Associations of apolipoprotein E exon 4 and lipoprotein lipase S447X polymorphisms with acute ischemic stroke and myocardial infarction

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Abstract

Background: Because apolipoprotein E (apoE) and lipopoprotein lipase (LPL) polymorphisms interact with each other and with other factors to affect lipid metabolism, we sought to determine their separate and combined effects in association with ischemic vascular disease.

Methods: We performed a case-control study of 816 subjects: 246 acute ischemic stroke patients, 234 acute myocardial infarction patients, and 336 controls. *APOE* exon 4 and *LPL S447X* genotypes were determined.

Results: *APOE ε2* and *ε4* homozygotes were increased in stroke (4.5% vs. 1.0%, p = 0.008), while in myocardial infarction the *ε4* allele was increased (12.6% vs. 9.5%, p = 0.006) but *ε2* was decreased (3.7% vs. 12.1%, p = 0.000006). For subjects with either *APOE ε2* or *ε4* alleles, *LPL X* alleles were increased in vascular disease (OR = 2.2, p = 0.01). *LPL X* alleles displayed opposite tendencies toward association with disease when subjects were divided by sex, smoking, or *APOE* genotype. Meta-analysis and regression analysis of previous studies supported the sex and smoking dichotomies.

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Conclusion: This is the first report of an association of vascular disease with an interaction of *APOE* exon 4 and *LPL S447X* genotypes. Therefore, *APOE* genotypes and *LPL S447X* interactions with apoE, sex, and smoking may affect the risk of myocardial infarction and ischemic stroke.

Keywords: apolipoprotein E; ischemic; lipoprotein lipase; polymorphism; smoking.

Introduction

Polymorphisms in the lipid metabolic genes apolipoprotein E (*APOE*) and lipoprotein lipase (*LPL*) have been associated with ischemic cerebrovascular and cardiovascular disease (1–14). A polymorphism in exon 4 of *APOE* has three common alleles – *ε2*, *ε3*, and *ε4* – coding for E2, E3, and E4 isoforms, respectively. *APOE ε4* increases serum cholesterol and vascular disease risk, while *apoE ε2* lowers cholesterol levels (8–12, 15). LPL helps to clear triglyceride (TG)-rich particles from the circulation (16, 17). The LPL S447X polymorphism, which truncates two C-terminal amino acids from the LPL protein, is associated with increased LPL protein secretion and plasma postheparin activity, as well as decreased plasma TG (17–20). The *X* allele has been associated with reduced risk of vascular disease (1–3, 5–7, 17).

Because apoE and LPL polymorphisms interact with each other to affect TG and high-density lipoprotein-cholesterol (HDL-C) levels (19, 20), as well as interacting with other factors such as sex or smoking to influence lipid levels (5, 14, 18–22), we conducted a case-control study of these genetic and clinical factors in acute ischemic stroke and acute myocardial infarction (MI) patients. Associations suggested by the resulting data led us to analyze previous studies for confirmation or refutation of these results.

Materials and methods

Subjects

Research with humans was carried out according to the principles of the Declaration of Helsinki. The hospital's institutional Review Board approved the study, and patients or their relatives gave informed consent. All subjects were Hong Kong Chinese. Following admission to the acute stroke unit of a general regional hospital, the Prince of Wales Hospital, Shatin, Hong Kong, 246 consecutive acute ischemic stroke patients were recruited from January 2002 to November 2003. Patients were hospitalized for cerebral infarction

within 7 days of symptom onset. Brain computed tomography (CT) was performed within 24 h of admission. CT evidence of stroke was a requirement for inclusion in the study. Patients with past history or CT feature of primary intracerebral hemorrhage were excluded. In addition, magnetic resonance imaging was performed for 90% of patients. Ultrasound carotid duplex, extracranial and transcranial Doppler (TCD) ultrasound, and electrocardiography were performed routinely for cerebral infarction patients at this hospital. Patients were assessed by a neurologist to confirm the diagnosis of stroke.

From community centers for the elderly, 225 elderly people (aged 65–74 years) were recruited, originally for a study of the effects of exercise. Subjects with known cardiovascular disease or stroke, dementia, uncontrolled/newly diagnosed hypertension, or impaired mobility were excluded from the controls, as were those who were regularly performing exercise. An additional 111 elderly controls (aged at least 60 years) without known cardiovascular disease, stroke, or dementia were recruited from outpatient clinics of the Prince of Wales Hospital and United Christian Hospital.

Also recruited were 234 acute MI patients admitted to the coronary care unit of the Prince of Wales Hospital between 1998 and 2002. Patients were not excluded on the basis of age or sex. Diagnostic criteria for acute MI were as follows: characteristic clinical presentation of precordial pain for more than half an hour; pathological Q wave, ST elevation or T wave inversion in the electrocardiograms, with subsequent evolutionary changes; and an unequivocal rise in the aspartate aminotransferase (SGOT) level to over 45 IU/mL on one of the three successive days, with the highest level at least twice the lowest one in those with borderline elevation.

Age, sex, serum lipid concentrations, and vascular risk factors, including hypertension, diabetes mellitus, and smoking habit, were collected from controls or during acute admission for patients. Status as a current, former, or never smoker was determined by asking each subject for his/her self-assessment. To measure lipids, fasting blood samples were taken from patients the day after admission or from controls. Hypertension was defined as systolic blood pressure (SBP) >140 mm Hg or diastolic blood pressure (DBP) >90 mm Hg on at least two occasions, or treatment with blood pressure-lowering medication. Diabetes was defined as fasting plasma glucose >7.0 mmol/L. Levels of TG (enzymatic method without glycerol blanking), total cholesterol (TC) (enzymatic method), and HDL-C (dextran sulfate-magnesium chloride precipitation) were measured on a Hitachi analyzer (Hitachi, Tokyo, Japan), and low-density lipoprotein-cholesterol (LDL-C) was calculated using Friedewald's formula, except when TG was greater than 4.5 mmol/L. The precision performance of these assays was within the manufacturer's specifications. Blood pressure was measured in stroke patients after they had settled in the wards. Data were not available on passive smoking for all subjects or on SBP and DBP for MI patients.

Cholesterol and blood pressure are typically reduced following MI (23, 24). In addition, many coronary artery disease (CAD) patients took lipid-lowering statins, and some non-hypertensive CAD patients took CAD drugs that may also lower blood pressure (β-blockers and nitrate). Therefore, we did not use blood pressure or lipid data gathered from MI patients.

Analysis

LPL gene exon 9 was amplified by polymerase chain reaction (PCR) using an annealing temperature of 55°C and primers

TATTCACATCCATTTTCTTC and GTCAGCTTTAGCCCAGAA-TG (25). MnlI restriction-enzyme digestion overnight at 37°C and separation by 12% polyacrylamide gel electrophoresis revealed fragments of 28 and 127 bp for the *447X* allele, allowing determination of the three possible genotypes (*SS*, *SX*, *XX*).

The fourth exon of *APOE* was amplified by a two-step nested PCR using primers as previously described (26). The six possible *APOE* genotypes (*ε2/ε2*, *ε2/ε3*, *ε2/ε4*, *ε3/ε3*, *ε3/ε4*, *ε4/ε4*) were determined by digestion with HhaI restriction enzyme digestion overnight at 37°C and separation by 12% polyacrylamide gel electrophoresis as previously described (27).

SPSS version 11.5 and Epi6 (World Health Organization, Geneva, Switzerland) were used for data analysis. Differences in continuous variables were examined using Student's t-test or (for Table 4) ANCOVA. Because the distribution of TG levels was skewed, $\log_{10}TG$ was used for comparisons. χ^2 Analysis was used to compare distributions of categorical variables. p-Values were not corrected for multiple comparisons, and therefore readers should interpret results with due caution until confirmed by other studies.

For multiple logistic regression, factors with the highest p-value or which caused deviation from a good fit (i.e., $p < 0.05$ in the Hosmer and Lemeshow test) were removed until a value of $p < 0.1$ was reached for the remaining factors. Factors that were then used for further adjustments shown in Table 5 are listed in the legend to Table 5.

For meta-analysis, all published case-control studies of ischemic heart disease or stroke were collected by searching PubMed using the keywords lipoprotein lipase, polymorphism, and any of the following: stroke, cardiovascular, infarction, and cerebrovascular. Relevant articles identified were also examined for citations to relevant case-control studies. Meta-analysis was performed using the random effect model of DerSimonian and Laird, with odds ratios weighted by the number of subjects in each group.

Results

Patient characteristics

Mean age was matched between stroke patients and controls, although MI patients were younger (Table 1). Stroke patients were more often male, current smokers, and had higher TG, more diabetes, and higher blood pressure than controls. MI patients were more often male, current smokers, or former smokers than were controls.

Genotypes

Stroke, MI, and control groups were tested for Hardy-Weinberg equilibrium. For each subject group, both *APOE* and *LPL* genotypes were in equilibrium.

In stroke, examination of combinations of genotypes suggested that *APOE ε2* and *ε4* homozygotes were increased (Table 2). MI patients had a different distribution, with more *ε4* and fewer *ε2* alleles. Among subjects with hypertension, *ε4* alleles were increased in both stroke and MI: p=0.008, OR=2.2 (1.2–4.2). Both sexes exhibited similar allele associations with MI, with *ε4* tending to be positively associated with MI in men (OR=1.8, p=0.04) and women (OR=1.9, p=0.10), and *ε2* tending toward a negative

Table 1 Characteristics of stroke, control, and MI subjects.

	Stroke (n = 246)	p	Controls (n = 336)	p	MI (n = 234)
Age, years	70.7 ± 12	0.24	71.0 ± 5.9	<0.001	58.3 ± 9.6
Men, %	54.5	0.03	45.2	<0.001	81.6
SBP, mm Hg	166 ± 28	<0.001	142 ± 20		–
DBP, mm Hg	85.4 ± 19	<0.001	71.9 ± 13		–
Hypertension, %	65.9	0.20	60.2		–
Diabetes, %	30.5	<0.001	17.5	0.99	17.4
Former smoker, %[a]	21.1	0.61	26.3	<0.001	43.5
Current smoker, %[a]	22.7	<0.001	11.3	<0.001	28.7
TC, mmol/L	5.58 ± 1.14	0.32	5.47 ± 0.91		–
HDL, mmol/L	1.35 ± 0.36	0.32	1.31 ± 0.34		–
LDL, mmol/L	3.48 ± 1.00	0.96	3.48 ± 0.81		–
TG, mmol/L[b]	1.55 (1.44–1.67)	<0.001	1.30 (1.22–1.39)		–

Values are mean ± SD, number of subjects, or percentage. –, no data. [a] p for χ^2 comparison with never smokers; [b] geometric mean of TG (95% confidence interval).

association with MI in men (OR = 0.3, p = 0.00002) and women (OR = 0.4, p = 0.11). Excluding $\varepsilon2/\varepsilon4$ carriers, lipid values or mean age did not differ between carriers of $\varepsilon3/\varepsilon3$ and either $\varepsilon2$ or $\varepsilon4$ in either stroke or control subjects, while in MI patients carrying $\varepsilon4$ compared to $\varepsilon3/\varepsilon3$, mean age was younger (55.2 vs. 59.0 years, p = 0.01).

LPL genotype and allele distributions did not differ between patients and controls (Table 3). However, opposite associations between *X* alleles and disease were observed in men vs. women, smokers vs. non-smokers, and *APOE* $\varepsilon3/\varepsilon3$ carriers vs. non-carriers (Table 3): men, OR = 0.75 (95% CI: 0.49–1.2), p = 0.18; women, OR = 1.7 (1.0–3.0), p = 0.04; current smokers, OR = 0.84 (0.30–2.7), p = 0.72; never smoked, OR = 1.3 (0.84–2.2), p = 0.20; *APOE* $\varepsilon3/\varepsilon3$, OR = 0.81 (0.54–1.2), p = 0.30; *APOE* non-$\varepsilon3/\varepsilon3$, OR = 2.2 (1.1–4.3), p = 0.01.

To compare the effects of smoking and *LPL X* alleles on vascular disease, odds ratios for disease were calculated relative to *LPL SS* never smokers: *X*+ never smokers, OR = 1.3 (0.84–2.2), p = 0.20; *SS* current smokers, OR = 3.5 (2.6–4.9), p < 0.00001; *X*+ cur-

rent smokers, OR = 3.0 (1.1–9.3), p = 0.02. Since most smokers were men, subjects were re-analyzed after segregating by smoking and sex. Among never smokers, the opposite odds ratios of men and women remained nearly the same, suggesting that the sex effect is not secondary to a smoking effect. There were too few current smokers to obtain reliable odds ratios after stratifying by sex and genotype. Although MI and stroke were associated with different *APOE* genotypes, both diseases tended to associate with the combination of *LPL X*+ and *APOE* non-$\varepsilon3/\varepsilon3$ genotypes.

Several parameters differed between carriers of *LPL X*+ and *SS* (Table 4). Among stroke patients, lipid levels did not differ significantly between *X*+ and *SS* carriers.

Logistic regression

In various subject groups, multiple logistic regression identified different variables associated with disease. These variables, listed in the legend to Table 5, were

Table 2 Distribution of *APOE* genotypes and alleles in stroke and MI patients and controls.

APOE genotype	Controls (n = 311)	Stroke (n = 243)	MI (n = 231)
$\varepsilon2/\varepsilon2$	2 (0.6%)	7 (2.9%)	0 (0.0%)
$\varepsilon2/\varepsilon3$	60 (19.3%)	39 (16.0%)	13 (5.6%)
$\varepsilon2/\varepsilon4$	6 (1.9%)	6 (2.5%)	4 (1.7%)
$\varepsilon3/\varepsilon3$	203 (65.3%)	155 (63.8%)	164 (71.0%)
$\varepsilon3/\varepsilon4$	39 (12.5%)	32 (13.2%)	46 (19.9%)
$\varepsilon4/\varepsilon4$	1 (0.3%)	4 (1.6%)	4 (1.7%)
		$\varepsilon2/\varepsilon2 + \varepsilon4/\varepsilon4$: p = 0.008, OR = 4.9 (95% CI 1.3–27)	$\varepsilon3/\varepsilon4 + \varepsilon4/\varepsilon4$: p = 0.007, OR = 1.9 (95% CI 1.2–3.0) $\varepsilon2/\varepsilon3 + \varepsilon2/\varepsilon2$: p = 0.000002, OR = 0.24 (95% CI 0.1–0.5)
Allele	Controls (n = 622)	Stroke (n = 486)	MI (n = 462)
$\varepsilon2$	70 (11.3%)	59 (12.1%)	17 (3.7%)
$\varepsilon3$	505 (81.2%)	381 (78.4%)	387 (83.8%)
$\varepsilon4$	47 (7.6%)	46 (9.5%)	58 (12.6%)
			$\varepsilon4$: p = 0.006, OR = 1.8 (95% CI 1.2–2.7) $\varepsilon2$: p = 0.000006, OR = 0.30 (95% CI 0.2–0.5)

Table 3   Distribution of *LPL S447X* genotypes and alleles: p-values and ORs (95% CIs) on the left are for *X+* genotypes, and on the right are for *X* alleles.

	n	Genotype			Allele	
		XX	XS	SS	X	S
Stroke patients	243	8 (3.3%)	44 (18.1%)	191 (78.6%)	60 (12.3%)	426 (87.7%)
MI patients	231	0 (0.0%)	51 (22.1%)	180 (77.9%)	51 (11.0%)	411 (89.0%)
All patients	474	8 (1.7%)	95 (20.0%)	371 (78.3%)	111 (11.7%)	837 (88.3%)
Controls	313	1 (0.3%)	64 (20.4%)	248 (79.2%)	66 (10.5%)	560 (89.5%)
		OR_{All}=1.1 (0.7–1.5), p=0.75			OR_{All}=1.1 (0.8–1.6), p=0.47	
Female, all patients	153	4 (2.6%)	29 (19.0%)	120 (78.4%)	37 (12.1%)	269 (87.9%)
Female, controls	176	0 (0.0%)	26 (14.8%)	150 (85.2%)	26 (7.4%)	326 (92.6%)
		OR=1.6 (0.9–2.9), p=0.11			OR=1.7 (1.0–3.0), p=0.04	
Male, all patients	320	4 (1.3%)	65 (20.3%)	251 (78.4%)	73 (11.4%)	567 (88.6%)
Male, controls	137	1 (0.7%)	38 (27.7%)	98 (71.5%)	40 (14.6%)	234 (75.4%)
		OR=0.69 (0.43–1.1), p=0.11			OR=0.75 (0.49–1.2), p=0.18	
Current smoker, all patients	117	0 (0.0%)	17 (14.5%)	100 (85.5%)	17 (7.3%)	217 (92.7%)
Current smoker, controls	35	0 (0.0%)	6 (17.1%)	29 (82.9%)	6 (8.6%)	64 (91.4%)
		OR=0.82 (0.28–2.8), p=0.71			OR=0.84 (0.30–2.7), p=0.72	
Ever smoker, all patients	262	2 (0.8%)	50 (19.0%)	210 (80.2%)	54 (10.3%)	470 (89.7%)
Ever smoker, controls	114	1 (0.9%)	25 (21.9%)	88 (77.2%)	27 (11.8%)	201 (88.2%)
		OR=0.84 (0.48–1.5), p=0.52			OR=0.86 (0.51–1.5), p=0.53	
Never smoked, all patients	196	5 (2.6%)	40 (20.4%)	151 (77.0%)	50 (12.8%)	342 (87.2%)
Never smoked, controls	198	0 (0.0%)	39 (19.7%)	159 (80.3%)	39 (9.8%)	357 (90.2%)
		OR=1.2 (0.7–2.0), p=0.43			OR=1.3 (0.84–2.2), p=0.20	
APOE ε3/ε3, all patients	318	5 (1.6%)	58 (18.2%)	255 (80.2%)	68 (10.7%)	568 (89.3%)
APOE ε3/ε3, controls	199	1 (0.5%)	49 (24.6%)	149 (74.9%)	51 (12.8%)	347 (87.2%)
		OR=0.74 (0.47–1.2), p=0.15			OR=0.81 (0.54–1.2), p=0.30	
APOE non-*ε3/ε3*, all patients	154	3 (1.9%)	36 (23.4%)	115 (74.7%)	42 (13.6%)	266 (86.4%)
APOE non-*ε3/ε3*, controls	104	0 (0.0%)	14 (13.5%)	90 (86.5%)	14 (6.7%)	194 (93.3%)
		OR=2.2 (1.1–4.6), p=0.02			OR=2.2 (1.1–4.3), p=0.01	

used to adjust the association of genotypes with disease (Table 5). The association of *APOE* genotypes with MI remained highly significant after adjustment. The odds ratio for association of *APOE* genotypes with stroke was even greater than that for MI, although this association lost its significance after adjustment, perhaps because of the small number of subjects with *ε2/ε2* or *ε4/ε4*. The associations of *LPL X+* genotypes with vascular disease in women, never smokers, and *APOE* non-*ε3/ε3* genotypes were somewhat less significant after adjustment, although the association among *APOE* non-*ε3/ε3* genotypes remained significant.

Interactions of genotypes with other factors were included in the multivariate models of genotypes and clinical characteristics for stroke and for MI. No significant interactions were associated with MI. *LPL* genotype interacted with never smoking and with hypertension to associate with stroke.

Meta-analysis

We combined results of published case-control studies of ischemic heart disease or stroke to attempt to confirm the tendencies we observed toward opposite associations between *LPL* alleles and disease in men vs. women and in smokers vs. non-smokers. In men, *X* alleles were decreased in disease: OR=0.83 (95% CI 0.73–0.93), p=0.002 (Figure 1). *XX* genotypes displayed a non-significant tendency toward a greater reduction: OR=0.67 (0.41–1.09, p=0.10). However, in

Table 4   Comparison of various measures in *LPL X+* vs. *SS* carriers in subsets of subjects.

Test	Subjects	Measure	LPL X+	p	LPL SS
a	MI	Age, years	61.1±9.2	0.04	57.2±9.1
b	Stroke	NIH Stroke Scale	5.3±3.9	0.02	7.7±6.4
c	Control	HDL, mmol/L	1.41±0.29	0.02	1.29±0.36
d	Control	LDL, mmol/L	3.69±0.81	0.05	3.43±0.80
e	Control	TG, mmol/L	1.09 (0.96–1.22)	0.01	1.35 (1.25–1.46)
f	Control: men	TG, mmol/L	1.10 (0.93–1.30)	0.13	1.40 (1.21–1.63)
g	Control: women	TG, mmol/L	1.06 (0.88–1.28)	0.03	1.31 (1.20–1.44)
h	Control: *APOE ε3/ε3*	TG, mmol/L	1.07 (0.93–1.24)	0.05	1.32 (1.19–1.47)
i	Control: *APOE* non-*ε3/ε3*	TG, mmol/L	1.14 (0.87–1.49)	0.10	1.40 (1.22–1.60)

Mean±SD are shown for rows (a)–(d), and geometric mean (95% confidence interval) are shown for rows (e)–(i). p-Values were determined by ANCOVA after adjusting for the following factors: a) sex, diabetes, hypertension, and smoking, coded as 1 for never smoker, 2 for ex-smoker, and 3 for current smoker; b) age, sex, diabetes, hypertension, SBP, DBP, and smoking coded as above; c) age, sex, diabetes, hypertension, SBP, DBP, TC, LDL, and smoking coded as above; d) age, sex, diabetes, hypertension, SBP, DBP, HDL, log_{10}TG, and smoking coded as above; e–i) age, sex, diabetes, hypertension, SBP, DBP, TC, LDL, and smoking coded as above.

Table 5 Logistic regression analysis.

Genotype	Subjects	Adjusted OR (95% CI) for disease	p
a) *APOE*	Stroke and control	3.0 (0.6–16) for stroke	0.19
b) *APOE*	MI and control	2.4 (1.7–3.6) for MI	0.000005
c) *LPL X+*	Women	1.4 (0.7–2.5) for stroke or MI	0.3
d) *LPL X+*	Never smokers	1.1 (0.6–1.8) for stroke or MI	0.8
e) *LPL X+*	*APOE* non-$\varepsilon3/\varepsilon3$	2.2 (1.1–4.4) for stroke or MI	0.03
f) Never smoker$\times$*LPL X+*	Stroke and control	3.2 (1.3–7.9)	0.02
Hypertension$\times$*LPL X+*		0.42 (0.17–1.0)	0.05

In subsets of subjects, multiple logistic regression was used to find factors associated with disease at $p < 0.1$. These factors were then used to adjust the associations of genotypes with diseases. The following factors were used: a) age, SBP, DBP, diabetes, current smoking, and $\log_{10}$TG, for *APOE* $\varepsilon2/\varepsilon2 + \varepsilon4/\varepsilon4$; b) sex, current smoking, and former smoking, for *APOE* coded as −1 for $\varepsilon2/\varepsilon2$ or $\varepsilon2/\varepsilon3$, 0 for $\varepsilon2/\varepsilon4$ or $\varepsilon3/\varepsilon3$, and 1 for $\varepsilon4/\varepsilon4$ or $\varepsilon3/\varepsilon4$; c) diabetes, current smoking, and former smoking; d) sex and diabetes; e) sex and current smoking; f) age, SBP, DBP, diabetes, current smoking, and $\log_{10}$TG.

women, neither *X* alleles ($p = 0.52$) nor *XX* genotypes ($p = 0.63$) showed a negative association with disease. Since most studies did not present genotype results separately by sex, we attempted to gain further information by using regression of weighted *X* allele odds ratios vs. the proportion of males for all studies, including this report, with studies divided into independent male and female parts when possible. The slope of the regression was −0.16 (OR/proportion male), $p = 0.15$.

Since no papers presented genotype results separately by smoking status, we used regression analysis to compare weighted *X* allele odds ratios against proportion of current smokers (Figure 2) (4, 28–32). The slope of the regression was −0.88 (OR/proportion smokers), $p = 0.02$. Using the regression line, the predicted odds ratio was 0.4 for current smokers and 1.3 for never smokers.

Discussion

Our findings are consistent with reports of a positive association of the *APOE* exon 4 $\varepsilon4$ allele with MI, but only some studies reported a significant negative association between MI and $\varepsilon2$ (9, 15). ApoE, as a constituent of very low-density lipoprotein particles, binds LDL receptor (LDLR), LDLR-related protein (LRP), and other receptors, thus aiding the catabolism of TG-rich lipoproteins and cholesterol homeostasis (8, 9). ApoE E2, the protein isoform coded by the $\varepsilon2$ allele, binds LDLR poorly, which may increase the number of LDLRs, thereby lowering cholesterol levels (8, 10). The E4 form exhibits enhanced transfer from HDL to TG-rich lipoproteins, accelerating hepatic remnant clearance by apoE receptors and decreasing LDLR numbers, thereby increasing cholesterol levels (8, 10).
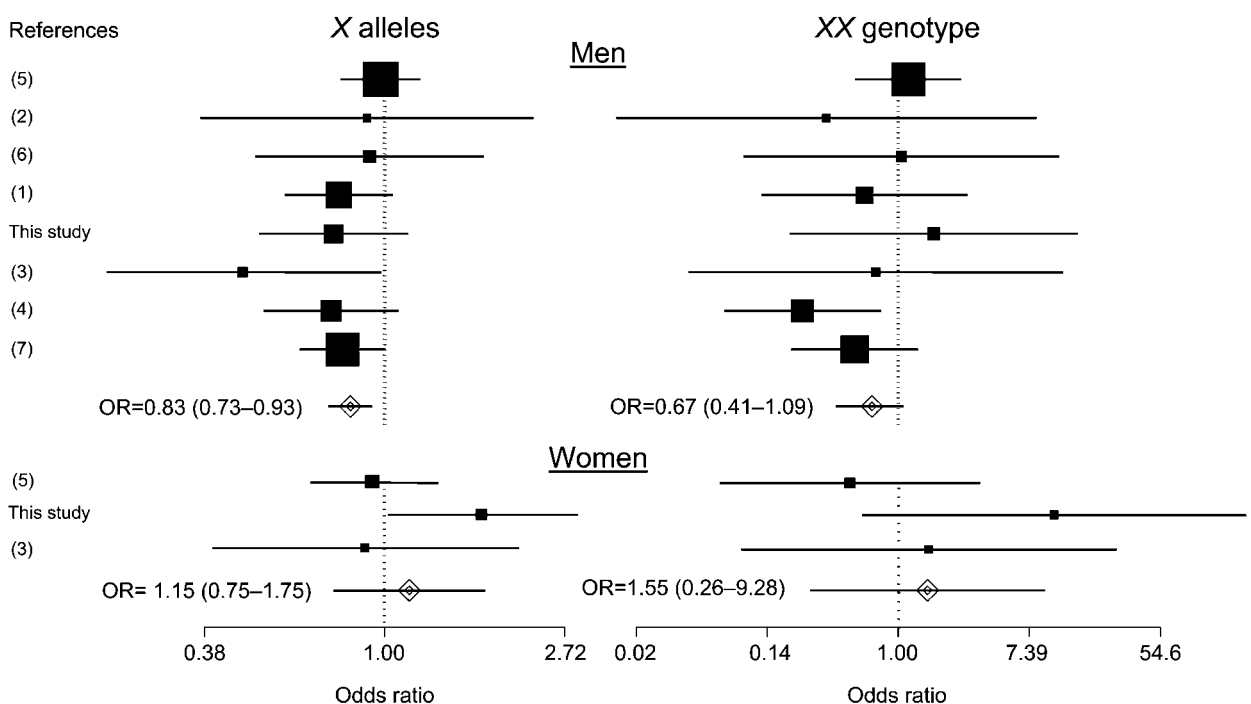

Figure 1   Meta-analysis of LPL. Meta-analysis of the association of *LPL* alleles and genotypes with vascular disease in men (top) and women (bottom).
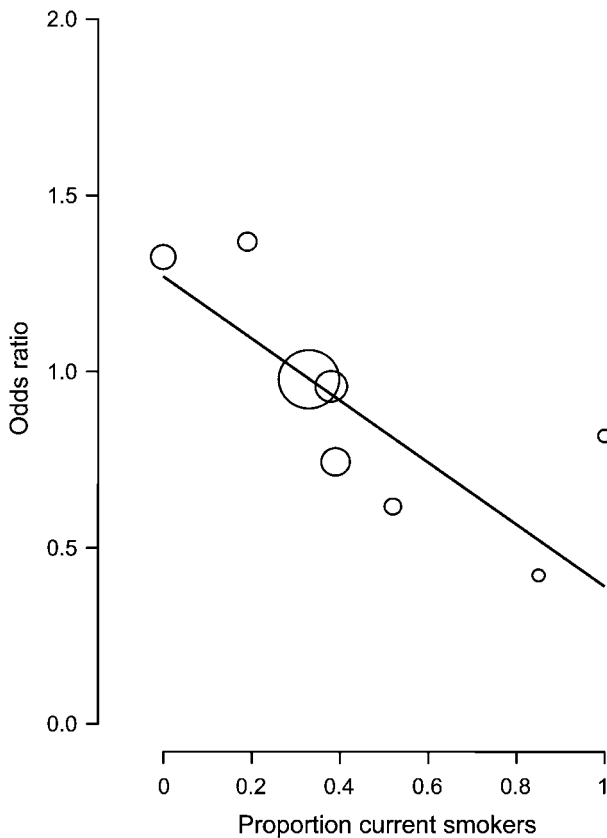
Figure 2 Regression of smoking and *LPL*. Regression analysis for association of *LPL X* alleles with vascular disease by proportion of current smokers among patients in published studies (4, 28–32). Circle area is proportional to the number of subjects in the study. Regression: slope −0.88, p=0.02.

The greater stroke frequency we observed not only for $\varepsilon 4$, but also for $\varepsilon 2$ homozygotes has not been generally reported, perhaps because of the rarity of the $\varepsilon 2/\varepsilon 2$ genotype (11). One possibility is that our observation is due to chance, since the p-value we observed (0.008) would not meet a Bonferroni-corrected significance level of 0.0016 (0.05 divided by 31, or the number of possible ways to dichotomize the six *APOE* genotypes). Meta-analysis of similar studies might provide the additional statistical power needed to confirm or refute this observation. If confirmed, a possible physiological explanation could be that the reduced receptor binding of E2 can increase circulating chylomicron remnant levels, especially after a meal (10), which might for unknown reasons increase the risk of stroke more than the risk of MI. Alternatively, the contrasting associations of $\varepsilon 2$ with MI and stroke might reflect the younger age of MI than stroke patients, rather than a difference in etiologies. In a study comparing young and old CHD patients, $\varepsilon 4$ was increased in young patients, while $\varepsilon 2$ was increased in old patients (12).

Among subjects with hypertension, we found an increased risk of stroke or MI associated with the $\varepsilon 4$ allele. It may be speculated that the combination of hypertension and $\varepsilon 4$ has a multiplicative effect, because these two risk factors might act through different pathogenic mechanisms.

The current results associated *LPL X+* carriers with several factors that suggested protection from vascular disease: lower TG, delayed age of MI, and less severe strokes. In meta-analysis, *X* alleles were indeed significantly negatively associated with vascular disease, although our own data showed associations of *X* alleles with vascular disease only in subsets of subjects. In a meta-analysis that expanded on one performed 3 years ago (5), *X* alleles were significantly negatively associated with stroke or cardiovascular disease in men, but not in women. It is possible that our result for women is an outlier that appears to eliminate the protective effect of *X* alleles. However, if the sexual divergence is confirmed in further studies, then our failure, and that of some other studies, to see a significant effect of *X* in all subjects (combined sexes) may be due to a small sample size or to the mix of subjects. For example, a largely male study population may yield a significant effect, while studies with a high proportion of women may not.

In multiple logistic regression analysis, we observed interactive associations of *LPL X+* genotypes with stroke that may give insight into the role of this polymorphism in vascular disease. *X+* genotypes in conjunction with hypertension appeared to be protective, but *X+* in conjunction with never smoking appeared to be harmful. The effects of these two interactions could be interpreted in a consistent way: *X+* protecting those at high risk of ischemic vascular disease, such as smokers or those with hypertension or other risk factors, and conversely harming those at low risk. The apparent protective effect of *X+* in men but not women might reflect the fact that men generally have greater risk of ischemic vascular disease.

Although *X+* genotypes showed decreased TG levels in both female and male controls, the lack of a protective effect against vascular disease in women might be due to the lower TG levels in women than men, in both genotypes (19), with reduced benefit to women from any further TG-lowering by *X+* genotypes. An alternative explanation could be that the *X* allele raises LPL activity, and that the net effect of *X* on vascular risk is a balance between the resulting reduction in circulating TG (good) and the accelerated atherosclerosis caused by increased LPL expression by macrophages in plaques (bad) (16). Estrogen increases LPL expression by macrophages, thus perhaps pushing the balance relatively more toward the harmful end in women (33). However, estrogen also increases HDL levels (34), which might counter this harmful effect.

This is the first report of an association of vascular disease with an interaction of smoking and *LPL S447X* genotype. Although the type of regression analysis that revealed this interaction may be less powerful than meta-analysis (as shown, for example, by the lack of significance in regression of sex vs. odds ratio of disease with *X*, despite the significant effect revealed by the corresponding meta-analysis), the fact that regression, despite its weakness, did reveal a significant interaction of smoking with *LPL S447X* genotype suggests that this effect may be strong. In

general, regression may be a useful tool for examination of published studies for potential association of disease with a factor that was not stratified.

The association with vascular disease increased in the order *SS* non-smoker<*X+* non-smoker<*X+* smoker<*SS* smoker. A similar interaction order was reported for the *LPL* D9N polymorphism: *N+* non-smoker<*DD* non-smoker<*DD* smoker<*N+* smoker (14). N9 and S447 reduce LPL activity and raise TG levels (14, 17–19). Smoking decreases LPL activity and raises TG (21), perhaps lowering risk by reducing endothelial-located, pro-atherogenic LPL and raising risk by reducing non-endothelial, anti-atherogenic LPL (16). Thus, polymorphisms may affect risk via the latter effect in smokers and the former in non-smokers, resulting in opposite effects of genotypes in smokers vs. non-smokers. N9 LPL binds more LDL, and thus may recruit oxidized LDL and monocytes to smoking-induced endothelial lesions, exacerbating atherosclerosis in smokers, although why N9 reduces risk in non-smokers remains unexplained (14, 16). To exclude the possibility that sex confounds these smoking interactions (because most smokers are men), subjects can be analyzed separately by smoking and sex, but our study had too few current smokers to do so reliably.

This is the first report of an association between vascular disease and an interaction of *APOE* exon 4 and *LPL S447X* genotypes. The greater odds ratio with the *APOE* non-*ε3/ε3* and *LPL X+* combination did not correspond to a greater TG level in ours or a previous study (20). It is not clear why both *APOE ε2* and *ε4* alleles would induce similar effects, but perhaps the same destination is reached via different routes. ApoE protein levels and LPL activity are higher in *ε2* carriers, and LDL-C is raised in *ε4* carriers (8, 18, 20). The combination of either *ε2* or *ε4* with the increased LPL activity in *X* carriers (17, 18, 20) might bring more oxidized LDL and macrophages into atherosclerotic plaques (16). LPL from macrophages can be atherogenic, perhaps by binding lipoproteins to sites of endothelial damage, converting TG-rich lipoproteins into remnants and creating toxic products of lipolysis, or affecting macrophage function (16, 35). However, apoE expression protected against atherosclerosis induced by macrophage-specific LPL in mice (22), and thus the effect of *APOE ε2* in our study might not be due to increased apoE protein levels, but rather due to another factor, such as delayed remnant clearance (15).

This study has limitations. Lack of body mass index or lipoprotein(a) data precluded adjusting for these factors in multivariate logistic regression. Multiple comparisons may produce false-positive associations. Rather than reducing the chance of their occurrence by Bonferroni correction of significance levels, we chose to present the data and let readers interpret the results with appropriate caution, allowing new associations to be uncovered that may otherwise remain hidden. However, it will be necessary to confirm or refute these findings in independent populations in studies performed by other investigators.

Limitations for the MI portion of the study include lack of some data for MI patients, and a control population older than the MI patients. This lack of age matching is a possible source of bias. Another limitation is that the study design is case-control, which may be prone to bias in subject selection and matching. On the other hand, case-control studies provide good statistical power compared to prospective studies of similar size. Ideally, findings of case-control studies will be confirmed or refuted by prospective studies.

Because S447X is the most common LPL coding polymorphism, the effects of interactions with smoking or the apoE exon 4 polymorphism on population risk would be great, and should spur authors of previous studies of this polymorphism and vascular disease to stratify their results by smoking status, sex, and *APOE* genotype to confirm or refute our findings. However, when stratified data are absent, regression can be attempted as an alternative method of combining results of multiple studies to discover interactions.

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