Genetic variants at the APOE, lipoprotein lipase (LpL), cholesteryl ester transfer protein (CETP), and endothelial nitric oxide (eNOS) genes and coronary artery disease (CAD): CETP Taq1 B2B2 associates with lower risk of CAD in Asian Indians

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Received: 5 October 2009 / Accepted: 23 February 2010 / Published online: 25 March 2010
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Abstract Coronary artery disease (CAD) arises due to a complex interplay between the environment and genetic factors. Alterations in many of the biomarkers such as lipids and lipoprotein levels are characteristic of CAD. The phenotypes themselves have genetic determinants, and many single-nucleotide polymorphisms (SNPs) have been identified which influence them. The current study aims to evaluate the effect of six common polymorphisms at four loci, lipoprotein lipase (D9N, N291S, S447X), apolipoprotein E (APOE), cholesteryl ester transfer protein (C277T), and endothelial nitric oxide synthase (E298D), on lipid and lipoprotein levels and its association with CAD. Genotyping for the SNPs was done in 240 Indians of which 90 had proven CAD. The other 150 were clinically free from CAD and acted as controls. Relation of genetic variants, clinical history, and biochemical parameters with CAD were analyzed by multiple regression analysis. The frequency of the B2 allele in the CETP gene was significantly lower in cases than in controls (0.40 vs 0.49, $P=0.042$). Significant association of CETP Taq1B SNP was seen with total cholesterol and low density lipoprotein cholesterol. Multivariate analysis accounting for clinical and metabolic predictors of CAD showed smoking to be a significant risk

factor (odds ratio (OR) 4.347, 95% confidence interval (CI) 1.888–10.012, $P=0.001$) and the CETP B2 variant imparting atheroprotection (OR 0.312, 95% CI 0.116–0.841, $P=0.021$) possibly through a favorable lipid profile. None of the other SNPs were associated with the risk of CAD.

Keywords Apolipoprotein E · Lipoprotein lipase · Cholesteryl ester transfer protein · Endothelial nitric oxide synthase · Single-nucleotide polymorphisms · Asian Indians

Introduction

Coronary artery disease (CAD) like many common disorders results from a complex interaction between environment and genetic factors (Ellsworth et al. 1999). Alterations in lipid metabolism including high: total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL-C), apolipoprotein B (ApoB), and lipoprotein a Lp (a) levels and low: high density lipoprotein cholesterol (HDL-C) are all important risk factors for CAD. All these lipid abnormalities themselves have genetic determinants (Galton 1990; Hayden et al. 1991; Sankaranarayanan et al. 1999). Many of the circulating biomarkers with CAD behave as heritable quantitative traits, and common single-nucleotide polymorphisms (SNPs) influencing their variance are now being identified both by candidate gene and genomewide studies (Sandhu et al. 2008; Melzer et al. 2008; Wallace et al. 2008; Willier et al. 2008).

The lipoprotein lipase (LPL) represents one such gene; lipoprotein lipase (LpL) is a multifunctional protein that hydrolyses core TG from circulating chylomicrons and very low density lipoprotein (VLDL), which are either degraded

Electronic supplementary material The online version of this article (doi:10.1007/s12687-010-0005-1) contains supplementary material, which is available to authorized users.

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or converted to LDL particles by hepatic lipases. The common variants Asp9Asn (D9N) and Asn291Ser (N291S) are associated with decreased LpL activity, and Ser447Stop (S447X) with increased activity (van Bockxmeer et al. 2001; Hokanson 1999; Zhang et al. 1996). These variants, therefore, may affect levels of TG and HDL and consequently could represent one of the most important influences on CAD (van Bockxmeer et al. 2001; Hokanson 1999; Wittrup et al. 1999).

Apolipoprotein E (ApoE) has a pivotal role in the metabolism of chylomicrons, chylomicron remnants, VLDL cholesterol, and HDL-cholesterol (HDL-C), and as a ligand, it binds both LDL-cholesterol (LDL-C) and ApoE receptors (Mahley et al. 1984). Polymorphisms at the APOE locus are some of the most important genetic determinants of LDL-cholesterol concentrations in the general population. The APOE gene has three main allelic variants: ϵ 2, ϵ 3, and ϵ 4; ϵ 2 and ϵ 4 have a strong effect on the level of its gene product; * ϵ 2 is associated with higher concentrations of ApoE, and * ϵ 4 with lower concentrations (Siest et al. 1995; Weisgraber et al. 1981).

Cholesteryl ester transport protein (CETP) mediates the exchange of lipids between high density lipoprotein (HDL) to the ApoB-containing lipoproteins, providing an avenue for the uptake of cholesteryl esters by hepatic receptors (Fielding and Fielding 1995). Among the most widely studied is the Taq1B SNP, a silent base change affecting the 277th nucleotide in the first intron of the CETP gene, and absence of the Taq1B restriction site (B2 allele) is associated with decreased CETP activity and in turn increased HDL levels in normolipidemic individuals (Freeman et al. 1994; Hannuksela et al. 1994; Kondo et al. 1989). The CETP Taq1B variant is in strong linkage disequilibrium with the promoter polymorphism -629A>C providing a potential explanation for the observed associations between the former variant and plasma CETP activity and lipid levels (Dachet et al. 2000). Alternatively, it has also been suggested that the effect of the CETP Taq1B variant may be due to another functional polymorphism in the CETP gene yet to be discovered (Eiriksdottir et al. 2001).

Nitric oxide from the endothelium is considered an important atheroprotective mediator, and acquired defects in the generation of nitric oxide are associated with increases in CAD (Moncada and Higgs 1993). Associations have been described between Glu298Asp polymorphism and nitric oxide synthesis and endothelial function (Leeson et al. 2002).

The Indian population has a tendency for dyslipidaemia and high incidence of CAD (Ravi et al. 2004). In the current study, six genetic variants potentially related to CAD were evaluated in univariate and multivariate models in an Indian CAD population. These include variants at loci lipoprotein lipase (D9N, N291S, S447X), apolipoprotein e

(APOE), cholesteryl ester transfer protein (Taq1B), and endothelial nitric oxide synthase (Glu298Asp).

Materials and methods

Subjects

The study was performed on a total of 240 unrelated individuals which consisted of 90 patients with CAD confirmed by coronary angiography: >50% stenosis in one or more arteries and stable or unstable angina, and 150 controls: examined clinically and investigated by electrocardiography to exclude CAD. The same cohort has also been used to investigate the frequency and role of SNPs in APOA5 and APOC3 (communicated for publication). Informed consent was obtained from all the subjects. The study is in accordance with the Helsinki Declaration and was approved by the local ethical committee. A detailed case record form pertaining to information on demographics, medical history, and coronary risk factors such as presence of diabetes, hypertension, smoking, lifestyle, and current medication was completed for each participant through personal interviews and through perusal of their medical records.

Blood specimens were collected after an overnight fast of 12 h by venipuncture using the vacutainer system from Becton Dickinson (Franklin Lakes, NJ, USA) in the anticoagulant EDTA as well as plain bulb for serum. Serum EDTA plasma samples were separated by centrifugation, and aliquots were preserved at -20°C till analysis.

Biochemical parameters

The laboratory parameters, namely serum TC, TG, and HDL-C levels, were determined by routine enzymatic end point methods (X Imola; Randox Laboratories Ltd, UK). LDL-C and VLDL cholesterol were calculated according to Friedwald's formula. Serum apolipoproteins A1 and B were measured by the immunoturbidimetric immunoassay (Daytona, Randox Laboratories Ltd, UK).

SNP genotyping

The genomic DNA was isolated from peripheral blood using QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). DNA yield and purity were determined by measuring absorbance at 260/280 nm.

APOE polymorphisms (ϵ 2 rs 429358, ϵ 3 rs 7412) and LPL polymorphisms D9N (rs 1801177), N291S (rs268), S447X (rs 328), CETP Taq1B (rs 708272), and eNOS Glu298Asp (rs 1799983) were genotyped. Details of polymerase chain reaction (PCR) primers, annealing con-

ditions, and restriction enzyme digestion have been described elsewhere (Kondo et al. 1989; Hixson and Vernier 1990; Masana et al. 2001; Stocks et al. 1992; Zhang et al. 1995a; Colombo et al. 2003). Post amplification, the products were restriction digested with appropriate restriction enzymes overnight, the digests resolved on 2.5% agarose gel, and for APOE genotypes, the products were loaded on 8% polyacrylamide gel and visualized under UV transilluminator imaging system.

Sequencing

Amplified products were purified using QIAquick PCR purification kit (Qiagen, Hilden, Germany) and directly sequenced to identify the polymorphic site by Automated ABI prism 3100 Avant Genetic Analyzer (Applied Biosystems Inc., Foster City, CA) using ABI prism BigDye terminator kit (version 3.1). Genotyping of 10% of samples was confirmed by sequencing.

Statistical analysis

Allele frequency was calculated as the number of occurrences of the test allele in the population divided by the total number of alleles. Any deviation of the genotype frequencies from the Hardy–Weinberg equilibrium was assessed by the chi-square test. Chi-square tests were used for comparison of binary variables across groups. The non-parametric Kolmogorov–Smirnov test was used as test of normality for the quantitative variables, and the values were subsequently log-transformed to normalize distribution. To determine if the variants under investigation were associated with lipid and lipoprotein subclasses concentrations, the following procedure was carried out: the major allele referred to as 1 and the minor allele as 2; the corresponding genotypes were assigned as 11, 12, or 22 for the homozygous normal, heterozygotes, and homozygous variants, respectively, and analyzed statistically by analysis of covariance (ANCOVA) technique. In the model, genotypes were used as predictors, and the potential confounding factors, i.e., smoking, diabetes, hypertension, and alcohol use, were used as covariates. Heterozygotes (12) and homozygote variants (22) were combined for the purpose of analysis for those polymorphisms where the minor allele frequency was <10% (APOE genotypes, LpL D9N, and LpL S447X) and ANCOVA performed. The genotype–phenotype correlation was performed only on controls since almost all the cases were on lipid-lowering medications. To determine risk for CAD, odds ratio adjusted for other covariables were determined using stepwise backward regression model. The analysis was performed by entering all relevant genes and risk factors followed by backward regression with “*P* to remove” and

“*P* to enter” of 0.05. SNPStats online software tool was applied to determine the genetic model of association and linkage disequilibrium (Sole et al. 2006). Routine statistical analysis was carried out with the SPSS version 15 software (SPSS Inc., Chicago, IL) and GraphPad StatMate 2.0 (GraphPad software, Inc). A *P* value <0.05 is considered significant for the data at 5% level of significance.

Results

Table 1 displays means and standard deviations for the study subjects for relevant biochemical characteristics as well as risk factors. Statistically significant differences were seen in the smoking status ($P<0.0001$) and presence of family history ($P=0.034$) as well as hypertension ($P=0.038$) in cases vs controls. Relative frequencies of the variant alleles of all the polymorphisms are shown in Table 2. There was no significant departure from the Hardy–Weinberg equilibrium for any of the polymorphisms studied. A higher frequency of the CETP Taq1 B2 allele was seen in controls as compared to the cases (0.49 vs 0.40), which was statistically significant (odds ratio (OR)=0.550, 95% confidence interval (CI)=0.314–0.960, $P=0.042$). The LpL N291S polymorphism was not detected

Table 1 General characteristics of cases and controls

Characteristics ^a	Cases <i>n</i> =90	Controls <i>n</i> =150
Age	47	48
Sex (% females)	9%	30%
BMI (kg/m ²)	25.1±3.6	24±3.5
Smoking status (%)		
Smoker <10 cigs/day (%)	26*	13
Past smoker (%)	15	6
Tobacco chewer (%)	4	4
Family history of CAD (%)	20*	13
History of hypertension (%)	32*	18
Alcohol status current drinker (%)	21	18
Plasma cholesterol (mg/dl)	180±58.5	192.8±35.3
HDL cholesterol (mg/dl)	36.6±17.5	40.8±11
LDL cholesterol (mg/dl)	114.2±53.1	125±30.2
VLDL cholesterol (mg/dl)	34±25	30±17.6
Plasma triglycerides (mg/dl)	175.7±124	152±88
Triple-vessel disease (%)	51	NA
Double-vessel disease (%)	9	NA
Single-vessel disease (%)	40	NA
Myocardial infarction (%)	39	NA

NA not applicable

^a Mean (standard deviation (SD)) for continuous variables

* $P<0.05$, cases significantly different from controls

Table 2 Genotype and allele frequencies of polymorphisms in APOE, CETP, ENOS, and LPL genes in cases and controls

SNP	Genotype frequencies (%)						Allele frequencies		OR	95% CI	P value
							Major	Minor			
CETP	B1B1	B1B2	B2B2				B1	B2			
Cases (90)	37	44	19				0.593	0.407	0.55	0.314–0.960	0.042*
Controls (150)	25	51	28				0.503	0.497			
ENOS	GG	GT	TT				G	T			
Cases (90)	64	35	1				0.815	0.185	1.13	0.658–1.939	0.679
Controls (150)	67	31	2				0.825	0.175			
S447X	CC	CG	GG				C	G			
Cases (90)	87	12	1				0.92	0.08	0.85	0.408–1.792	0.711
Controls (150)	85	14	1				0.92	0.08			
D9N (G280A)	GG	GA	AA				G	A			
Cases (90)	98.7	1.3	0				0.993	0.007	1.35	0.329–5.535	0.704
Controls (150)	97	3	0				0.99	0.1			
ApoE	E3E3	E3E4	E3E2	E4E4	E2E2	E4E2	E3	E4	E2		
Cases (90)	86.4	12.5	0	1.1	0	0	0.921	0.079	0	0.650 (E4)	0.29 (E4)
Controls (150)	78.6	18	2.7	0.7	0	0	0.892	0.095	0.013	0–1.502 (E2)	0.160 (E2)

Allele frequencies were estimated by direct counting

SNP single-nucleotide polymorphism, OR unadjusted odds ratio, 95% CI 95% confidence interval

* $P < 0.05$, statistically significant

in the 240 samples analyzed. The LD between polymorphic sites was calculated using genotypes from the 240 unrelated subjects (see supplementary Table 1) with SNPStats software. None of the polymorphisms were in strong LD except: CETP Taq1B (rs 708272) and LpL S447X (rs 328) which showed a borderline significance ($P = 0.048$). In the UNI-ANCOVA, total plasma cholesterol showed significant association with CETP Taq1B across all the covariates as shown in Table 3. Interestingly, CETP Taq1B also showed association with LDL level across all covariates. APOE genotypes were associated with TC ($P = 0.027$) and LDL ($P = 0.041$) when smoking was the covariate. However, the

association was lost for diabetes and hypertension. None of the other variants showed any association with the lipid parameters that were being investigated. Stepwise backward regression analysis was performed to evaluate the association of the various genotypes as well as other variables such as smoking, alcohol consumption, diabetes, and hypertension with risk of CAD. As shown in Table 4 in the final model, smoking was significantly associated with the risk of CAD (OR 4.347, 95% CI 1.888–10.012, $P = 0.001$), and CETP B2B2 allele was atheroprotective (OR 0.312, 95% CI 0.116–0.841, $P = 0.021$). None of the other genotypes LpL D9N (OR 0.255, 95% CI 0.024–2.690, $P =$

Table 3 Association of CETP genotypes on lipids and lipoproteins: ANCOVA

	TC <i>P</i> values	TG <i>P</i> values	HDL <i>P</i> values	LDL <i>P</i> values	VLDL <i>P</i> values	ApoA1 <i>P</i> values	ApoB <i>P</i> values
CETP Taq1B ^a	0.041*	0.727	0.286	0.014*	0.723	0.965	0.914
CETP Taq1B ^b	0.027*	0.766	0.169	0.011*	0.780	0.695	0.396
CETP Taq1B ^c	0.045*	0.803	0.363	0.014*	0.802	0.764	0.308
CETP Taq1B ^d	0.021*	0.670	0.321	0.018*	0.675	0.928	0.560
APOE genotypes ^a	0.018*	0.276	0.115	0.041*	0.254	0.190	0.732
APOE genotypes ^b	0.027*	0.278	0.217	0.058	0.257	0.263	0.634
APOE genotypes ^c	0.197	0.211	0.179	0.459	0.196	0.320	0.207
APOE genotypes ^d	0.164	0.234	0.064	0.475	0.218	0.272	0.983

The ANCOVA model was adjusted for age, sex, and BMI. In addition, smoking^a, alcohol use^b, diabetes^c, and hypertension^d were included as covariates

* $P < 0.05$, statistically significant

Table 4 Stepwise logistic regression analysis of risk of coronary artery disease in case-control studies

		OR	95% CI	P
1	Smoking	4.347	1.888–10.021	0.001
2	CETP Taq1B2	0.312	0.116–0.841	0.021

Backward stepwise regression analysis was performed including the following variables: smoking, hypertension, diabetes, genotypes LpL D9N, LpL S447X, CETP Taq1B, eNOS G298A, and APOE. Regression models were additionally adjusted by age

0.256), LpL S447X (OR 0.970, 95% CI 0.404–2.331, $P=0.946$), eNOS Glu298Asp (OR 1.137, 95% CI 0.601–2.151, $P=0.693$) or hypertension (OR 1.585, 95% CI 0.689–3.645, $P=0.279$) and diabetes (OR 1.232, 95% CI 0.403–3.764, $P=0.279$) were associated with the risk of CAD. The association can be explained by a dominant model of inheritance of the protective allele (Table 5).

Discussion

Indians worldwide demonstrate a triad of high TG with high LDL-C and low HDL-C levels (Ravi et al. 2004). The present study was undertaken with the aim to understand the genetic factors that may affect these variables and to see their association with CAD.

Frequency of the polymorphisms and comparison with different populations

The frequency of CETP Taq1B SNP is distinct in Indians. A higher frequency of the CETP B2 allele (49.7%) was seen in the present study compared to 25.5% in African-Americans (Cuchel et al. 2002) and 46% in Caucasians (Freeman et al. 1994). Our results corroborate the findings of other studies on Indian population (Mukherjee and Shetty 2004; Dixit and Mittal 2005). The APOE ϵ 2, ϵ 3, and ϵ 4 allele frequencies are significantly heterogeneous

among world populations. The frequency of the ϵ 4 allele in the present study (0.093) is lower than that seen in most studies on Caucasians (Volcik et al. 2006; Luc et al. 1994; Ruixing et al. 2008) and African-Americans (Volcik et al. 2006), and closer to studies on other Asian (Ruixing et al. 2008) and Indian population (Venkatramana et al. 2001). The genotype frequency of eNOS Glu298Asp was: GG=67%, GT=31%, TT=2%. Homozygosity for the Asp298 or “T” allele of the eNOS gene was rare in our study (0.02) similar to other Asian population (Hibi et al. 1998) and unlike that in Caucasians where a relatively higher frequency (15.3%) of homozygous TT genotype was observed (Agema et al. 2004). The LpL N291S variant which predisposes to more severe dyslipidaemia with increasing age and weight gain and is also associated with coronary heart disease (CHD) and T2DM (Yaomin et al. 2006) was not seen in the current study and may be extremely rare or absent in the Indian population; the allelic frequency of 447X is also low (0.08) as compared to the Mediterranean population where the frequency is 0.14 (Corella et al. 2002). The D9N frequency in normal population reported to be 2–4% (Wittrup et al. 1999) was 1% in our study.

Effect of the variants on lipid parameters

Several studies have reported an association of Taq1 B polymorphism with lipid variation; B2 allele has been shown to be associated with higher HDL-cholesterol levels (Bernard et al. 1998; Kuivenhoven et al. 1997). Tai *et al.*, in his study on multiethnic groups in Singapore comprising Chinese, Malays, and Asian Indians, have reported that Asian Indians have the lowest HDL-cholesterol levels even though the highest frequency of B2 allele was seen (Tai et al. 2003). In their study, dietary cholesterol showed a significant interaction with the Taq1B polymorphism in determining HDL-C concentrations in Indians and Malays but not in Chinese. In the present study, significant association of CETP Taq1B SNP was seen with total

Table 5 Assessment of genetic models of CETP Taq1B rs708272

	Model	Genotype	OR (95% CI)	P value	AIC
<p><i>P</i> values were obtained from logistic regression modeling after adjustment for age, gender, diabetes, and hypertension</p> <p>OR odds ratio, CI confidence interval, AIC Akaike information criterion</p> <p>*$p<0.05$, statistically significant</p>	Codominant	B1/B1	1.00	0.099	317
		B1/B2	0.56 (0.31–1.03)		
		B2/B2	0.50 (0.24–1.05)		
	Dominant	B1/B1	1.00	0.034*	315.1
		B1/B2-B2/B2	0.54 (0.31–0.95)		
	Recessive	B1/B1-B1/B2	1.00	0.28	318.5
		B2/B2	0.70 (0.36–1.35)		
	Overdominant	B1/B1-B2/B2	1.00	0.28	318.5
		B1/B2	0.75 (0.44–1.27)		
	Log-additive			0.69 (0.047–1.00)	315.7

cholesterol and LDL-C across all covariates indicating that the atheroprotective effect of the CETP Taq1B polymorphisms may be due to a more favorable lipid profile.

The Atherosclerosis Risk in Communities (ARIC) Study (Volcik et al. 2006) has reported an association of APOE genotype and LDL and HDL cholesterol levels in both white and African-Americans. In the present study, an association of APOE genotypes with TC as well as LDL-C was seen, but the significance was lost when diabetes and hypertension were the covariates. The state of insulin resistance that is seen in diabetes also contributes to dyslipidaemia.

In the study by Masana *et al.*, none of the LpL polymorphisms D9N, N291S, and S447X showed any differences between upper and lower tertiles for any of the plasma lipid levels (Masana et al. 2001). Similarly, the Wandsworth Heart and Stroke Study has found no association between serum TG and HDL-cholesterol concentrations and these variants in the three ethnic groups being studied, i.e., whites, South Asians, and blacks of African origin coresident in South London (Hall et al. 2000). Whereas, 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 (Wittrup et al. 2002). Among the populations of Asian ethnicity, the X447 allele showed borderline (McGladdery et al. 2001) or no association with HDL-C concentration (Hall et al. 2000), which is also reflected in the current study.

Effect of the variants on CAD

The study by Kathiresan et al. (Kathiresan et al. 2008) emphasizes that multiple common alleles influence variation for quantitative traits like LDL and HDL cholesterol levels, and these lipid-associated SNPs may provide incremental information about cardiovascular risk beyond mere lipid levels. In the Framingham Offspring Study, the B2B2 genotype was associated with decreased CETP activity, increased concentrations of HDL-C, and decreased CHD risk (Ordovas et al. 2000). In the Veterans Affairs HDL-C Intervention Trial, carriers with the B2 allele had a lipoprotein profile more favorable than that of noncarriers. Men with the B2B2 genotype also had fewer CHD end points than did noncarriers suggesting that the favorable lipoprotein profile was atheroprotective (Brousseau et al. 2002). That a significant interaction exists between the CETP Taq1B genotype and the progression of CHD in men has been reported from the Regression Growth Evaluation Statin Study (Kuivenhoven et al. 1998), whereas Eiriksdottir et al. (Eiriksdottir et al. 2001) have reported that the significantly delayed occurrence of first myocardial infarction in men with CHD having the B2B2 genotype. In the

current study, the CETP B2B2 allele was found to confer atheroprotection, possibly due to the more favorable lipid profile seen with the genotype. Another study on Asian Indians (Poduri et al. 2009) indicates CETPTaq1B1B1/405II/APOAI-75GA to be best model of CAD risk prediction in our population. Our results corroborate their findings with the CETP B2B2 allele being atheroprotective. Regarding the APOE polymorphism, in the Coronary Artery Risk Development in Young Adults Study (Howard et al. 1998) on 3485 African-Americans and whites in the USA, it was suggested that APOE phenotype could be a risk factor for cardiovascular diseases in both the populations as it was associated similarly with an adverse lipoprotein profile. In the Multiple Risk Factor Intervention Trial (Eichner et al. 1993) and the Fabry Outcome Survey (FOS; Wilson et al. 1994), the association of the ϵ 4 allele and CHD persisted after adjustment by traditional coronary risk factors. There have also been many studies where no association (Kolovou et al. 2000) has been reported. In the current study, we did not find any differences in the ϵ 4 allele frequency between the groups nor decreased risk with the ϵ 2 allele.

In the FOS (Kastelein et al. 1999), both LpL genotypes D9N and N291S were associated with lipoprotein changes compatible with increased atherosclerosis risk. The LpL D9N has also been associated with progression of carotid artery plaque (Spence et al. 2003), whereas S447X was associated with magnetic resonance imaging-detected strokes but not with changes in plasma lipoproteins. Yang et al. found that the frequency of the X447 allele was significantly lower in cases than in controls (6.2% vs 7.6%; $P<0.01$) and suggested that the X447 allele of the *LPL* gene may protect from myocardial infarction, although this effect is small (Yang et al. 2004); however, literature is inconsistent regarding the LpL polymorphisms and the association with CAD (Yang et al. 2004; Zhang et al. 1995b). Overall, we found no significant difference in D9N and S447X carrier frequencies between subjects with and without CAD.

No association between eNOS, Glu298Asp, and CAD was also seen (OR 1.130, 95% CI 0.658–1.939, $P=0.679$); this is in agreement with a previous study from South India (OR=0.66, 95% CI 0.11–4.04, $P=1.0$; Mathew et al. 2008).

In summary, though this study has limitations, the most important one is a limitation of sample size and, consequently, statistical power to examine multiple interactions, but these preliminary findings could serve as a reference point for further studies with larger sample sizes. There are significant geographical variations in the risk for CAD, with India gearing to develop the highest incidence of CAD. In this context, it is important to monitor the differences in allele frequencies. The frequency of the polymorphisms studied is distinct in the Indian population.

The CETP B2 allele is associated with a decrease of CAD risk; however, none of the other variants can be used as markers for CAD in Asian Indians.

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