3.10 (1.33)	2.90 (1.30)	3.35 (1.72)	NS	NS
TG, mmol/l	1.17 (1.09)	0.95 (0.63)	0.95 (0.62)	0.01	0.001
ApoA-I, g/l	1.61 (0.26)	1.63 (0.27)	1.59 (0.20)	NS	NS
ApoB, g/l	1.07 (0.26)	1.04 (0.25)	1.08 (0.29)	NS	NS
ApoE, mg/l	47.0 (15.0)	45.2 (12.1)	48.6 (4.0)	NS	NS

NS, not significant; F test, Fisher-Snedecor test; K-W test, Kruskal-Wallis test.

The distributions of apoE (ε2 (9.4%), ε3 (77.6%), ε4 (13.0%)), and LPL (G (11.9%)) genotype frequencies did not deviate from the Hardy-Weinberg expectation and were similar to those observed in other Caucasian populations.

The first aim of this study was to investigate a possible association between LPL or apoE genetic polymorphisms and serum lipid levels. The relationship between ε2ε4, ε3ε2, ε3ε3, ε3ε4, ε4ε4 genotypes of apoE polymorphism and concentrations of lipid parameters are shown in **Table 2.** ApoE polymorphism was significantly linked to Tchol, LDLc, apoB, and apoE concentrations, as well as to the ratio of LDLc/HDLc. ε3ε4 and ε4ε4 genotypes were associated with high levels of Tchol, LDLc, apoB, and LDLc/HDLc ratio. ε3ε2 was associated with low serum concentration of Tchol, LDLc, apoB, and LDLc/HDLc ratio, and with high serum concentration of apoE.

LPL polymorphism was significantly associated with TG whereas all other parameters studied were not influenced by this polymorphism (**Table 3**). The G allele was significantly associated with lower TG levels as compared to the C allele.

The main aim of this study was to investigate the impact of the apoE polymorphism in conjunction with the (C/G)LPL[447] polymorphism on lipid levels. We formed subgroups according to the apoE and LPL polymorphisms (**Table 4**).

CC/ε3ε4 and CG/ε3ε4 were both associated with high levels of Tchol, LDLc, and apoB due to ε4 allele, and CC/ε3ε2 or CG/ε3ε2 were linked to low serum concentrations of Tchol and apoB, and to high serum concentration of apoE effect due to ε2 allele. Subjects with G allele (CG/ε3ε2, CG/ε3ε3, CG/ε3ε4) had lower TG levels in comparison with subjects carrying the C allele (CC/ε3ε2, CC/ε3ε3, CC/ε3ε4).

Results of multiple regression analysis are given in **Table 5.** Two classes of models were considered: model A included interactions of apoE and LPL genotypes, whereas model B considered the effects of each polymorphism separately. The models also included apoE levels, age, BMI, and gender. Determination coefficients for models A and B were very similar, indicating that both genotypes seem to act independently on lipid variables. Even when no significant difference between models A and B was observed, when we examined variance attributed to each covariate, a small but significant part of TG levels and of LDLc/HDLc ratio was explained by CC/ε3ε4 subgroup. Therefore, CC/ε3ε4 genotypes were associated with higher TG levels and higher ratio of LDLc/HDLc than subjects with CG/ε3ε4 genotypes.

TABLE 4. Population mean (SD) of plasma lipid and lipoprotein levels for six subject groups formed according to the LPL/apoE genotypes (994 subjects)

	Genotype (n)						*P* Value	
	CC/ε3ε2 (115)	CC/ε3ε3 (505)	CC/ε3ε4 (160)	CG/ε3ε2 (33)	CG/ε3ε3 (144)	CG/ε3ε4 (37)	F Test	K-W Test
Tchol, mmol/l	5.40 (1.03)	5.73 (1.01)	5.99 (1.08)	5.12 (0.86)	5.63 (0.97)	6.06 (1.14)	<0.001	<0.001
HDLc, mmol/l	1.43 (0.36)	1.36 (0.40)	1.36 (0.41)	1.47 (0.35)	1.43 (0.44)	1.43 (0.30)	NS	NS
LDLc, mmol/l	3.49 (1.01)	3.87 (0.93)	4.08 (1.03)	3.23 (0.75)	3.77 (0.90)	4.21 (1.03)	<0.001	<0.001
LDLc/HDLc	2.67 (1.29)	3.14 (1.29)	3.31 (1.43)	2.33 (0.82)	2.96 (1.33)	3.15 (1.27)	<0.001	<0.001
TG, mmol/l	1.08 (0.60)	1.13 (0.93)	1.31 (1.68)	0.95 (0.57)	0.94 (0.58)	0.95 (0.84)	0.03	0.01
ApoA-I, g/l	1.68 (0.24)	1.60 (0.26)	1.59 (0.26)	1.64 (0.23)	1.63 (0.28)	1.61 (0.21)	NS	NS
ApoB, g/l	0.96 (0.26)	1.08 (0.25)	1.15 (0.27)	0.88 (0.18)	1.05 (0.23)	1.16 (0.30)	<0.001	<0.001
ApoE, mg/l	55.7 (13.3)	44.9 (10.9)	44.4 (17.2)	51.5 (12.4)	43.8 (10.4)	40.8 (12.1)	<0.001	<0.001

NS, not significant; F test, Fisher-Snedecor test; K-W test, Kruskal-Wallis test.

TABLE 5. Inter-individual variations of serum lipid and apolipoprotein levels (994 subjects)

Model A			Model B		
Covariates	RC	$R^2 \times 100$	Covariates	RC	$R^2 \times 100$
TG					
[ApoE]	+0.307	34.66	[ApoE]	+0.308	34.66
BMI	+0.040	5.63	BMI	+0.040	5.63
Men	+0.209	2.15	ε3ε2	−0.298	2.44
CC/ε3ε2	−0.269	1.31	Men	+0.206	1.95
CC/ε3ε4	+0.180	0.85	CG	−0.135	0.65
CG/ε3ε2	−0.296	0.49	ε3ε4	+0.118	0.38
Total $R^2 \times 100$		45.10			45.72
Tchol					
[ApoE]	+0.365	17.39	[ApoE]	+0.369	17.39
CC/ε3ε2	−0.683	4.12	ε3ε2	−0.721	6.74
Age	+0.028	2.42	Age	+0.028	2.29
CG/ε3ε2	−0.798	2.10	ε3ε4	+0.387	1.94
Men	+0.253	1.37	Men	+0.254	1.37
CC/ε3ε4	+0.374	1.37			
CG/ε3ε4	+0.435	0.55			
Total $R^2 \times 100$		29.32			29.73
LDLc					
[ApoE]	+0.090	9.01	[ApoE]	+0.092	9.01
Men	+0.156	6.09	ε3ε2	−0.248	6.56
CC/ε3ε2	−0.224	3.27	Men	+0.156	5.39
CG/ε3ε2	−0.293	2.09	ε3ε4	+0.136	1.81
CC/ε3ε4	+0.127	1.10	Age	+0.008	1.02
Age	+0.008	1.07	BMI	+0.007	0.45
CG/ε3ε4	+0.162	0.61			
BMI	+0.007	0.48			
Total $R^2 \times 100$		23.72			24.24
HDLc					
Men	−0.259	12.79	Men	−0.259	12.79
BMI	−0.030	7.52	BMI	−0.030	7.52
Age	+0.008	0.89	Age	+0.008	0.89
Total $R^2 \times 100$		21.20			21.20
LDLc/HDLc					
Men	+0.825	15.87	Men	+0.823	15.87
[ApoE]	+0.272	06.30	[ApoE]	+0.283	6.30
BMI	+0.072	04.34	ε3ε2	−0.725	4.49
CC/ε3ε2	−0.613	01.92	BMI	+0.070	3.72
CG/ε3ε2	−0.907	01.53	ε3ε4	+0.313	0.81
CC/ε3ε4	+0.330	00.74			
Total $R^2 \times 100$		30.72			31.19
ApoB					
[ApoE]	+0.064	10.25	[ApoE]	+0.066	10.25
Men	+0.112	7.03	ε3ε2	−0.185	8.10
CC/ε3ε2	−0.163	3.93	Men	+0.112	6.20
CG/ε3ε2	−0.222	2.59	ε3ε4	+0.107	2.36
BMI	+0.010	1.88	BMI	+0.009	1.77
CC/ε3ε4	+0.107	1.79	Age	+0.005	0.79
Age	+0.005	0.84			
CG/ε3ε4	+0.103	0.49			
Total $R^2 \times 100$		28.79			29.46
ApoA-I					
Men	−0.143	7.80	Men	−0.143	7.80
BMI	−0.014	3.54	BMI	−0.014	3.54
Age	+0.006	1.23	Age	+0.006	1.23
[ApoE]	+0.013	0.43	[ApoE]	+0.013	0.43
Total $R^2 \times 100$		12.99			12.99
ApoE					
CC/ε3ε2	+0.991	5.45	ε3ε2	+0.922	6.28
BMI	+0.063	4.25	BMI	+0.062	4.28
Men	+0.370	1.90	Men	+0.369	1.94
CG/ε3ε4	−0.655	0.75	ε3ε4	−0.304	0.77
CG/ε3ε2	+0.593	0.68			
CC/ε3ε4	−0.230	0.36			
Total $R^2 \times 100$		13.39			13.27

Variables, regression coefficients (RC), and percentage of variance ($R^2 \times 100$) explained by the combination (LPL/apoE) model A and by the model B considering LPL and apoE genotypes separately. Table represents the significant effects only.

TABLE 6. Inter-individual variation of serum triglyceride levels

Men			Women		
Covariates	RC	$R^2 \times 100$	Covariates	RC	$R^2 \times 100$
[ApoE]	+0.422 (0.023)	40.27	[ApoE]	+0.269 (0.021)	17.94
WHR	+2.529 (0.540)	06.81	BMI	+0.025 (0.005)	09.23
ε2 × [apoE]	−0.071 (0.011)	04.77	OC	+0.372 (0.042)	07.98
G × [apoE]	−0.037 (0.012)	00.85	ε2 × [apoE]	−0.124 (0.027)	02.09
BMI	+0.024 (0.010)	00.53	ε4 × [apoE]	+0.032 (0.008)	01.59
ε4 × [apoE]	+0.023 (0.011)	00.39	G × [apoE]	−0.104 (0.030)	01.13
			ε2 × BMI	+0.020 (0.006)	00.79
			G × WHR	+0.486 (0.178)	01.01
			WHR	+0.806 (0.357)	00.55
Total $R^2 \times 100$		53.62	Total $R^2 \times 100$		42.31

Regression coefficients (RC) and percentage of variance explained ($R^2 \times 100$) for men (508 subjects) and for women (550 subjects). Table represents the significant effects only. Independent variables: polymorphisms of LPL and apoE, [apoE], age, alcohol consumption, OC used, BMI, and WHR. X × Y represents the interaction of these two factors. All subjects, even those with rare genotypes, are included. However, subjects with no data for use of oral contraceptives and consumption of alcohol are excluded.

ApoE levels were strongly linked with TG levels and accounted for 34.66% of their variance. Genotype ε3ε4 was associated with elevated TG levels and accounted for 0.38% of their variance. ε3ε2 and CG genotypes were associated with low TG levels and accounted for 2.44% and 0.65% of their total variance, respectively. Therefore, independent of its concentration, apoE influenced TG by its polymorphism. Regression analysis (Table 5) was also performed to estimate the percentage of phenotypic variation, for other lipid parameters, that was determined by genetic variation and by apoE levels. Total variance values for Tchol, LDLc, apoB, and apoA-I were 1.10, 0.14, 0.68, and 0.15, respectively. Variation in apoE concentration and polymorphism accounted for 26.07%, 17.38%, 20.71%, and 0.43% of the variation in Tchol, LDLc, apoB, and apoA-I, respectively. The variances of these parameters were not influenced by (C/G) LPL^{447} polymorphism.

Gender had a significant effect on all lipid levels, reflecting the usual finding that men have higher Tchol, LDLc, apoB and TG levels and lower HDLc and apoA-I levels than women in the general population. Other findings are presented in Table 5. BMI was linked with a high concentration of TG (5.63% of total variance), LDLc, apoB, apoE levels, and LDLc/HDLc ratio. Age was associated with a high concentration of Tchol, LDLc, apoA-I, and apoB.

Table 6 presents multiple regression analysis of TG levels for men and women separately. In men, the total variance of TG was 0.68 and 53.62% of this variance was explained by apoE levels (40.27%), WHR (6.81%), interaction ε2 × [apoE] (4.77%), G × [apoE] (0.85%), BMI (0.53%), and ε4 × [apoE] (0.39%). The unexplained portion of total variance was 46.38%. In women, the total variance of TG was 0.26 and 42.31% of this variance was explained by apoE levels (17.94%), BMI (9.23%), OC use (7.98%), ε2 × [apoE] (2.09%), ε4 × [apoE] (1.59%), G × [apoE] (1.13%), ε2 × BMI (0.79%), G × WHR (1.01%), and WHR (0.55%). The unexplained portion of total variance was 57.69%.

DISCUSSION

Apolipoprotein and lipid levels may be determined by gene–gene or gene–environment interactions. The allelic variation at a locus can influence a biological trait and might be modulated by the effect of another polymorphic gene. Studies of gene × gene combination and gene × environmental interaction could explain a part of the variability of a quantitative trait or the prevalence of a disease better than the study of one single gene. Therefore, we studied the combined effects of apoE and LPL polymorphisms.

LPL and apoE are known to be rate-determining proteins in the catabolism of lipoproteins, significantly influencing the metabolism of both plasma triglycerides and plasma cholesterol levels. Both genes contribute to the multifactorial control of lipoprotein metabolism. Because of their involvement in lipid metabolism, the LPL and apoE genes are considered to be important candidate genes for cardiovascular risk (30, 37–40). Numerous population studies have reported the effects of apoE alleles and LPL^{447} (C/G) alleles on lipid concentrations.

In this work we have confirmed the average effect of the ε4 allele to increase significantly the concentrations of Tchol, apoB, LDLc, and TG levels and the average effect of the ε2 allele to decrease these constituents and to increase the apoE levels (1). The association of apoE polymorphism with Tchol, apoB, LDLc levels can be explained by the receptor-mediated pathway (41). We have also confirmed the effect of the (C/G) LPL^{447} polymorphism on TG levels. Individuals with the G allele presented lower TG levels than subjects with the C al-

lele. The functional significance, if any, of the LPL446 isoform remains undefined. A higher frequency of the G allele in controls than in hypertriglyceridemic subjects has been reported (24, 35). An increase of total LPL activity and protein mass in the presence of the truncated form has been reported in vitro (42, 43), even when specific LPL activity was moderately diminished (43), which is in agreement with a TG-lowering effect of the mutant allele (44).

A possible increase of the enzyme activity of the LPL446 form accompanied by a down-regulation of LDL receptor in the case of the ϵ4 allele (41) might result in an increase of LDL in the subgroup of G/ϵ4 individuals. Steady-state concentrations of LDL depend on rates of conversion of VLDL to LDL and the fractional catabolic rate of LDL by specific receptors. Both LPL and apoE proteins could theoretically be related to these processes. Usually, about two-thirds of VLDL remnants are taken up by the liver, and one third goes to LDL (45). The VLDL conversion to LDL depends on LPL activity. A possible increase in lipolytic activity by LPL446 could accelerate this conversion. Interestingly, apoE is implicated in this pathway by its differential affinities for the LDL-receptor depending on the three isoforms (E2, E3, E4). ApoE4 isoform presents a higher affinity for LDL receptors resulting in high binding of lipoproteins, and, therefore, in down-regulation of LDL receptors (41) due to the high cholesterol cellular content, and consequently to high LDL, Tchol, and apoB levels in serum. However, LDL levels in the carriers of the ϵ4 allele were not changed when we considered G allele, which does not agree with an elevation of LPL activity in the presence of this mutation. Moreover, observations in vitro (24, 46, 47) showed or little no diminution effect of this mutation on LPL activity. As the above hypothesis could not explain the results observed here, another hypothesis is proposed. The decrease of TG (with G allele) could be the consequence of an increased rate of clearance of triglyceride-rich lipoproteins by receptor-mediated pathways. ApoE associated with VLDL or other lipoproteins serves as a ligand for the LDL receptor, LDL receptor related protein (LRP), lipolysis-stimulated receptor, VLDL receptor, and human apolipoprotein E receptor 2 (apoE R2) (2–5,48–51).

As in other studies (52, 53), our results show that apoE concentration was very significantly correlated to TG levels. These results could be related to LPL metabolism. Previously, Gomez-Coronado et al. (29) observed that apoE-rich VLDL are less hydrolyzed by LPL than apoE-poor VLDL. Binding of LPL to VLDL is required for hydrolysis of TG anchored into these lipoproteins. VLDL enrichment by apoE could inhibit the enzyme binding or the correct association with these lipoproteins. ApoE has been found to exhibit inhibitory activity to LPL (54, 55).

As the preferential location of apoE4 isoform is in VLDL (41, 56–59), apoE4 isoform could influence VLDL metabolism and therefore TG levels. In fact, a large fraction of apoE4 is found in VLDL. Therefore, we propose that the apoE concentration and polymorphism could physico-chemically interact with LPL for their effects on triglycerides.

Men and women were analyzed separately in the last part of this study. Men had higher mean TG levels than women. This result is in agreement with other works (60, 61). Recently, Li et al. (61) have shown that men and postmenopausal women have significantly higher levels of TG than premenopausal women, and suggested that higher estrogen levels in premenopausal women may play a significant role in this difference. In conclusion, this study, using a large sample of healthy subjects, confirms that the ϵ4 allele is associated with increased LDLc, apoB, and Tchol levels. One important finding of this study is the clear independent effects of LPL and apoE polymorphisms, in interaction with apoE levels, on triglycerides. By adding these three parameters to classical factors regulating TG, we accounted for 42.31% and 53.62% of total variance of TG levels in women and men, respectively. We have shown that combined ϵ4/C alleles and high apoE levels were associated with high TG levels in addition to high Tchol, LDLc, apoB levels, and high ratio of LDLc/HDLc.

This study gives an example of the complexity of multiple genetic and environmental effects. Such linkage needs to be clarified for better understanding of the inter-individual variability of lipids and for determination of the genetic susceptibility of an individual to cardiovascular risk. In addition to the genes considered here, other candidate genes should be taken into account in the future, using as large a sample as the Stanislas Cohort.

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