Influence of genetic variants in the apolipoprotein A5 and C3 gene on lipids, lipoproteins, and its association with coronary artery disease in Indians

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Received: 4 November 2009 / Accepted: 27 September 2010 / Published online: 17 October 2010
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Abstract Indians worldwide demonstrate a triad of elevated triglyceride (TG) with high low-density lipoprotein cholesterol and low high-density lipoprotein cholesterol levels. In the present study, we aim to investigate the effect of −1131T>C, −3A>G, c.56 C>G, and c.553 G>T SNPs in the apolipoprotein A5 (APOA5) gene and 1100C>T and 3238C>G in the apolipoprotein C3 (APOC3) on plasma lipid and lipoprotein levels and risk of coronary artery disease (CAD) in Indians. Genotyping and lipid assays were carried out using standard protocols in a study population that included 150 controls and 90 cases with CAD. Significant associations between minor alleles and higher TG levels were seen for −1131T>C ($P<0.001$), −3A>G ($P<0.001$), c.56C>G ($P=0.026$), and c.553G>T ($P=0.003$) SNPs in the APOA5 gene and 1100C>T ($P=0.001$) and 3238C>G ($P=0.009$) in the APOC3 gene. The haplotypes 11211, 22111, 11112, and 22112 were significantly associated with TG levels ($P=0.025$, $P=0.017$, $P=0.027$, and $P<0.001$, respectively) and very-low-density lipoprotein cholesterol levels ($P=0.025$, $P=0.017$, $P<0.001$, and $P=0.002$) in males. The 22111, 11112, and 22112 were associated with elevated TG ($P=0.030$, $P=0.036$, and $P=0.024$) but not VLDL-C levels in females. No association with other lipid parameters was seen. In the logistic regression model, the rare S2 allele was a significant risk factor ($P=0.030$, 95% CI 1.186–31.432) along with smoking ($P<0.0001$, 95% CI 2.018–10.397) for CAD. The APOA5 and APOC3 locus is a

strong determinant of plasma TG levels in Indians. The APOC3 3238G is a risk factor for CAD and a higher frequency was also seen with type 2 diabetes mellitus.

Keywords Coronary artery disease · Apolipoprotein A5 · Apolipoprotein C3 · Haplotypes · Triglycerides · India

Introduction

Several studies have shown that hypertriglyceridemia is an independent risk factor for cardiovascular disease (CVD) (Austin 1991; Assmann et al. 1996). A recent meta-analysis performed on >10,000 cases and a population of >250,000 participants indicated that high triglyceride (TG) increases the risk of suffering an adverse CVD event by 72% in addition to that carried by traditional risk factors (Sarwar et al. 2007).

The apolipoprotein A5 (APOA5) gene is a key gene involved in TG and high-density lipoprotein (HDL) level determination (Pennacchio et al. 2001; Pennacchio and Rubin 2003; Groenendijk et al. 2001). Three common haplotypes have been identified in the APOA5 gene: wild-type haplotype APOA5 *1; APOA5 *2, defined by rare alleles of −1131 T>C, −3A>G, IVS3+476G>A, and 1259T>C; and APOA5 *3, defined by a rare allele of S19W (Pennacchio et al. 2002). Since the APOA5 gene is located near the APOA1-C3-A4 gene cluster, there have been studies suggesting that the effect of APOA5 on TG levels may reflect linkage disequilibrium (LD) with functional variants of other genes in the cluster. Evidence from a double-knockout mouse model supports the notion that the APOA5 and APOC3 genes independently influence plasma TG concentrations but in an opposing manner (Ito et al. 1990; Maeda et al. 1994). There have been studies indicating that the −1131T>C in the APOA5 gene is in

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strong LD with the $-482\text{C}>\text{T}$ variant in the apolipoprotein C3 (APOC3) gene (Martinelli et al. 2007). The SstI variant has previously been shown to be associated with elevated levels of TG and is in strong linkage disequilibrium with the $-455\text{T}>\text{C}$ and $-482\text{C}>\text{T}$, both located in the insulin-response site in the promoter of APOC3 (Dammerman et al. 1993).

The strong association between APOA5, APOC3, and TG and the establishment of TG as an independent coronary artery disease (CAD) risk factor (Hokanson and Austin 1996) by meta-analysis suggest that variants in these genes might be associated with atherogenesis. Association studies have been previously reported on the Asian Indian population, mostly on healthy volunteers, to elucidate the role of specific genetic variants in the APOC3 gene (Chhabra et al. 2004) or the APOA5 gene (Chandak et al. 2006) in regulating plasma lipid levels and few studies on individuals with CAD (Shanker et al. 2008).

Hence, given that Indians are more sensitive to the adverse effects of hypertriglyceridemia, in the present study, we examined four SNPs in the APOA5 gene ($-1131\text{T}>\text{C}$, $-3\text{A}>\text{G}$, S19W, G185C) and two in the APOC3 gene ($1100\text{C}>\text{T}$, $3238\text{C}>\text{G}$) to determine whether haplotypes defined by these variants are associated with the risk of premature CAD. To the best of our knowledge, this is the first report that investigates the clinical association of those six polymorphisms in the APOC3-A5 cluster. Also for the first time, we report here the effects of the G185C ($553\text{G}>\text{T}$) polymorphisms on the plasma TG levels in Indian population.

Materials and methods

Subjects

Blood samples were collected from a total of 240 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 subjects were predominantly from Western India. The study was approved by the local ethical committee and informed consent obtained from all the subjects. 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.

Biochemical parameters

Serum total cholesterol, TG, and high-density lipoprotein cholesterol (HDL-C) levels were determined by routine

enzymatic methods (X Imola; Randox Laboratories Ltd, UK). Low-density lipoprotein cholesterol (LDL-C) and very-low-density lipoprotein cholesterol (VLDL-C) were calculated according to Friedwald's formula. Serum apolipoproteins A1 and B were measured by the immunoturbidimetric immunoassay (Daytona, Randox Laboratories Ltd, UK). All the biochemical parameters were performed on fasting serum samples.

Genotyping

Genomic DNA was isolated from peripheral blood using QIAamp® DNA Blood Mini Kit (Qiagen, Hilden, Germany). APOA5 polymorphisms $-1131\text{T}>\text{C}$ (rs 662799), $-3\text{A}>\text{G}$ (rs651821), S19W (rs3135506), and G185C (rs 2075291) and APOC3 polymorphisms $1100\text{C}>\text{T}$ (rs 4520) and $3238\text{C}>\text{G}$ (rs5128) were genotyped. Details of PCR primers, annealing conditions, and restriction enzyme digestion have been described elsewhere (Kao et al. 2003; Tang et al. 2006; Hodoglugil et al. 2006; Waterworth et al. 1999).

Sequencing

Genotypes of 10% of samples were confirmed by 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).

Statistical analysis

Allele frequency was calculated and Hardy–Weinberg equilibrium applied. Chi-square tests were used for comparison of binary variables across groups. The Kolmogorov–Smirnov test was used as test of normality for quantitative variables, and the values were subsequently log-transformed to normalize distribution. We tested for overall difference between genotypes by ANOVA. Independent *t* test was performed to analyze the effect of genotypes on lipid parameters. Odds ratio adjusted for other covariables were determined using logistic regression models. The genotype–phenotype correlation was performed only on controls since almost all the cases were on lipid lowering medications. All statistical tests were performed using the program SPSS (SPSS Inc., Chicago, IL).

Results

Table 1 displays means and standard deviations for the study subjects for relevant biochemical characteristics as

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%	29%
BMI kg/m ²	25.1±3.6	24±3.5
Smoking status (%)		
Smoker <10 cigarettes/day (%)	26*	13
Past smoker (%)	15*	6
Tobacco chewer (%)	4	4
History of diabetes (%)	20*	13
History of hypertension (%)	32*	18
Diabetes + Hypertension (%)	12*	6
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

^aMean±SD for continuous variables*Statistically significant $P<0.01$

well as risk factors. Statistically significant differences were seen in the smoking status ($P<0.0001$), presence of family history ($P=0.034$), as well as hypertension ($P=0.038$) in cases vs controls.

Minor allele frequencies of four SNPs at the APOA5 locus and two SNPs at the APOC3 are shown in Table 2. Hardy–Weinberg proportions were observed for all the SNPs. The –1131T>C and –3A>G in the APOA5 gene are in complete linkage disequilibrium in the Indian population. Minor allele of APOC3 3238C>G was much more frequent in cases (0.34) as compared to controls (0.27) but did not reach statistical significance ($P=0.067$) in the univariate analysis. However, in the logistic regression model, the rare S2 allele was a significant risk factor ($P=0.030$, 95% CI 1.186–31.432) along with smoking ($P<0.0001$, 95% CI 2.018–10.397).

To determine if the allelic variants under investigation associated with lipid and lipoprotein subclasses concentrations, heterozygotes (i.e., 12), and homozygotes (i.e., 22) were combined and tested for association between individual SNPs and plasma lipid levels. Tables 3 and 4 display pattern of association of SNPs with lipid and lipoprotein levels in the APOA5 and APOC3 gene, respectively.

Table 3 displays significant correlation between the –1131T>C polymorphism and plasma TG and VLDL-C concentrations for men ($P=0.002$ and $P=0.047$), women ($P=0.029$ and $P=0.049$), and both genders combined ($P<0.001$ and $P=0.008$). Heterozygotes exhibited 58% and homozygotes exhibited 68% increase in the TG levels. Similar trend emerged with the –3A>G polymorphism. In contrast to certain other studies where the association between c.56C>G and TG levels was not clear, in the

Table 2 Allelic relative frequencies of polymorphisms in APOC3 and APOA5 genes in cases and controls

Allele Frequencies	Controls	Cases	OR	95% CI	<i>P</i> value
APOA5 –1131C	0.2	0.18	0.77	0.444–1.350	0.401
APOA5 –3G	0.2	0.18	0.77	0.444–1.350	0.401
APOA5 c.56G	0.05	0.05	0.56	0.220–1.442	0.267
APOA5 c.553T	0.02	0	0	0–1.053	0.087
APOC3 3238G	0.27	0.34	2.34	0.980–5.566	0.067
APOC3 1100T	0.45	0.41	1.41	0.775–2.576	0.288

Allele frequencies were estimated by direct counting

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

Table 3 Plasma lipids and lipoprotein subclasses according to the polymorphism in the APOA5

	Men		P value	Women		P value	Both		P value
	11	12+22		11	12+22		11	12+22	
−1131T>C									
TC (mg/dl)	183.4±34.7	191±35.6	0.396	190.5±39.5	194.5±47.1	0.810	186±36	192±39.3	0.500
TG (mg/dl)	114.83±52.3	169.97±61.9	0.002	120±58.5	171.23±103.2	0.029	117±54	187±146	0.0002
HDL-C (mg/dl)	36.2±6.68	37.46±7.07	0.538	46.34±8.76	43.64±8.19	0.405	40±9	39.40±8	0.650
LDL-C (mg/dl)	128.6±31.67	128.1±29.5	0.959	123.2±33	124.29±35.17	0.895	126±32	126±31	0.950
VLDL (mg/dl)	22.97±10.45	33.99±12.4	0.047	25.5±12.45	34.27±20.8	0.049	24±11	37±29	0.008
ApoA1 (g/l)	1.07±0.21	1.12±0.18	0.425	1.13±0.18	1.18±0.17	0.567	1.05±0.18	1.14±0.17	0.380
ApoB (g/l)	0.96±0.23	0.98±0.20	0.821	0.90±0.20	0.95±0.24	0.608	0.89±0.23	0.97±0.21	0.560
ApoB/ApoA1 ratio	0.90±0.16	0.88±0.19	0.714	0.80±0.19	0.82±0.24	0.876	0.91±0.14	0.86±0.21	0.210
−3A>G									
Total cholesterol (mg/dl)	183.4±34.7	191.54±35.81	0.430	190.5±39.5	194.5±47.11	0.810	186±36	192±39.9	0.470
TG (mg/dl)	114.83±52.3	169.63±62.8	0.002	120±58.5	171.23±103.9	0.029	117±54	187.35±147.8	0.0004
HDL-C (mg/dl)	36.2±6.68	37.64±6.96	0.473	46.34±8.76	43.69±8.19	0.405	40±9	39.53±7.8	0.690
LDL-C (mg/dl)	128.6±31.67	122.7±32.4	0.526	123.2±33	124.3±35.17	0.895	126±32	123.17±32.9	0.890
VLDL (mg/dl)	22.97±10.45	38.95±32.9	0.051	25.5±12.45	34.24±20.8	0.049	24±11	37.5±29.5	0.009
ApoA1 (g/l)	1.07±0.21	1.10±0.17	0.553	1.13±0.18	1.18±0.18	0.567	1.05±0.18	1.21±0.17	0.490
ApoB (g/l)	0.96±0.23	0.98±0.20	0.840	0.90±0.20	0.95±0.24	0.608	0.89±0.23	0.97±0.21	0.580
ApoB/ApoA1 ratio	0.90±0.16	0.88±0.19	0.812	0.80±0.19	0.82±0.24	0.874	0.91±0.14	0.86±0.21	0.720
56C>G									
Total cholesterol (mg/dl)	183.4±34.7	188.76±60	0.953	190.5±39.5	184.76±60	0.853	186±36	187±52.7	0.840
TG (mg/dl)	114.83±52.3	205±190	0.021	120±58.5	111.6±54	0.984	117±54	169±169.08	0.026
HDL-C (mg/dl)	36.2±6.68	37.23±6.96	0.787	46.34±8.76	45.2±11.4	0.784	40±9	39.2±8.85	0.679
LDL-C (mg/dl)	128.6±31.67	111.98±36.9	0.202	123.2±33	117.8±20.89	0.920	126±32	113.6±32.7	0.208
VLDL (mg/dl)	22.97±10.45	41.06±38.4	0.021	25.5±12.45	22.32±10.08	0.615	24±11	35.9±33.8	0.070
ApoA1 (g/l)	1.07±0.21	1.16±0.22	0.322	1.13±0.18	1.27±0.16	0.438	1.05±0.18	1.19±0.20	0.292
ApoB (g/l)	0.96±0.23	1.01±0.26	0.722	0.90±0.20	0.99±0.041	0.378	0.89±0.23	1.00±0.22	0.414
ApoB/ApoA1 ratio	0.90±0.16	0.86±0.19	0.469	0.80±0.19	0.78±0.92	0.867	0.91±0.14	0.84±0.17	0.529
553G>T									
Total cholesterol (mg/dl)	183.4±34.7	219.75±30.8	0.069	190.5±39.5	122		186±36	203±46.3	0.330
TG (mg/dl)	114.83±52.3	200.25±55.9	0.008	120±58.5	191		117±54	348±370	0.003
HDL-C (mg/dl)	36.2±6.68	38.75±5.8	0.494	46.34±8.76	26		40±9	36.3±6.83	0.319
LDL-C (mg/dl)	128.6±31.67	141.23±23	0.470	123.2±33	58		126±32	115±44.2	0.450
VLDL (mg/dl)	22.97±10.45	40.05±11.19	0.008	25.5±12.45	38.2		24±11	69.7±74.04	0.005
ApoA1 (g/l)	1.07±0.21	0.95±0.36	0.470	1.13±0.18	0.87		1.05±0.18	1.035±0.23	0.690
ApoB (g/l)	0.96±0.23	1.03±0.09	0.718	0.90±0.20	0.68		0.89±0.23	0.91±0.21	0.830
ApoB/ApoA1 ratio	0.90±0.16	1.15±0.34	0.086	0.80±0.19	0.78		0.91±0.14	0.84±0.09	0.990

Mean±SEM. *P* values and means and SEM were determined after adjusting for age, BMI, smoking, diabetic status, and alcohol use. TG and VLDL were natural log-transformed before analysis. Values of 11 genotype are common to all since it includes only those controls, which did not have any of the SNPs, to rule out confounding results

Table 4 Plasma lipids and lipoprotein subclasses according to the polymorphism in the APOC3

	Men		<i>P</i> value	Women		<i>P</i> value	Both		<i>P</i> value
	11	12+22		11	12+22		11	12+22	
Sst1 (C3238C>G)									
TC (mg/dl)	183.4±34.7	181.6±39.21	0.864	190.5±39.5	200.3±36.6	0.466	186±36	188.3±39.29	0.808
TG (mg/dl)	114.83±52.3	157±66.7	0.019	120±58.5	170.9±83.08	0.025	117±54	161.95±72.3	0.001
HDL-C (mg/dl)	36.2±6.68	37.47±7.28	0.533	46.34±8.76	44.6±8.9	0.574	40±9	40.01±8.54	0.890
LDL-C (mg/dl)	128.6±31.67	117.7±32.9	0.234	123.2±33	126.36±28.9	0.680	126±32	120.76±31.4	0.410
VLDL (mg/dl)	22.97±10.45	34.5±12.9	0.001	25.5±12.45	39.7±14.15	0.005	24±11	36.4±13.6	<0.0001
ApoA1 (g/l)	1.07±0.21	1.11±0.18	0.512	1.13±0.18	1.21±0.19	0.321	1.05±0.18	1.14±0.18	0.300
ApoB (g/l)	0.96±0.23	0.96±0.22	0.928	0.90±0.20	1.0±0.18	0.213	0.89±0.23	0.98±0.20	0.466
ApoB/ApoA1 ratio	0.90±0.16	0.89±0.23	0.972	0.80±0.19	0.85±0.23	0.632	0.91±0.14	0.88±0.23	0.540
1100C>T									
TC (mg/dl)	183.4±34.7	190.29±39.19	0.504	190.5±39.5	189.9±38.43	0.964	186±36	190.16±38.7	0.620
TG (mg/dl)	114.83±52.3	170.8±110.2	0.041	120±58.5	153.56±77.03	0.109	117±54	174±136.03	0.009
HDL-C (mg/dl)	36.2±6.68	37.9±7.5	0.381	46.34±8.76	43.9±8.31	0.389	40±9	40±8.29	0.920
LDL-C (mg/dl)	128.6±31.67	120.8±31.9	0.363	123.2±33	121.18±30.9	0.868	126±32	120.93±31.38	0.403
VLDL (mg/dl)	22.97±10.45	37.2±31.9	0.067	25.5±12.45	30.9±15.38	0.182	24±11	34.95±27.25	0.020
ApoA1 (g/l)	1.07±0.21	1.11±0.19	0.489	1.13±.18	1.19±0.20	0.494	1.05±.18	1.13±0.20	0.370
ApoB (g/l)	0.96±0.23	0.98±0.19	0.700	0.90±0.20	0.94±0.21	0.638	0.89±0.23	0.97±0.19	0.510
ApoB/ApoA1 ratio	0.90±0.16	0.91±0.22	0.787	0.80±0.19	0.80±0.22	0.972	0.91±0.14	0.87±0.22	0.667

Mean±SEM. *P* values and means and SEM were determined after adjusting for age, BMI, smoking, diabetic status, alcohol use. TG and VLDL were natural log-transformed before analysis. Values of 11 genotype are common to all since it includes only those controls, which did not have any of the SNPs, to rule out confounding results

Indian population, c.56G was associated with plasma lipids and the mean TG levels were 54% elevated in heterozygotes. The association was statistically significant ($P=0.021$) in males as well as in the combined genders but not in females ($P=0.98$). The c.553G>T SNP also showed a strong association with TG and VLDL-C concentrations in men ($P=0.008$ and $P=0.008$) and in both genders combined ($P=0.003$ and $P=0.005$). The allelic frequency of c.553T was low, i.e., 0.029 ($n=240$). Only five males and one female with heterozygosity 553GT and one male homozygous for the minor variant were identified. All had very high plasma triglyceride levels of 372 ± 320 mg/dl for males and 191 mg/dl for the female. Table 4 depicts association between SNPs in the APOC3 gene and plasma lipids. Significant association was seen with plasma TG and VLDL-C levels in males ($P=0.019$ and $P=0.001$), in females ($P=0.025$ and $P=0.005$), and in both genders ($P=0.001$ and $P<0.001$) with the 3238G>C SNP. The 1100C>T SNP showed association with TG levels in males ($P=0.041$) and in both genders ($P=0.001$), but not in females.

To understand the combined effects of all the SNPs, we constructed haplotypes. The most common haplotypes 11111, 11112, and 22112 represented 23%, 20%, and 25%, respectively of all variants, and the other haplotypes

11211, 22111, and 22121 account for 10%, 16%, and 4%, respectively (haplotypes in the following order –1131T>C, –3A>G, c.56C>G, c. 553G>T, and 3238C>G).

The effects of haplotypes on plasma lipid variables are shown in Fig. 1.

Haplotype 22111 was significantly associated with TG and VLDL-C levels in males ($P=0.018$ and $P=0.018$) and in both genders combined ($P=0.001$ and $P=0.018$) and with TG but not with VLDL-C in females ($P=0.030$ and $P=0.224$). ANOVA performed between groups was highly significant for TG ($P<0.001$), VLDL-C ($P=0.002$), and ApoB ($P<0.0001$), (data not shown) indicating significant variations between the groups. Additive effect of the c.56C>G was seen when combined with –1131T>C; an increase of 70 mg/dl was seen in the TG levels with the 22211 haplotype (not represented) vs the 50 mg/dl increase seen with 22111. The 22112 haplotype showed a highly significant association with TG levels in males, females, and both combined ($P=0.002$, $P=0.02$, and $P<0.001$, respectively). Due to the low frequency of c.553T, the haplotype 22121 has not been represented, as it is unlikely to have a significant impact.

The frequency of the SNPs in diabetics vs non-diabetics is given in Table 5. Significant increase of the S2 allele was seen in diabetics ($\chi^2=10.991$, $P<0.01$). Interactions be-

tween APOC3/A5 haplotypes and the presence of diabetes on the determination of triglyceride concentrations are represented in Table 6. Differences of 2 mg/dl were seen between the 11111 haplotype of diabetics vs the non-diabetics. The haplotype 11112 in diabetics showed an

increase in TG of 20 mg/dl as compared to the non-diabetics. Haplotype 22112 showed a mean increase of 38 and 70 mg/dl in TG in diabetics and non-diabetics, respectively, which was statistically significant when compared with the wild-type haplotype.

Fig. 1 Effect of interaction between *APOC3/A5* haplotypes on changes in plasma lipid and lipoprotein subclasses in the population-based controls. Mean effects and standard errors (*bars*) for each haplotype on lipid and lipoprotein subclasses. Single nucleotide polymorphisms were arranged in the haplotype descriptions from 5 to 3 as follows: –1131T>C, –3A>G, c.56C>G, c.553G>T, and 3238C>G. Haplotype effects were log-transformed and compared to the effect of the 11111 haplotype (reference haplotype). Haplotypes 11111, 11211, 22111, 11112, and 22112 have been represented. Asterisks indicate a significant effect at $P<0.05$. **a** Effect of haplotypes on total cholesterol (TC). **b** Effect of haplotypes on triglycerides (TG). **c** Effect of haplotypes on high-density lipoprotein cholesterol (HDL-C). **d** Effect of haplotypes on low-density lipoprotein cholesterol (LDL-C). **e** Effect of haplotypes on very-low-density lipoprotein cholesterol (VLDL). **f** Effect of haplotypes on apolipoprotein A1 levels (*ApoA1*). **g** Effect of haplotypes on apolipoprotein B levels (*ApoB*)

Discussion

Indians worldwide demonstrate a triad of high TG with high LDL-C and low HDL-C levels. A study conducted on migrant Indians has stressed the role of elevated serum TG as a risk factor for CVD in this ethnic group (Mc Keigue et al. 1989).

Global comparison of *APOA5* and *APOC3* allelic frequency The frequency of the –1131T>C polymorphism varies greatly among populations; it was found to be around 26–30% in East Asians (Hsu et al. 2006; Lai et al. 2003), 6–9% in Caucasians (Lai et al. 2004; Vaessen et al. 2006) and 12.8% in Turks (Hodoglugil et al. 2006). We observed a marked difference in the minor allele frequency of this SNP, when compared with other populations (Table 7). Moreover, we have found that the –1131T>C is in complete linkage disequilibrium (LD) with the –3A>G, which echoes the findings and haplotype structures in the European population (Pennacchio et al. 2002; Lai et al. 2004). This is in contrast to the findings in Turks (Hodoglugil et al. 2006) and the Costa Rica population

(Ruiz-Narváez et al. 2005) where the LD among these two sites is not complete. The c.56C>G polymorphism has a frequency of <0.1% in Chinese (Lai et al. 2003), 4–8% in Europeans (Pennacchio et al. 2002; Lai et al. 2004; Ruiz-Narváez et al. 2005; Martin et al. 2003; Talmud et al. 2002), 10% in the Costa Rica population (Ruiz-Narváez et al. 2005), and 6% in Turks (Hodoglugil et al. 2006). In our study, the minor allele of c.56C>G has a frequency of 5%, akin to the Caucasians, but in contrast to another study on Western Indians where it was 3% (Chandak et al. 2006). The c.553T SNP is very rare in the Indian population with a frequency of 0.02.

The S2 allele of 3238C>G SNP in the *APOC3* gene is highly prevalent in Indian population with a frequency (0.27) higher than that observed for most of the Caucasians (0.02–0.11) (Paul-Hayase et al. 1992; Rees et al. 1983) but within the same range as reported for different non-Caucasian populations (0.15–0.39) (Ko et al. 1997; Zeng et al. 1995). The 1100T>C SNP shows a minor allele frequency of 0.45. In contrast, the Second Northwick Park Heart Study (NPHSII) has revealed a frequency of 0.23–0.25 (Waterworth et al. 2000) of the 1100C SNP. Overall, we observed that almost 80% of Indian population harbored at least one of the minor variants of the polymorphisms being investigated and 28% had multiple SNPs.

Effect of the *APOA5* and *APOC3* variants on lipid parameters The polymorphisms –1131T>C and –3A>G in the *APOA5* gene, the 3238 C>G in the *APOC3* gene, and the haplotypes formed by them are significantly associated with elevated plasma triglyceride levels in Indian males and females. The c.56C>G and 1100C>T have shown gender differences in lipid metabolism, and no association was seen with elevated levels of TG in females. The *APOA5* SNP 553G>T was associated with hypertriglyceridemia in a Taiwanese population (Kao et al. 2003) and subsequently with CAD in Chinese population (Tang et al. 2006); although the c.553T was very rare in the Indian population, all five heterozygotes and the one homozygote for the minor allele had very high plasma triglyceride levels. Similar association of the SNPs was also seen with the VLDL-C values as expected. No association with other lipid parameters HDL-C, LDL, total cholesterol, apolipoprotein A1, and apolipoprotein B were seen in our study. The study by Lai et al. (2003) on ethnic groups across Singapore has yielded similar results where significant inverse associations were observed between the minor alleles of *APOA5* and HDL cholesterol concentrations for Chinese and Malays but not for Indians. The polymorphisms in *APOA5* and *APOC3* have been analyzed in different populations and the findings have supported their influence in some populations with the HDL-C (Dammerman et al. 1993; Grallert et al. 2007; Russo et al. 2001) and LDL-C (Lai et al. 2004) levels

Table 5 Allelic relative frequencies of polymorphisms in *APOC3* and *APOA5* genes in diabetics and non-diabetics

Allele Frequencies	Diabetics	Non-diabetics
<i>APOA5</i> –1131C	0.26	0.18
<i>APOA5</i> –3G	0.26	0.18
<i>APOA5</i> c.56G	0.1	0.05
<i>APOA5</i> c.553T	0	0.02
<i>APOC3</i> 3238G	0.45*	0.27
<i>APOC3</i> 1100T	0.54	0.38

SNP, single-nucleotide polymorphism. Allele frequencies were estimated by direct counting

*Significantly different between diabetics and non-diabetics $P<0.05$

Table 6 Effect of interact between APOC3/A5 haplotypes and diabetes on changes in triglyceride plasma levels in controls

ApoA5 -1131T>C	ApoA5 -3A>G	ApoA5 c.56C>G	ApoA5 c.553G>T	ApoC3 3238C>G	Triglyceride levels mg/dl		
					Non-diabetic Mean±SEM	Diabetic	P values
1	1	1	1	1	121±8.03	123±31.1	0.95
1	1	1	1	2	141±11.3	166±21.09	0.47
1	1	2	1	1	190±72.5	173±2.01	0.91
2	2	1	1	1	155±16.28	#/-	#/-
2	2	1	1	2	186±18.3	154±30.77	0.4
2	2	2	1	1	#/-	178±13.5	#/-

Mean±SEM. P values and means and SEM were determined after adjusting for age, BMI, smoking, diabetic status, alcohol use

#/- indicates genotype not seen in the cohort

but not for others (Chandak et al. 2006; Hodogluligil et al. 2006).

In our study, the haplotypes 11112, 22111, and 22112 show highly significant association with TG and VLDL-C levels irrespective of gender. Interestingly, it was observed that the 11112 which includes the APOC3 3238C>G on the wild-type background of APOA5 was significant for elevated TG levels. Similar results have been found by Ruiz-Narvaez et al. (2005) who state that though the APOC3 and APOA5 regulate triglyceride metabolism but in opposite directions, the TG-raising effect of -3238C>G was not counteracted by upregulation of the APOA5 gene, probably because this triglyceride increase did not depend on transcription.

The 22112 which includes the APOA5 -1131T>C and -3A>G and the APOC3 3238 C>G showed elevated TG levels that were highly significant in males, females, as well as both genders combined. An additive increase of 20 mg/dl TG was observed in the 22112 haplotype when compared with the 22111 haplotype.

Effect of the APOA5 and APOC3 variants on CAD In our study, none of the APOA5 polymorphisms were associated with CAD. There have been a few controversial reports regarding the association between CAD and APOA5 polymorphism. The NPHSII study revealed that men who carried the haplotype APOA5 -1131C/APOC3 -482T

showed CAD prevalence lower than in men who carried the common haplotype defined by nine common alleles (Wong et al. 2003), whereas a near doubling of CAD was found with -1131C carriers by Szalai et al. (2004), similarly the EPIC-Norfolk prospective study (Vaessen et al. 2006) found that -1131C variant was slightly, yet significantly, overrepresented in future CAD cases. No evidence that the APOA5/A4/C3 genetic polymorphisms are major risk factors of CAD was seen in French men (Dallongeville et al. 2006), however Talmud et al. (2004) showed a trend toward increased progression of atherosclerosis in carriers of the c.56G variant. The Framingham offspring study (Russo et al. 2001) did not find any significant increase in CHD risk associated with the S2 allele in the APOC3 gene despite its association with increased TG and ApoC3 non-HDL and lower HDL-C and HDL2-C. However, another study in the Indian population as reported that APOC3 3238C>G is an important genetic variant that is associated with CAD through its interaction with plasma lipids and other standard factors (Shanker et al. 2008). In our study also we have found the S2 allele to be a risk factor for CAD; overrepresentation of the APOC3 3238G allele in individuals with type 2 diabetes (chi-square=10.74, df=2, $P<0.001$) was also seen.

Overall, our results demonstrate that common variants at the APOA5 and the APOC3 and their haplotypes are significantly associated with plasma TG and VLDL-C

Table 7 Frequency of APOA5 -1131T>C polymorphism in different populations

Population (ref)	Sample size	C allele	χ^2 ^a	P values ^a
Costa Rica (38)	1,703	0.032	88.40	<0.0001
Caucasians (36)	2,031	0.056	46.85	<0.0001
Caucasians (37)	2,391	0.069	33.85	<0.0001
Turkish (28)	3,020	0.128	6.4485	0.011
Chinese (34)	317	0.297	5.161	0.023
Western Indians (24)	557	0.20	0.0339	0.853
Current Study	150	0.20		

Each population has been compared with respect to the Indian population and the chi-square and P values calculated

^a χ^2 tabulated at 5% level of significance (i.e., 0.05) and P values have been calculated

concentrations in the Indian population, and the frequencies of these variants are very different as compared to other populations. That gender-specific differences exist is a fact; although men and women share most genetic information, they have significantly different disease susceptibilities that go well beyond the expected gender-specific diseases (Ordovas 2007). This new knowledge would require a more careful and complete elucidation of the complex interactions predisposing to common diseases.” The presence of these SNPs could serve as useful tools for the direct quantitative measurement of variation in lipid and lipoprotein levels.

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