The lipoprotein lipase S447X polymorphism and plasma lipids: interactions with *APOE* polymorphisms, smoking, and alcohol consumption

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Abstract We studied 4,058 subjects from a representative sample of the Singapore population *1*) to determine the association between the S447X polymorphism at the *LPL* locus and serum lipid concentration in Chinese, Malays, and Asian Indians living in Singapore and *2*) to explore any interactions with apolipoprotein E (*APOE*) genotype, exercise, obesity, cigarette smoking, and alcohol intake. Information on obesity, lifestyle factors (including smoking, alcohol consumption, and exercise frequency), glucose tolerance, and fasting lipids was obtained. Male and female carriers of the X447 allele had lower serum triglyceride concentrations and higher HDL cholesterol (HDL-C) concentrations. The association between the X447 allele and serum HDL-C concentration was modulated by *APOE* genotype in males and cigarette smoking and alcohol intake in females. The effect of the X447 allele was greatest in men who carried the E4 allele and women who smoked or consumed alcohol. The X447 allele at the *LPL* locus is common and associated with a less atherogenic lipid profile in Asian populations. Interactions with *APOE* genotype, cigarette smoking, and alcohol intake reinforce the importance of examining genetic associations, such as this one, in the context of the population of interest.—Lee, J., C. S. Tan, K. S. Chia, C. E. Tan, S. K. Chew, J. M. Ordovas, and E. S. Tai. **The lipoprotein lipase S447X polymorphism and plasma lipids: interactions with *APOE* polymorphisms, smoking, and alcohol consumption.** *J. Lipid Res.* 2004. **45:** 1132–1139.

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LPL increases the uptake and catabolism of triglyceride-rich lipoproteins (TRLs) by hydrolyzing triglyceride (TG) in chylomicrons and VLDLs and also by serving as a "bridge" to mediate the binding of TRLs to the cell surface via heparan sulfate proteoglycans (1), leading to their uptake by a variety of receptors (2–5). In turn, this results in enhanced transfer of surface material from TRLs to HDL (6) and reduced cholesteryl ester transfer from HDL (7). In agreement with these in vitro findings, variation in plasma postheparin LPL activity has been found to be associated with changes in serum lipid concentrations, especially HDL cholesterol (HDL-C) (8–10), and the risk of coronary heart disease (CHD) (11).

Several polymorphisms at the *LPL* locus have been described that are associated with variations in LPL activity, serum lipid concentrations, and the risk of CHD (12). Of these, the *Hin*dIII polymorphism is the most common. The presence of the *Hin*dIII polymorphism is associated with increased LPL activity, lower serum TG concentration, and higher HDL-C concentration. Haplotype analyses suggest that the variations in serum lipid concentrations and the risk of myocardial infarction associated with the *Hin*dIII polymorphism are mediated through linkage disequilibrium with another polymorphism, the S447X polymorphism (13–15).

The S447X polymorphism results in the premature truncation of LPL (16). In vitro, this polymorphism is associated with increased levels of LPL secretion (17, 18). This translates to higher plasma postheparin LPL activity in vivo (19). A meta-analysis of studies carried out in Caucasian populations showed a significant 8% decrease in plasma TG and a 4% increase in HDL-C associated with the X447 allele (20). However, among populations of Asian ethnicity, the X447 allele showed borderline (21) or no (22) association with HDL-C concentration. It is possible that these latter studies may have failed to detect the

Abbreviations: ANCOVA, analysis of covariance; apoE, apolipoprotein E; BMI, body mass index; CHD, coronary heart disease; CV, coefficient of variation; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; TG, triglyceride; TRL, triglyceride-rich lipoprotein.

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associations between the X447 allele and serum lipid concentrations because they were underpowered. Alternatively, true differences in the associations between the S447X polymorphism and serum lipid concentrations between ethnic groups may result from differing prevalences of other factors involved in gene-gene and gene-environment interactions (23). In support of this hypothesis, significant interactions have been observed between the S447X polymorphism and other genetic [apolipoprotein E (*APOE*) polymorphisms] and environmental (cigarette smoking) factors (24). Other polymorphisms at this locus have also been shown to interact with smoking (25, 26), physical activity (27, 28), and obesity (29–31).

The aim of this study was *1*) to determine the frequency of the S447X polymorphism at the *LPL* locus and its association with serum lipid concentrations in an Asian population living in Singapore, and *2*) to examine the potential effect modification of these associations by interactions with polymorphisms at the *APOE* locus, cigarette smoking, alcohol intake, physical activity, and obesity.

MATERIALS AND METHODS

Subjects

The 1998 National Health Survey was a population-based survey carried out to assess the levels of risk factors for CHD in the Singapore population. The major findings and methodology have been previously reported in detail (32). Briefly, a representative sample of the Singapore population was identified through systematic sampling according to household types, followed by disproportionate stratified sampling, to give an ethnic distribution of 64% Chinese, 21% Malays, and 15% Asian Indians. A total of 4,723 individuals participated in the survey. Anthropometric data were collected, as were data on lifestyle factors (exercise and alcohol intake). The latter were collected using an interviewer-administered questionnaire. Subjects who smoked daily were classified as smokers. Frequent exercise was defined as three or more sessions per week each lasting at least 20 min. Subjects were considered to consume alcohol if they drank alcoholic beverages at least once a month. All subjects had fasting blood taken after a 10 h fast. Subsequently, those who were not on oral hypoglycemic agents or insulin were subjected to a 75 g oral glucose tolerance test. Diabetes mellitus was diagnosed in those who were on oral hypoglycemic agents or insulin or who had fasting plasma glucose $\geq$ 7.0 mmol/l or 2 h postchallenge glucose $\geq$ 11.1 mmol/l. All participants gave prior consent to the use of their blood samples for this research. Also, ethical approval was obtained from the Singapore General Hospital Institutional Review Board.

Laboratory methods

Total cholesterol [intra-assay coefficient of variation (CV), 0.8%; interassay CV, 1.7%], TG (intra-assay CV, 1.5%; interassay CV, 1.8%), and glucose (intra-assay CV, 0.9%; interassay CV, 1.8%) were measured on a BM/Hitachi 747/737 analyzer using the enzymatic colorimetric method. HDL-C (intra-assay CV, 2.9%; interassay CV, 3.6%) was measured using a homogenous colorimetric assay, whereas LDL cholesterol (LDL-C) (intra-assay CV, 0.9%; interassay CV, 2.0%) was measured using a homogenous turbidimetric assay.

Genetic analyses

Genotyping at the *APOE* and *LPL* loci was carried out by single nucleotide extension using the ABI Prism SNaPshot multiplex system (Applied Biosystems, Foster City, CA). *APOE* genotyping was carried out as previously described (33). In this study, carriers of the ε2 allele (2/2 and 2/3) were classified as E2, homozygotes for the ε3 allele (3/3) were classified as E3, and carriers of the ε4 allele (3/4 and 4/4) were classified as E4.

To determine the presence of the S447X polymorphism, a DNA fragment from the *LPL* locus was amplified using the following primer pairs: 5′-TAC ACT AGC AAT GTC TAG CTG AAG GCA GA-3′ (forward) and 5′-TCA GCT TTA GCC CAG AAT GCT CAC C-3′ (reverse). PCR amplification was carried out in a 10 μl reaction volume containing 0.1 mmol/l of each deoxy nucleoside triphosphate (dNTP), 1.5 mmol/l magnesium chloride, 0.4 μmol/l of each primer, and 0.06 U of Qiagen Hotstar Taq polymerase. PCR cycling conditions were 95°C for 12 min followed by 50 cycles of 95°C for 30 s, 65°C for 30 s, and 72°C for 40 s. A final extension phase of 5 min at 72°C was included at the end of the protocol. Subsequently, the PCR products were incubated for 90 min at 37°C followed by 15 min at 75°C (to inactivate the enzymes) with 5 U each of Exo I (USB Corp., Cleveland, OH) and calf intestinal phosphatase (New England Biolabs, Inc., Beverly, MA) to remove unincorporated primers and dNTPs. The single nucleotide polymorphism-specific probe 5′-CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC CAT GAC AAG TCT CTG AAT AAG AAG T-3′ was then annealed to the cleaned-up PCR product. In the presence of fluorescent-ddNTPs (each labeled with a different fluorescent dye) and DNA polymerase, a single base complementary to the polymorphic base in the targeted site of the PCR sample was extended at the 3′ end of the probe. This extension reaction was carried out in a 5 μl reaction volume that contained 2.5 μl of the SNaPshot Ready Reaction Mastermix, 0.5 μl of water, 1.5 μl of the mixed PCR products, and 1 μl of the probe. This was placed in a PCR thermocycler for 35 cycles of 96°C for 30s, 50°C for 30s, and 60°C for 30s. Unincorporated ddNTPs were removed by 90 min of incubation with 2 U of calf intestinal phosphatase at 37°C followed by 15 min at 75°C. The final products were then electrophoresed on an ABI Prism 3100 genetic analyzer (Applied Biosystems, Foster City, CA), and the genotype was determined by the color and the electrophoretic mobility of the extended probe. Genotyping was carried out using Genotyper version 3.7 (Applied Biosystems). Complete data were available for 4,058 individuals (1,882 males: 1,252 Chinese, 356 Malays, and 274 Asian Indians; 2,176 females: 1,494 Chinese, 393 Malays, and 289 Asian Indians).

Statistical analyses

SPSS version 11 (SPSS, Inc., Chicago, IL) was used for all analyses. The data were analyzed separately for each gender. A *P* value <0.05 was considered statistically significant. Allele frequency was determined by direct counting, and the standard goodness-of-fit test was used to test the Hardy-Weinberg equilibrium for each gender/ethnic group. Analysis of covariance (ANCOVA) was used to estimate the effect of the S447X polymorphism on serum lipid concentrations adjusted for age, body mass index (BMI), smoking status (current smoker or nonsmoker), the presence of diabetes mellitus (determined by oral glucose tolerance test), alcohol intake (drinker or nondrinker), exercise frequency (0–2 or $\geq$3 times per week), and ethnic group. Because of the skewed distribution of TG, log transformation was used. The means and their confidence intervals presented for TG correspond to the antilog of the log-transformed values. Because of the low numbers of individuals homozygous for the rare allele, they were pooled with heterozygotes and subsequently

classified as carriers or noncarriers of the rare allele (X447). The homogeneity of allele effects between ethnic groups was examined by introducing the corresponding interaction term into the model. No statistically significant heterogeneity between ethnic groups was noted. For that reason, analyses were conducted for the population as a whole and adjusted for ethnic group. Interactions between genetic and environmental factors were assessed by considering a two-factor interaction term in the model. These interaction terms were individually added to the ANCOVA model and their significance determined. When considering BMI's interaction with the S447X polymorphism at the *LPL* locus, BMI was analyzed as a categorical variable. It was grouped into three levels according to the tertiles in each gender. Where statistically significant interactions were identified, we conducted analyses using the same ANCOVA model, stratified by the interacting factors (*APOE* genotype, smoking or alcohol consumption), and presented the estimated means for HDL-C concentration in each stratum. Because the presence of diabetes mellitus is generally associated with low serum HDL-C concentration, we performed the analyses with and without the inclusion of subjects with diabetes mellitus. The exclusion of those with diabetes mellitus had no significant effect on the results of the analyses compared with the models in which diabetes mellitus was adjusted for. As such, the analyses are presented with diabetic subjects included.

RESULTS

Adjusted mean lipid levels for males and females are presented in **Table 1**. In general, Asian Indians had a less favorable lipid profile with higher serum concentrations of total cholesterol, LDL-C, and TG and lower HDL-C concentrations. Malays had lipid profiles that were intermediate between those of Chinese and Asian Indians, and they had an increased prevalence of obesity.

Frequencies of the S447X polymorphism and association with serum lipids

Genotypes and rare allele frequencies in men and women for the three ethnic groups combined are shown in **Table 2**. These did not deviate from the Hardy-Weinberg equilibrium.

No statistically significant interactions among ethnic groups, the S447X polymorphism, and serum lipid concentrations were detected (**Table 3**). As such, the assessment of associations between X447 allele carrier status and lipid levels was carried out for males and females after adjusting for ethnic group and other factors as indicated for each specific analysis. The presence of the X447 allele was associated with lower serum TG concentration and higher HDL-C concentration in both males and females. The presence of the X447 allele was also associated with lower total cholesterol and LDL-C, but only in females.

Interactions between the S447X polymorphism and polymorphisms at the apoE locus, smoking, alcohol consumption, obesity, and physical activity

Analyses for gene-gene or gene-environment interactions were carried out in relation to serum lipid concentrations (HDL-C, LDL-C, and TG). Moreover, we examined interactions between the S447X and apoE polymorphisms (E2, E3, and E4), BMI, cigarette smoking, alcohol intake, and exercise.

Among men, but not in women, a statistically significant interaction was found between *APOE* genotype, the S447X polymorphism, and serum HDL-C concentration (**Fig. 1**). The S447X polymorphism had the greatest effect on serum HDL-C concentration in males with the E4 genotype ($P < 0.0001$). A smaller, although statistically significant,

TABLE 1. Characteristics of the study population

| Variable | Males (n = 1,881) | | | | | | Females (n = 2,176) | | | | | |
	Chinese (n = 1,252)	Malay (n = 356)	Indian (n = 273)	P[a] C vs. M	C vs. I	M vs. I	Chinese (n = 1,494)	Malay (n = 393)	Indian (n = 289)	P[a] C vs. M	C vs. I	M vs. I
Age (years)	38.1 (37.4–38.8)	39.7 (38.4–41.0)	41.0 (39.6–42.4)	0.015	<0.001	0.205	37.7 (37.1–38.3)	38.3 (37.1–39.6)	40.2 (38.78–41.52)	0.305	<0.001	0.029
TC (mmol/l)	5.50 (5.45–5.56)	5.87 (5.76–5.98)	5.70 (5.57–5.84)	<0.001	0.011	0.025	5.32 (5.27–5.38)	5.74 (5.62–5.86)	5.34 (5.22–5.46)	<0.001	0.728	<0.001
LDL-C (mmol/l)	3.54 (3.49–3.59)	3.96 (3.86–4.07)	3.89 (3.76–4.02)	<0.001	<0.001	0.201	3.24 (3.20–3.29)	3.76 (3.65–3.87)	3.54 (3.43–3.65)	<0.001	<0.001	0.02
HDL-C (mmol/l)	1.26 (1.25–1.28)	1.15 (1.12–1.18)	1.07 (1.03–1.10)	<0.001	<0.001	<0.001	1.56 (1.54–1.58)	1.43 (1.54–1.58)	1.22 (1.19–1.26)	<0.001	<0.001	<0.001
TG (mmol/l)	1.39 (1.35–1.43)	1.65 (1.56–1.75)	1.72 (1.61–1.83)	<0.001	<0.001	0.220	1.01 (0.99–1.04)	1.20 (1.14–1.27)	1.19 (1.13–1.26)	<0.001	<0.001	0.787
BMI (kg/m²)	23.6 (23.4–23.8)	24.9 (24.4–25.3)	24.5 (24.0–25.0)	<0.001	<0.001	0.334	22.1 (21.9–22.2)	26.2 (25.7–26.8)	25.5 (24.95–26.09)	<0.001	<0.001	0.264
DM (%)	8.2	12.4	19.8		<0.001[b]		6.9	16.5	18.3		<0.001[b]	
Frequent exercise (%)	18.8	21.1	28.2		0.002[b]		11.2	14.2	18.7		0.002[b]	
Smokers (%)	23.4	44.1	29.7		<0.001[b]		3.3	4.1	0.3		0.012[b]	

BMI, body mass index; DM, diabetes mellitus; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride. Values shown are means and (95% confidence intervals). TG was log-transformed for the analysis and back-transformed to obtain the mean.

[a] Pairwise comparisons were carried out using the Mann-Whitney U test. Population abbreviations are as follows: C, Chinese; I, Indian; M, Malay.

[b] Compared using the Chi-square test.

TABLE 2. Genotype distribution and rare allele carrier frequency for S447X polymorphism at the *LPL* locus in Singapore

Sample	Genotype Frequency			Rare Allele Carrier Frequency	*P* Value for the Test of Hardy-Weinberg Equilibrium[a]
	S447S	S447X	X447X	X447	
Male					
Chinese	0.779 (975)	0.207 (259)	0.014 (18)	0.118 (277)	0.866
Malay	0.843 (300)	0.152 (54)	0.006 (2)	0.081 (56)	0.797
Asian Indian	0.791 (216)	0.198 (54)	0.011 (3)	0.110 (57)	0.854
Female					
Chinese	0.801 (1,196)	0.183 (274)	0.016 (24)	0.108 (298)	0.074
Malay	0.845 (332)	0.148 (58)	0.008 (3)	0.081 (61)	0.790
Asian Indian	0.813 (235)	0.170 (49)	0.017 (5)	0.102 (54)	0.202

Values shown are frequency and (number of subjects).
[a] Tested using a standard goodness-of-fit test.

effect was noted in those with the E3 genotype, and no effect was observed in E2 subjects.

The interaction between cigarette smoking, the S447X polymorphism, and serum HDL-C concentration is shown in **Table 4**. In both men and women, the effect of the X447 allele was greater in smokers than in nonsmokers. This interaction was statistically significant in women ($P = 0.027$) and of borderline significance in men ($P = 0.067$). In a similar manner, the difference between S447 homozygotes and X447 carriers was greatest in those who consumed alcohol, and the interaction was statistically significant in women (**Table 5**).

Neither of these interactions was significant for TG or LDL-C. Furthermore, no statistically significant interac-

tions were noted between the S447X polymorphism and exercise or obesity with respect to any lipid parameters.

DISCUSSION

Our study includes the largest number of Asians examined to date in terms of examining the impact of genetic variation at the *LPL* locus on plasma lipid levels (21, 22). Another important feature of our study relates to decreasing the potential confounding effect in earlier studies of differences in socioeconomic status and geographical location (rural vs. urban) between ethnic groups. Singapore is a small island that is completely urbanized, and the

TABLE 3. Comparison of plasma lipids by carrier status of the X447 mutation for males and females

Variable	Noncarriers	Carriers	*P* Value for S447X	*P* Value for Interaction S447X*Ethnicity
Male	n = 1,491	n = 390		
TC (mmol/l)	5.60 (5.51–5.69)	5.67 (5.54–5.79)	0.255	0.132
LDL-C (mmol/l)	3.71 (3.63–3.79)	3.72 (3.61–3.84)	0.756	0.283
HDL-C (mmol/l)	1.13 (1.11–1.16)	1.21 (1.18–1.25)	<0.001	0.784
TG (mmol/l)	1.66 (1.59–1.73)	1.58 (1.49–1.68)	0.057	0.157
Female	n = 1,763	n = 413		
TC (mmol/l)	5.44 (5.30–5.58)	5.35 (5.19–5.52)	0.08	0.128
LDL-C (mmol/l)	3.53 (3.41–3.66)	3.41 (3.26–3.55)	0.006	0.118
HDL-C (mmol/l)	1.36 (1.31–1.41)	1.43 (1.37–1.48)	<0.001	0.678
TG (mmol/l)	1.23 (1.15–1.31)	1.10 (1.02–1.19)	<0.001	0.470

Values shown are means and (95% confidence intervals). Marginal means for serum HDL-C concentrations were estimated by analysis of covariance (ANCOVA) stratified by gender and current smoking according to the following regression equations. In men, TC = 3.34 + 0.027 (age) + 0.05 (BMI) − 0.094 (Chinese) + 0.172 (Malay) + 0.097 (current smoker) − 0.088 (diabetes present) + 0.038 (alcohol drinker) − 0.101 (frequent exercise) + 0.64 (X447 allele present); LDL-C = −1.426 + 0.024 (age) + 0.061 (BMI) − 0.243 (Chinese) + 0.059 (Malay) + 0.134 (current smoker) − 0.107 (diabetes present) + 0.026 (alcohol drinker) − 0.127 (frequent exercise) + 0.016 (X447 allele present); HDL-C = 1.787 − 0.001 (age) − 0.028 (BMI) + 0.156 (Chinese) + 0.128 (Malay) − 0.117 (current smoker) − 0.037 (diabetes present) + 0.038 (alcohol drinker) − 0.033 (frequent exercise) + 0.08 (X447 allele present); Ln(TG) = −1.12 + 0.01 (age) + 0.048 (BMI) − 0.115 (Chinese) − 0.065 (Malay) + 0.172 (current smoker) + 0.134 (diabetes present) − 0.002 (alcohol drinker) − 0.053 (frequent exercise) − 0.05 (X447 allele present). In women, TC = 3.024 + 0.036 (age) + 0.034 (BMI) + 0.207 (Chinese) − 0.443 (Malay) − 0.062 (current smoker) + 0.118 (diabetes present) − 0.002 (alcohol drinker) − 0.029 (frequent exercise) − 0.09 (X447 allele present); LDL-C = 1.073 + 0.028 (age) + 0.051 (BMI) − 0.011 (Chinese) + 0.236 (Malay) − 0.026 (current smoker) + 0.276 (diabetes present) − 0.014 (alcohol drinker) − 0.003 (frequent exercise) − 0.127 (X447 allele present); HDL-C = 1.744 + 0.009 (age) + 0.004 (BMI) + 0.236 (Chinese) − 0.242 (Malay) − 0.055 (current smoker) − 0.162 (diabetes present) + 0.02 (alcohol drinker) + 0.03 (frequent exercise) + 0.064 (X447 allele present); Ln(TG) = −1.057 + 0.009 (age) + 0.034 (BMI) + 0.007 (Chinese) − 0.009 (Malay) + 0.069 (current smoker) + 0.257 (diabetes present) − 0.038 (alcohol drinker) − 0.006 (frequent exercise) − 0.112 (X447 allele present).
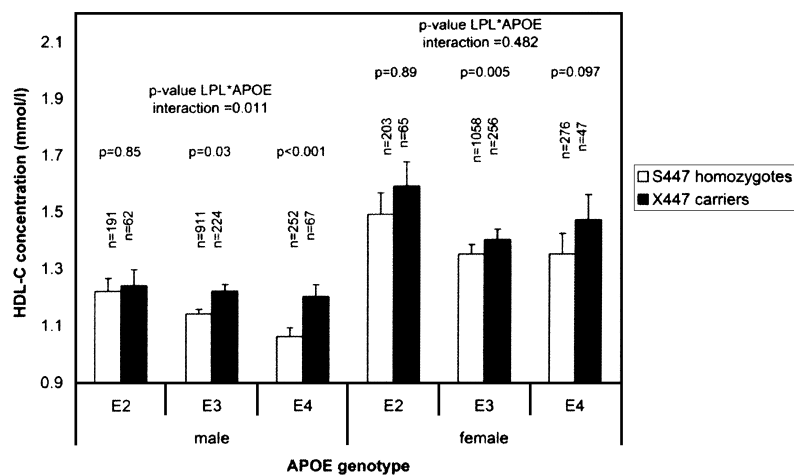
Fig. 1. Estimated means for high density lipoprotein cholesterol (HDL-C) concentration for X447 allele noncarriers and carriers by apolipoprotein E (apoE) genotype in males and females. Analysis of covariance, stratified on gender and apoE genotype, was used to estimate mean HDL-C values adjusted for age and body mass index as continuous variables and ethnicity, the presence of diabetes mellitus, alcohol consumption, exercise frequency, and cigarette smoking as categorical variables. The *P* value for interaction term ethnicity*apoE*S447X was not statistically significant, with values of 0.161 in men and 0.16 in women. The error bars represent the SEM.

three major ethnic groups live in a similar environment. As such, large differences in environmental exposure between those living in rural and urban areas do not exist in our study. Furthermore, even though some differences in socioeconomic status exist, the population in Singapore enjoys a high standard of living and uniform access to health care. These features, coupled with the marked differences in CHD risk and levels of CHD risk factors between ethnic groups (Table 1), have led some to suggest that Singapore represents a population laboratory in which to examine the effect of genetic and environmental factors contributing to CHD (34).

We have shown that the S447X polymorphism at the *LPL* locus is common in all three ethnic groups living in Singapore (Table 2). The frequency of the X447 allele in Chinese (0.118) is similar to that seen in Chinese living in Canada (0.133) (21). On the other hand, the frequency of the X447 allele among Asian Indians living in Singapore (0.109) is a little higher than that reported for southern Asians living in the United Kingdom (0.087) (22), which may be attributable to the different migratory patterns to these two countries (primarily from the south in the case of Singapore and from the north in the United Kingdom).

The presence of the X447 allele was associated with significantly higher HDL-C concentrations in both men and women (Table 3), thereby replicating (albeit with greater statistical power) the findings in Chinese Canadians (21). In addition, the X447 allele was also associated with lower serum TG concentrations. Although this association reached statistical significance only in women, we do not believe that our study supports an earlier hypothesis that the effects of the X447 allele are gender specific (20, 35). The direction of the association was similar between men and women, and interaction term gender*S447X polymorphism was not statistically significant ($P = 0.164$; data not shown). Instead, our data raises the possibility that the presence of gene-gene and/or gene-environment interactions in our population, which differ from those present in the reported white populations, may be responsible for the gender differences seen in the latter populations.

The observed interaction between the *APOE* locus, the *LPL* locus, and HDL-C concentrations is one such interaction that may be at play. This interaction was of particular interest for several reasons. First, our findings replicate those of a recent study conducted in a Spanish population. Corella et al. (24) noted an interaction between *APOE* genotype, the S447X polymorphism, and HDL-C

TABLE 4. HDL-C for X447 noncarriers and carriers by cigarette smoking in males and females

Cigarette Smoking	Noncarriers		Carriers		*P* Value for Noncarriers vs. Carriers	*P* Value for Interaction Smoking*S447X	*P* Value for Interaction Ethnicity*Smoking*S447X
	n	HDL	n	HDL			
Males							
Not current	1,058	1.19 (1.17–1.22)	292	1.24 (1.19–1.29)	0.057	0.067	0.649
Current	433	1.07 (1.04–1.11)	98	1.20 (1.14–1.27)	0.001		
Females							
Not current	1,708	1.39 (1.35–1.42)	402	1.46 (1.41–1.51)	0.003	0.027	0.143
Current	55	1.28 (1.06–1.51)	11	1.77 (1.52–2.02)	0.005		

Values shown are means and (95% confidence intervals). Marginal means for serum HDL-C concentrations were estimated by ANCOVA stratified by gender and current smoking according to the following regression equations: male nonsmokers, HDL-C = 1.783 − 0.001 (age) − 0.028 (BMI) + 0.18 (Chinese) + 0.162 (Malay) − 0.05 (diabetes present) + 0.032 (alcohol drinker) + 0.042 (frequent exercise) + 0.065 (X447 allele present); male smokers, HDL-C = 1.632 − 0.0003 (age) − 0.027 (BMI) + 0.098 (Chinese) + 0.07 (Malay) − 0.019 (diabetes present) + 0.058 (alcohol drinker) + 0.004 (frequent exercise) + 0.124 (X447 allele present); female nonsmokers, HDL-C = 1.753 + 0.004 (age) − 0.027 (BMI) + 0.233 (Chinese) + 0.244 (Malay) − 0.165 (diabetes present) + 0.023 (alcohol drinker) + 0.03 (frequent exercise) + 0.058 (X447 allele present); female smokers, HDL-C = 1.899 − 0.0003 (age) − 0.022 (BMI) + 0.087 (Chinese) − 0.029 (Malay) + 0.042 (diabetes present) − 0.063 (alcohol drinker) − 0.035 (frequent exercise) + 0.269 (X447 allele present).

TABLE 5. HDL-C for X447 noncarriers and carriers stratified by alcohol consumption in males and females

Alcohol Consumption	Noncarriers		Carriers		P Value for Noncarriers vs. Carriers	P Value for Interaction Alcohol*S447X	P Value for Interaction Ethnicity*Alcohol*S447X
	n	HDL	n	HDL			
Males							
Drinker	721	1.14 (1.10–1.18)	205	1.27 (1.20–1.35)	0.057	0.111	0.495
Nondrinker	770	1.11 (1.08–1.14)	185	1.16 (1.11–1.22)	0.001		
Females							
Drinker	447	1.29 (1.21–1.37)	106	1.62 (1.38–1.85)	0.003	0.034	0.319
Nondrinker	1,316	1.36 (1.31–1.41)	307	1.42 (1.35–1.48)	0.005		

Values shown are means and (95% confidence intervals). Marginal means for serum HDL-C concentrations were estimated by ANCOVA stratified by gender and alcohol consumption according to the following regression equations: male drinkers, HDL-C = 1.861 + 0.003 (age) − 0.03 (BMI) + 0.122 (Chinese) + 0.064 (Malay) − 0.1 (current smoker) − 0.064 (diabetes present) + 0.044 (frequent exercise) + 0.1 (X447 allele present); male nondrinkers, HDL-C = 1.76 − 0.002 (age) − 0.027 (BMI) + 0.196 (Chinese) + 0.165 (Malay) − 0.134 (current smoker) − 0.017 (diabetes present) + 0.02 (frequent exercise) + 0.058 (X447 allele present); female drinkers, HDL-C = 1.711 + 0.004 (age) − 0.027 (BMI) + 0.305 (Chinese) + 0.204 (Malay) − 0.067 (current smoker) − 0.197 (diabetes present) + 0.066 (frequent exercise) + 0.114 (X447 allele present); female nondrinkers, HDL-C = 1.75 + 0.004 (age) − 0.026 (BMI) + 0.217 (Chinese) + 0.23 (Malay) − 0.026 (current smoker) − 0.158 (diabetes present) + 0.016 (frequent exercise) + 0.045 (X447 allele present).

concentration that is remarkably similar to that found in our study. In that study, as in ours, the presence of the X447 allele was associated with the greatest variation in HDL-C concentration in those who were also carriers of the E4 allele at the *APOE* locus. Such replication in an independent study population provides additional evidence that the observed association is causal and not the result of chance. Second, the statistical interaction observed in our study may reflect an interaction that is known to occur at the molecular level. This adds biological plausibility to our findings. LPL markedly increased the binding of apoE-containing lipoproteins to the cell surface (36). This occurs through a number of different mechanisms. *APOE* and LPL increase the binding of TRLs to heparan sulfate proteoglycans in an additive manner (37). Both apoE and LPL enhance the binding of VLDL to the VLDL receptor (5) and the degradation of VLDL via LDL receptor and the LDL receptor-related protein-mediated pathways (38). It has also been shown that apoE4 is preferentially distributed to TRLs, whereas apoE2 and apoE3 are preferentially distributed to HDL (39–41). It is our hypothesis that the enrichment of TRLs with apoE, seen in carriers of the E4 allele, enhances the impact of increased LPL activity associated with the X447 allele on the catabolism of TRLs. This, in turn, results in the finding that the impact of the X447 allele on HDL-C concentration is greatest in carriers of the E4 allele.

The S447X polymorphism at the *LPL* locus also interacted significantly with cigarette smoking and alcohol consumption in their association with serum HDL-C concentration. Cigarette smoking and alcohol consumption have opposing effects on serum HDL-C concentration. Cigarette smoking is associated with low serum HDL-C concentration, whereas moderate alcohol consumption is associated with high serum HDL-C concentration (42). The mechanisms for these changes in HDL-C concentrations are unclear. However, both smoking and alcohol consumption alter plasma LPL activity. Smoking is associated with lower plasma LPL activity (43), which would result in delayed metabolism of TRLs. The less efficient lipolysis of VLDL and chylomicrons will reduce the amount

of surface material available for incorporation into nascent HDL particles. In contrast, alcohol consumption is associated with increased plasma LPL activity (44). We believe that our findings provide additional evidence that variations in plasma LPL activity form an important mechanism by which these two environmental factors alter HDL metabolism.

Most studies that have examined the associations between the S447X polymorphism and serum lipid concentrations have focused on serum TG and HDL-C concentrations. Only a few have included the LDL-C concentration, and these showed variable results. Some studies showed similar LDL-C concentrations in X447 carriers compared with S447 homozygotes (15, 21, 24), whereas others showed higher LDL-C concentrations in carriers of the X447 allele (35). In contrast, we found lower LDL-C concentrations in carriers of the X447 allele in women but not in men. A possible explanation for this association is that increased catabolism of TRLs in carriers of the X447 allele results in less available substrate for conversion to LDL. However, the significance of this finding is uncertain at this time.

The only other study that has reported an interaction between obesity and the S447X polymorphism found that X447 carriers had significantly lower serum TG concentrations in those with BMI < 25 kg/m², whereas no significant difference was found for those in the high BMI group (30). In addition, the investigators reported that, among those with low plasma insulin concentrations, X447 carriers had significantly higher HDL-C, whereas no difference was found in those with high plasma insulin concentrations. In another study, the presence of the *Hin*dIII polymorphism was more strongly associated with hypertriglyceridemia in obese subjects (29). These findings were not replicated in our study.

The authors recognize that this study has limitations. The most important one is a limitation of sample size and, consequently, statistical power to examine multiple interactions as we have done. However, the facts that our findings replicate the results of previous studies and are biologically plausible increase the probability that these

associations are causal and not the result of chance. Nevertheless, further studies in independently collected samples, including larger numbers of Malays and Asian Indians, are required to confirm these findings.

In conclusion, this study has shown that the S447X polymorphism is common in Chinese, Malays, and Asian Indians. The presence of the X447 allele is associated with lower serum TG and higher serum HDL-C concentrations in males and females, a profile that would be expected to be less atherogenic. The presence of the X447 allele was also associated with lower LDL-C concentrations in women. The association between the X447 allele and serum HDL-C concentrations was modulated by genetic variation at the *APOE* locus and environmental factors (cigarette smoking and alcohol intake). This emphasizes the need to examine genetic associations in the context of the population of interest. Furthermore, our findings implicate variation in plasma LPL activity as an important mechanism by which cigarette smoking and alcohol consumption may alter HDL metabolism.▪

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