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ORIGINAL ARTICLE

Association of *CETP* and *LIPC* Gene Polymorphisms with HDL and LDL Sub-fraction Levels in a Group of Indian Subjects: A Cross-Sectional Study

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Abstract There is an increasing interest to understand the molecular basis of high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) subfractions and their association with coronary artery disease (CAD). The formation of these subfractions is greatly influenced by hepatic lipase (HL) and cholesteryl ester transfer protein (CETP) enzymes. To identify genetic markers influencing LDL and HDL subfractions and their role in CAD we performed a case–control genetic association study on 117 healthy controls and 119 angiographically verified CAD patients. Biochemical analysis was performed using standard assays. HDL-C and LDL-C subfractions were estimated using precipitation methods. Genotyping of C-514T (rs1800588) in the *LIPC* gene for HL and I405V (rs5882) in the *CETP* gene was done using PCR-based restriction enzyme analysis and sequencing. Both the polymorphisms were not associated with CAD. The C-514T was associated with increased HDL_3-C levels in controls ($P = 0.049$). The I405V polymorphism was found to be associated with low levels of small dense, LDL ($P = 0.038$). A multiple regression analysis showed that the effects were dependent on gender and triglyceride levels. We conclude that these polymorphisms are not associated with CAD but are important determinants of HDL-C and small dense LDL particles in our population.

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Introduction

Coronary heart disease (CHD) is a major cause of morbidity and mortality in India. While the prevalence of coronary artery disease (CAD) doubled in rural areas, in urban India it quadrupled over the past four decades [1]. This could be attributed to the high prevalence of metabolic syndrome in Indians, which is characterized by abdominal obesity, insulin resistance, hypertension and atherogenic dyslipidemia [1]. The common feature of atherogenic dyslipidemia includes elevated triglyceride, low high density lipoprotein cholesterol (HDL-C) and increased presence of small dense, low density lipoprotein (sdLDL) which is highly atherogenic [1, 2]. Earlier studies have focused on HDL subfractions, HDL_2 and HDL_3, and their role in CAD, however there is a controversy regarding which of these is more cardioprotective [3, 4]. The formation of these subfractions is influenced by polymorphisms in the hepatic lipase (*LIPC*) [5, 6] and cholesteryl ester (CE) transfer protein (*CETP*) [7] genes, both of which participate in lipoprotein metabolism.

CETP is known to transfer CE from HDL to very low density lipoprotein (VLDL) in exchange for triglyceride (TG). While the CE in the VLDL/LDL pool is delivered to the liver and eliminated from the body as a component of bile, the TG in low density lipoprotein (LDL) and HDL is hydrolyzed by hepatic lipase (HL) resulting in smaller, denser particles [8]. HL is involved in the metabolism of IDL (intermediate density lipoprotein) and large LDL to sdLDL particles and in the conversion of HDL_2 to HDL_3, in addition to its key role in reverse cholesterol transport [9]. The *CETP* and *LIPC* genes therefore are strong candidate genes to

influence the risk of CAD. The T allele of the C-514T (rs1800588) *LIPC* polymorphism is less transcriptionally active than the C allele and has been associated with low HL activity and increased HDL particle size [6]. Polymorphisms in the *CETP* gene have been associated with CETP activity, HDL and LDL particle size and risk of CAD [10]. The I405V missense polymorphism (rs5882) in the *CETP* gene has been associated with increase in HDL-C and lipoprotein subclasses [7]. The −629C/A promoter polymorphism (rs1800775), on the contrary affects 50 % of CETP activity and has showed HDL-C raising effect [11]. There have been other polymorphisms in the *CETP* (D442G) [12] and *LIPC* genes (−2T/C) [13] that have been associated with CAD risk. We hypothesize that polymorphisms in these genes may be responsible for high prevalence of atherogenic dyslipidemia (sdLDL and reduced HDL) and could be important determinants of CAD risk in our population.

With high incidence of premature CAD and atherogenic dyslipidemia in India it becomes imperative to identify genetic markers that influence the clinical outcome. With this aim we undertook this study to evaluate the influence of polymorphisms in these genes on lipoprotein particle size in our population and evaluate their role in CAD.

Materials and Methods

Subjects

The study was approved by the Institutional research ethics committee. Patients selected were those who came to our hospital with chest pain and were asked to undergo angiography. Subjects found to have ≥70 % stenosis even in one of the vessel were said to have angiographically verified CAD. These patients were selected for the study. For controls we selected subjects who came for health check up at our hospital. Subjects who were found to be non-diabetic, normotensive (<140/90) and with no past or family history of heart disease were included in the study.

Informed consent was obtained from both the patients and controls. Blood samples were collected from both patients and controls who were fasting overnight for 12 h. Additionally in patients the blood was drawn prior to the angiographic procedure. Serum samples were used for biochemical analysis whereas the EDTA anticoagulated blood was used for DNA extraction and subsequently for genotyping using PCR.

Biochemical Analyses

Serum sample was analyzed for total cholesterol (TC), TG, HDL-C, and LDL-C on automated analyzer Synchron Lx20 using enzymatic methods. Both HDL-C and LDL-C were estimated directly from serum using kits from Daichii Pure Chemicals, Japan. Apolipoprotein (apo) AI and apo B were measured by rate nephelometry method on IMMAGE. The kits for TC, TG, apo A1, and apo B were obtained from Beckman, USA. sdLDL was analyzed on Synchron Lx20 using the direct LDL kit, after precipitating serum using Heparin–$MgCl_2$ [14]. HDL-C subfraction was estimated using polyethylene glycol based method (reagents kindly provided by Gerhard Unzeitig, Technoclone, Austria). HDL_3-C was estimated from supernatant, and HDL_2-C calculated after subtracting HDL_3-C from HDL-C. High sensitive C-reactive protein was measured from serum by immunoturbidimetric method (Randox, India).

Genotyping

The C-514T and T-2C *LIPC* polymorphism was genotyped using PCR-based *Nla*III [15] and *Hae*III [13] restriction digestion method whereas I405V and D442G *CETP* polymorphism was genotyped using *Rsa*I and *Msp*I [7] restriction digestion method. The *CETP*–629C/A polymorphism was genotyped using allele-specific primers (C-allele: 5′-gat atg cat aaa ata act ctg gtg-3′; A-allele: 5′-gat atg cat aaa ata act ctg cgt-3′ and a common forward primer: 5′-gcc cca gct gta ggt aaa gta-3′) [11]. The genotypes of all these various polymorphisms were confirmed by bi-directional sequencing (Gene Om, and Bangalore Genei, India).

Statistical Analysis

SPSS version 17.0 (IBM Corp., Armonk, NY, USA) and Stat View software version 5.0 (SAS Institute Inc., Cary, NC, USA) were used to analyze the data. Mean and standard deviation were calculated. Pearson's χ^2 test was applied to test the relationship of categorized independent and dependent variables. Odds ratio and their 95 % confidence intervals were calculated. A P value <0.05 was considered statistically significant. Normality of the data was checked by Kolmogorov–Smirnov test. To study significant variation in the distribution of the various parameters (lipid, lipoproteins, and their subfractions (sdLDL, HDL_2, HDL_3) one-way analysis of variance (ANOVA) was used as the test of significance. If one-way ANOVA was significant, post hoc tests were used to see which pairs were significant. A multiple regression model was used to evaluate the gene effect on various lipid parameters with age, gender and TG levels included as covariates.

Results

The analysis was performed on 117 controls and 119 cases. One healthy control was excluded from the study due to

Table 1 Levels of biochemical analytes in controls and patients

Parameters[a]	Controls ($n = 117$)	Patients ($n = 119$)	P value
Age (years)	52.62 ± 9.31	54.14 ± 8.27	0.1784
TC (mg/dl)	193.41 ± 28.01	157.53 ± 39.03	<0.0001
TG (mg/dl)	112.22 ± 42.71	163.82 ± 90.33	<0.0001
HDL-C (mg/dl)	50.06 ± 9.87	34.92 ± 8.33	<0.0001
HDL_2-C (mg/dl)	12.58 ± 6.26	10.66 ± 4.53	0.018
HDL_3-C (mg/dl)	37.48 ± 7.26	24.26 ± 6.02	<0.0001
TC/HDL-C	4.04 ± 1.07	4.68 ± 1.20	<0.0001
VLDL (mg/dl)	22.58 ± 8.56	32.65 ± 18.1	<0.0001
hsCRP (mg/l)	0.26 ± 0.519	1.20 ± 2.281	<0.0001
Apo A1 (g/l)	1.26 ± 0.18	0.99 ± 0.17	<0.0001
Apo B (g/l)	1.00 ± 0.21	0.98 ± 0.24	0.506
LDL-C (mg/dl)	120.47 ± 24.38	101.59 ± 31.6	<0.0001
sdLDL (mg/dl)	20.26 ± 9.78	14.04 ± 7.7	<0.0001

[a] *TC* total cholesterol, *HDL_2-C* HDL–C fraction 2, *HDL_3-C* HDL-C fraction 3, *Chol:HDL-C* cholesterol/HDL-C ratio, *hsCRP* high sensitive C-reactive protein, *Apo A1* Apo lipoprotein A1, *Apo B* apolipoprotein B, *LDL-C* low density lipoprotein cholesterol

P values calculated using Students *t* test

unavailability of serum sample for biochemical measurements. Table 1 shows the biochemical measurements in controls and cases. The 2 groups were found to be similar for age with preponderance of male population in both the groups (69 % in controls and 75 % in cases). Of note, the hsCRP levels were significantly elevated in the cases.

Table 2 shows the genotype and allele frequency distribution of the polymorphisms included in the study. The allele frequencies for C-514T and I405V did not deviate from Hardy–Weinberg equilibrium. None of the *LIPC* and *CETP* polymorphisms were found to be associated with CAD or severity of CAD (data not shown). No homozygous mutants were observed for C-629A. For both T-2C and D442G only wild types were seen. Table 3 shows the rare allele frequencies seen in our population and other populations.

To evaluate the effect of polymorphisms on HDL and LDL subfractions we analyzed the effect of the genotypes on the biochemical measurements in healthy controls and patients (Tables 4, 5, and 6). The C-514T polymorphism was associated with an increase in HDL_3-C levels ($P = 0.04$) in healthy subjects (Table 4). On further categorizing the data according to gender, the T carriers were associated with increase in both HDL_3-C ($P = 0.04$) and apo A1 ($P = 0.047$) in healthy males. No such association was observed in healthy females and in patient group. Genotype effect was seen for the I405V polymorphism of the *CETP* gene. The V carriers were found to be associated with reduced sdLDL in healthy controls ($P = 0.03$) (Table 5) that remained significant with HDL-C ($P = 0.01$) in healthy men along with significantly elevated HDL_2-C levels ($P = 0.018$) and sdLDL levels ($P = 0.011$). A similar genotype effect was seen in patients

Table 2 Genotype and allele frequencies in controls and patients for LIPC, and CETP polymorphisms

Polymorphisms	Genotypes			Alleles	
C-514T	CC (%)	CT (%)	TT (%)	C	T
Controls ($n = 117$)	66 (56.4)	40 (34.2)	11 (9.4)	0.73	0.26
Patients ($n = 119$)	66 (55.5)	40 (33.6)	13 (10.9)	0.72	0.27
	$\chi^2 = 0.15$, df = 2, NS			$\chi^2 = 0.091$, df = 1, NS	
T-2C	TT (%)	TC (%)	CC (%)	T	C
Controls ($n = 117$)	117 (100)	–	–	1.000	0.000
Patients ($n = 119$)	119 (100)	–	–	1.000	0.000
I405V	AA (%)	AG (%)	GG (%)	A	G
Controls ($n = 117$)	32 (27.4)	66 (56.4)	19 (16.2)	0.55	0.44
Patients ($n = 119$)	39 (32.8)	60 (50.4)	20 (16.8)	0.57	0.42
	$\chi^2 = 0.985$, df = 2, NS			$\chi^2 = 0.283$, df = 1, NS	
C-629A	CC (%)	CA (%)	AA (%)	C	A
Controls ($n = 117$)	20 (17.1)	97 (82.9)	–	0.58	0.41
Patients ($n = 119$)	24 (20.2)	95 (79.8)	–	0.60	0.39
	$\chi^2 = 0.368$, df = 1, NS			$\chi^2 = 0.115$, df = 1, NS	
D442G	TT (%)	TC (%)	CC (%)	T	C
Controls ($n = 117$)	117 (100)	–	–	1.000	0.000
Patients ($n = 119$)	119 (100)	–	–	1.000	0.000

P values calculated using Pearson's χ^2 test

NS non-significant

Table 3 Comparison of the rare allele frequencies in our population with various populations

Population (n) [Ref]	Rare allele		
	T	V	A
Indians ($n = 117$) [our study]	0.264	0.444	0.414
Indians ($n = 171$) [19]	–	0.530	0.640
Indians ($n = 375$) [23]	0.273	–	–
Chinese ($n = 1324$) [23]	0.374	–	–
Taiwan ($n = 283$) [26]	–	0.159	–
Japanese ($n = 136$) [7]	–	0.480	–
Asian ($n = 30$) [25]	–	0.617	0.500
White American ($n = 82$) [24]	0.170	–	–
Caucasians ($n = 2188$) [25]	–	0.318	0.482
African-American ($n = 84$) [24]	0.523	–	–
African-American ($n = 148$) [25]	–	0.611	0.573

wherein apo B and sdLDL levels were lowered in the V carriers ($P = 0.005$ and $P = 0.01$ respectively) (Table 5). Additionally, the total cholesterol, LDL-C levels and cholesterol/HDL ratio were also significantly lower indicating that individuals with V carriers may respond better to lipid-lowering treatment. The promoter polymorphism C-629A of the CETP gene was found to be significantly associated with elevated apo A1 levels ($P = 0.01$) in healthy controls (Table 6) that remained significant in healthy males. The patient population showed significant association of the CA genotype with elevated HDL-C, HDL subfractions and apo A1. Further analysis by gender showed an association with increased HDL ($P = 0.003$), HDL_3-C ($P = 0.006$), and apo A1 ($P = 0.03$) in male patients (Table 6) and HDL-C ($P = 0.04$), and HDL_2-C ($P = 0.004$) in female patients (data not shown).

A multiple regression analysis indicated that the effect of the C-514T on HDL subfraction and that of I405V on sdLDL levels were dependent on gender and triglyceride levels ($P < 0.0001$).

Discussion

CHD continues to be a major health problem in India and is influenced by both environmental and genetic factors. The high prevalence of coronary risk factors in urban Indian population is an indication for initiating programs for primary prevention of CHD. Genetic evidence from our earlier studies has highlighted the role of various gene polymorphisms in CAD and its risk factors [16–18]. There have been similar studies from Southern parts of India in the Tamilian population where polymorphisms (−629C/T and I405V) in the *CETP* gene have been evaluated among the general and CAD population [19, 20]. The overall goal of this research was to study the influence of *LIPC*, and *CETP* gene polymorphism on HDL and LDL subfractions in both healthy and CAD subjects.

The genotype and allele frequency distribution of the polymorphisms included in the study is shown in Table 2. The allele frequencies did not deviate from

Table 4 Effect of −514C>T LIPC Genotypes on biochemical analytes among Controls and Patients and according to gender within the 2 groups

Controls ($n = 117$)				
Parameters	CC ($n = 66$)	CT ($n = 40$)	TT ($n = 11$)	P value
HDL-C (mg/dl)	48.45 ± 10.43	51.63 ± 9.02	54.0 ± 7.81	0.105
HDL_2-C (mg/dl)	12.36 ± 5.99	12.7 ± 6.99	13.45 ± 5.39	0.859
HDL_3-C (mg/dl)	36.09 ± 8.14	38.92 ± 5.79	40.55 ± 4.25	0.049
LDL-C (mg/dl)	121.65 ± 27.08	118.60 ± 20.50	120.18 ± 21.50	0.825
sdLDL (mg/dl)	21.02 ± 10.94	19.2 ± 8.03	19.55 ± 8.50	0.635
Males ($n = 81$)				
	CC ($n = 51$)	CT ($n = 25$)	TT ($n = 5$)	
HDL_3-C (mg/dl)	34.51 ± 7.23	37.24 ± 5.22	41.4 ± 4.62	0.037
Apo A1 (g/l)	1.18 ± 0.16	1.26 ± 0.14	1.31 ± 0.92	0.047
Patients ($n = 119$)				
	CC ($n = 66$)	CT ($n = 40$)	TT ($n = 13$)	
HDL-C (mg/dl)	35.36 ± 7.69	34.23 ± 9.37	34.85 ± 8.56	0.795
HDL_2-C (mg/dl)	10.44 ± 4.12	11.2 ± 5.21	10.15 ± 4.49	0.645
HDL_3-C (mg/dl)	24.92 ± 5.67	23.03 ± 6.72	24.69 ± 5.27	0.281
LDL-C (mg/dl)	100.59 ± 31.97	104.68 ± 31.98	97.23 ± 30.02	0.710
sdLDL (mg/dl)	13.98 ± 7.26	14.45 ± 8.43	13.08 ± 8.09	0.854

P values calculated using one way analysis of variance (ANOVA)

Table 5 Effect of I405V CETP Genotypes on biochemical analytes among controls and patients and according to gender

Controls ($n = 117$)				
Parameters	II ($n = 32$)	IV ($n = 66$)	VV ($n = 19$)	P value
TC (mg/dl)	198.59 ± 25.11	193.27 ± 30.80	185.16 ± 20.62	0.255
HDL-C (mg/dl)	48.28 ± 11.59	50.29 ± 8.94	52.26 ± 9.84	0.367
HDL_2-C (mg/dl)	10.94 ± 7.32	13.14 ± 6.04	13.42 ± 4.68	0.217
HDL_3-C (mg/dl)	37.34 ± 7.95	37.15 ± 7.07	38.84 ± 6.91	0.669
TC/HDL-C	4.41 ± 1.38	3.97 ± 0.94	3.65 ± 0.70	0.033
Apo A1 (g/l)	1.27 ± 0.2	1.25 ± 0.18	1.25 ± 0.15	0.919
Apo B (g/l)	1.05 ± 0.18	1.02 ± 0.22	0.92 ± 0.14	0.097
LDL-C (mg/dl)	124.69 ± 22.87	121.05 ± 25.95	111.37 ± 19.42	0.162
sdLDL (mg/dl)	22.63 ± 10.44	20.48 ± 9.78	15.47 ± 7.04	0.038
Males ($n = 81$)				
	II ($n = 20$)	IV ($n = 47$)	VV ($n = 14$)	
HDL-C (mg/dl)	42.70 ± 7.42	48.0 ± 8.05	51.36 ± 10.27	0.01
HDL_2-C (mg/dl)	8.75 ± 4.31	12.06 ± 5.44	13.50 ± 4.96	0.018
sdLDL (mg/dl)	27.0 ± 10.64	21.89 ± 9.05	17.14 ± 7.43	0.011
Patients ($n = 119$)				
	II ($n = 39$)	IV ($n = 60$)	VV ($n = 20$)	
TC (mg/dl)	169.00 ± 44.23	156.2 ± 35.93	139.15 ± 30.11	0.018
HDL-C (mg/dl)	32.21 ± 8.00	36.82 ± 8.33	34.55 ± 7.78	0.025
HDL_2-C (mg/dl)	10.00 ± 4.32	10.85 ± 4.70	11.4 ± 4.44	0.484
HDL_3-C (mg/dl)	22.21 ± 5.16	25.97 ± 6.37	23.15 ± 5.17	0.006
TC/HDL-C	5.36 ± 1.16	4.37 ± 1.08	4.27 ± 1.11	<0.0001
Apo A1 (g/)	0.95 ± 0.14	1.03 ± 0.17	0.95 ± 0.14	0.022
Apo B (g/l)	1.07 ± 0.23	0.96 ± 0.24	0.86 ± 0.19	0.005
LDL-C (mg/dl)	112.72 ± 35.24	99.13 ± 28.58	87.30 ± 26.37	0.009
sdLDL (mg/dl)	16.90 ± 8.17	12.87 ± 7.34	12.00 ± 6.51	0.016

P values calculated using one way analysis of variance (ANOVA)

Hardy–Weinberg equilibrium. None of the polymorphisms included in this study were found to be associated with CAD. Similar findings have been reported among Indians and other populations [20–22]. However, in the study by Padmaja et al. [20] among Indians the −629CA was associated with CHD risk among men. Further analysis was performed to evaluate the effect of simultaneous presence of both rare alleles (−514T and 405V) on CAD, however, no significant association was observed in our study.

The allele frequencies of the polymorphisms studied showed ethnic specific distribution. Table 3 shows the comparison of major SNPs observed in this study to some of the other major ethnic groups across the globe. In the *LIPC* promoter polymorphism C-514T, the minor allele frequency in our control was lower than the Chinese and Malays from Singapore, similar to the Indians in Singapore [23] and African-Americans [24] but higher than white Americans [15]. The wild type A allele of I405V (A→G) polymorphism in our study was slightly lower than the earlier reports from Southern parts of India [19] but significantly lower as compared to African-Americans and Asians and significantly higher than the Caucasians [25]. However, on comparison with other Asian studies than the above the minor allele frequency was found to be almost similar to the Japanese [7], but lower than the Chinese [26]. Similarly, the −629A allele was found to be significantly lower than the earlier published reports from Southern India [19] and the African-Americans but slightly lower than the Caucasians [25]. Also, the A allele of C-629A polymorphism associated with elevated HDL-C levels in our study is lower than the Scottish [27] and the Netherland population [11]. This finding seems to be in agreement with the observation of low HDL-C levels seen in the Indian population [28]. Both the T-2C and the D442G polymorphisms in all the subjects studied were found to be homozygous for the wild type. The differences in the allele frequencies between the various populations and our population could be due to the differences in the sampling, sample size and may also be attributed to racial and ethnic variations. India consists of various distinct population groups that have remained isolated from each other due to various religious and cultural differences and population

Table 6 Effect of −629C>A CETP genotypes on biochemical analytes among controls and patients and according to gender

Controls ($n = 117$)			
Parameters	CC ($n = 20$)	CA ($n = 97$)	P value
HDL-C (mg/dl)	49.75 ± 11.89	50.12 ± 9.47	0.878
HDL_2-C (mg/dl)	13.2 ± 7.56	12.45 ± 5.99	0.629
HDL_3-C (mg/dl)	36.55 ± 7.27	37.67 ± 7.28	0.532
Apo A1 (g/l)	1.16 ± 0.18	1.28 ± 0.18	0.012
sdLDL (mg/dl)	19.75 ± 8.35	20.36 ± 10.09	0.801
Males ($n = 81$)			
	CC ($n = 15$)	CA ($n = 66$)	
Apo A1 (g/l)	1.13 ± 0.15	1.23 ± 0.15	0.015
Patients ($n = 119$)			
	CC ($n = 24$)	CA ($n = 95$)	
HDL-C (mg/dl)	29.71 ± 7.29	36.24 ± 8.08	0.001
HDL_2-C (mg/dl)	8.33 ± 3.77	11.25 ± 4.53	0.001
HDL_3-C (mg/dl)	21.38 ± 5.44	24.99 ± 5.97	0.006
TC/HDL-C	5.26 ± 1.36	4.53 ± 1.12	0.011
Apo A1 (g/l)	0.91 ± 0.13	1.01 ± 0.16	0.008
sdLDL (mg/dl)	12.50 ± 6.97	14.43 ± 7.87	0.274
Males ($n = 90$)			
	CC ($n = 18$)	CA ($n = 72$)	
HDL-C (mg/dl)	28.06 ± 7.06	34.44 ± 7.97	0.003
HDL_2-C (mg/dl)	8.33 ± 3.77	11.25 ± 4.53	0.001
HDL_3-C (mg/dl)	21.38 ± 5.44	24.99 ± 5.97	0.006
TC/HDL-C	5.26 ± 1.36	4.53 ± 1.12	0.011
Apo A1 (g/l)	0.91 ± 0.13	1.01 ± 0.16	0.008

P values calculated using Students t test

from Mumbai is heterogeneous and therefore a larger study would help in confirming these findings in our population.

The C-514T is known to be in linkage disequilibrium with 3 other promoter polymorphisms [29]. In our healthy controls the HDL-C, and HDL_3-C subfraction were elevated in −514T carriers. These subclasses stimulate cholesterol efflux from cells efficiently which represents the first step in the reverse cholesterol transport and therefore highlights the role of the T allele in having a cardio-protective role. Regression analysis showed that this effect was dependent on gender and triglyceride levels. The latter effect could be due to the association of decreased HL activity with T allele [5, 24, 30] and highlights the importance of triglycerides and gene-diet interactions in modulating lipid and lipoprotein levels [16]. The interaction between total fat intake and HL polymorphism is known to affect HDL-C levels [23]. The effect of gender may be due to preponderance of male population in our study. In Mexican-American population from San Antonio Family Heart Study, the authors [31] found no evidence for major locus effect on HDL-C levels after adjustment for apo A1 levels. While Carr et al. [32] found association of buoyant LDL among carriers of −514T allele in pre-menopausal women, in Framingham Offspring Study with 2,667 subjects no association was seen [15]. These discrepancies could have been due to differences in the methods used for estimating sdLDL. In our CAD group the HDL-C levels and its subfractions across the 3 genotypes in the CC carriers were almost the same as in TT carriers. Zambon et al. [33] found that lipid lowering therapy resulted in a decrease in HL activity which was more in CC carriers as compared to CT and TT carriers. This could probably be the reason why the HDL levels and its subfractions did not vary significantly across the genotypes in our study. Probably at baseline the levels would have been even lesser in the CC genotypes as compared to TT genotypes, and this may have been compensated for during the treatment.

In the *CETP* gene I405V and C-629A polymorphisms were studied. The VV genotype showed significantly improved cholesterol–HDL ratio and significantly reduced levels of sdLDL in our controls. The polymorphism seems to play an important role by influencing LDL and HDL subfractions and thereby assume cardioprotective role in our healthy population. Increased levels of sdLDL are highly atherogenic and increase the risk of CVD. A linkage of *CETP* locus to LDL particle size has been previously reported [34]. The I405V polymorphism has been reported to be associated with LDL size [7]. In our patients the TC, LDL-C and sdLDL levels were low in the V carriers. In a study by Bruce et al. [35] in a subpopulation with high plasma TG, the HDL-C levels were significantly higher in the VV group and so was the CHD prevalence. The V allele has been associated with decreased CETP activity as a result of which the transfer of CE from HDL-C to LDL and VLDL in exchange for TG would be affected. This would lead to an increase in HDL-C concentration and simultaneously increase the proportion of TG in VLDL and LDL. The latter being a good substrate for HL and would result in formation of sdLDL [7, 35]. The increased sdLDL concentration in our II carriers as compared to VV carriers in our controls could be due to (i) increased CETP activity and (ii) more CC carriers of the C-514T polymorphism (53 %). In Ashkenazi Jew families ascertained for exceptional longevity [36] subjects carrying the VV genotype had large LDL particle size. High HDL-C in VV patients was also seen by Okumura et al. [7] along with significantly lowered plasma CETP concentration and LDL particle size as against II + IV patients. In our patients, the TC, LDL-C and sdLDL levels were low in the V carriers suggesting that the VV carriers probably respond better to lipid lowering drugs. In a study from Taiwan by Wu et al. [26] both controls and the patients did not show any variation in the TC, LDL-C, HDL-C, and apo A1 levels across the 3 genotypes.

A recent meta-analysis [37] of 92 studies showed that overall, per A allele inherited, carriers of −629C>A variant had lower mean CETP mass, lower CETP activity, higher mean HDL-C levels, higher mean Apo A1 levels and lower

mean TG than CC homozygotes. In our study apo A1 levels were significantly increased in the CA carriers of the healthy controls and remained so in the healthy males. The frequency of A allele associated with increased HDL-C levels in our population is low as compared to other populations and is in agreement with low HDL-C seen in our population [28]. Patients were associated with significantly elevated HDL-C levels and its subfractions. Blakenberg et al. [38] studied the C-629A polymorphism and found it to lower CETP activity, increase HDL-C levels and more importantly, demonstrated for the first time, that the −629A allele was associated with a strong protective effect on future cardiovascular mortality. Mortality decreased from 10.8 % in CC carriers to 4.6 % in CA carriers and 4.0 % in AA carriers. Further the clinical benefit of statin was restricted to CC homozygotes.

Conclusion and Future Directions

None of the polymorphisms were found to be directly associated with CAD or its severity. The presence of wild type homozygous T-2C and D442G implies that these polymorphisms could either be absent or rare in our population. The feasibility of HDL-C fractions in determining CAD risk may be achieved by designing a prospective study with a larger number of healthy subjects. The difference in the HDL-C levels and its subfractions at baseline and the difference over a period of time in those subjects who go onto develop CAD would help in understanding the independent contribution of the 2 subfractions (which is more cardioprotective than the other). Likewise in CAD patients both CETP and hepatic lipase enzyme activity can be measured in order to find the influence of genotypes on enzyme activity and also the effect of statins with respect to genotype on the enzyme activity. Our previous study has shown high prevalence of phenotype B (qualitative presence of sdLDL) in healthy population [39]. The presence of high levels of sdLDL in our healthy males as compared to females suggests that it would be worthwhile to develop reference ranges for sdLDL among our Indian population. This would help in establishing a therapeutic threshold so that persons at risk can be treated with lipid lowering drugs and would definitely be very helpful considering the high risk of early MI in men.

Certain major limitations of the study include the cross-sectional design of this study with no measurements of HL or CETP enzyme activity, and the sample size. However, the findings of the study clearly demonstrate that the C-415T and I405V polymorphisms of the *LIPC* and *CETP* genes are not associated with CAD but are important determinants of levels of HDL-C and dense LDL particles respectively in our population and may have the potential to serve as markers for these traits. Considering the high prevalence of atherogenic dyslipidemia, a feature of metabolic syndrome, in our population, the −514C>T and I405V polymorphisms may have a large impact on our population by modifying these critical lipoproteins and their subfraction levels.

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Conflict of interest The authors have no conflict of interest to declare.

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