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Common Variants of *HMGCR*, *CETP*, *APOAI*, *ABCB1*, *CYP3A4*, and *CYP7A1* Genes as Predictors of Lipid-Lowering Response to Atorvastatin Therapy

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There is interindividual variation in lipid-lowering response to statins. The objective of this study was to investigate whether common variation in genes involved in lipid and statin metabolism modify the effect of statins on serum total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), and high-density lipoprotein-cholesterol concentration in coronary artery disease (CAD) patients. We studied the association between 18 single-nucleotide polymorphisms (SNPs) in six genes (*HMGCR*, *CETP*, *APOAI*, *ABCB1*, *CYP3A4*, *CYP7A1*) in response to atorvastatin therapy (20 mg/day) in 265 newly diagnosed CAD patients using multivariable adjusted general linear regression. Variant alleles of *ABCB1* (-41A/G), *HMGCR* SNP29 G/T, rs5908A/G, rs12916C/T, and *CYP7A1*-204A/C polymorphisms were significantly associated with attenuated LDL-C reduction and variant alleles of *CETP TaqI*, -629C/A, and *APOAI Pst*I polymorphisms were associated with higher increase in high-density lipoprotein-cholesterol. A three-loci interaction model consisting of *CYP7A1*rs 892871AA/*APOAI*PstIP1P1/*HMGCR* rs12916CT was a better predictor for LDL-C lowering, when compared with single polymorphisms analysis on statin response. Variant genotypes of *APOAI* −2500C/T, *CETP* 405I/V, and *ABCB1* 3435C/T showed higher risk of myocardial infarction events ($p < 0.05$) in a 1-year follow-up of CAD patients. These results suggest that SNPs in lipid and statin pathway genes are associated with reduced LDL-C lowering by statins and identify individuals who may be resistant to maximal LDL-C lowering by statins.

Introduction

HMG-CoA reductase inhibitors or statins are considered one of the most effective classes of drugs for reducing low-density lipoprotein-cholesterol (LDL-C) and total cholesterol (TC) (NCEP, 2002, ATP-III guidelines). Although clinical use of statins has consistently reduced risks of coronary heart disease and stroke, the degree of interindividual variability in lipid-lowering response to statins is marked (Maitland-van der Zee *et al*., 2002; Kajinami *et al*., 2005; Schmitz *et al*., 2007). For example, variable reduction in LDL-C levels has been reported in different ethnic populations in response to statin therapy (LaRosa, 2000; Puccetti *et al*., 2007). These differences have been attributed to genetic and environmental influences (Mangravite *et al*., 2006). Genetic variations in genes involved in statin and lipid metabolism have been proposed as important determinants of statin response (Hutz and Fiegenbaum, 2008). Polymorphisms in several genes such as those encoding 3-hydroxy 3-methylglutaryl coenzyme A (*HMGCR)*, ATP-binding cassette protein B1 (*ABCB1)*, and cholesterol-7-alpha-hydroxylase (*CYP7A1)* have shown inconsistent association with variability in statin efficacy (Wang *et al*., 1998; Chasman *et al*., 2004; Kajinami *et al*., 2004a, 2004b; Fiegenbaum *et al*., 2005; Rodrigues *et al*., 2005). Results of two studies, the Pravastatin Inflammation/CRP Evaluation study and the Atorvastatin Comparative Cholesterol Efficacy and Safety Study (ACCESS), suggest that compound effects of multiple genetic variants may be better predictors of statin response than any single gene variant (Chasman *et al*., 2004; Thompson *et al*., 2005). It has been also proposed that strategies that assess the simultaneous influence of multiple relevant susceptibility factors on disease risk (e.g., diet, lifestyle, and gene effects) may be of greater potential value for predicting the outcomes of statin treatment; however, the pharmacogenetic studies on association between gene–environment risk factors and statin response are limited (Mangravite *et al*., 2006). In this study, we studied the association of variants in genes encoding several common proteins involved in statin metabolism pathway and in pathways influenced by statins: *ABCB1*, a drug transporter; cholesterol ester transport protein (*CETP*), apolipoprotein AI (*APOAI*), and *CYP7A1* genes involved in lipid metabolism; *HMGCR*, rate-limiting enzyme involved in cholesterol metabolism; and cytochrome P450 3A4 (*CYP3A4*), a key enzyme involved in maintaining the drug metabolism with changes in lipid levels in response

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to atorvastatin therapy. We also examined the interactions between genes and environmental factors such as smoking and alcohol intake, sex, age, and family history in response to lipid-lowering therapy. We also analyzed whether genetic variants were associated with the future incidence of myocardial infarction (MI) in coronary artery disease (CAD) patients currently on atorvastatin therapy.

Materials and Methods

Two hundred sixty-five angiographically confirmed, unrelated CAD patients (=50% stenosis) attending the Cardiology Clinic of Nehru Hospital, Post Graduate Institute of Medical Education and Research, Chandigarh, between July 2005 and June 2008, who were not receiving any lipid-lowering therapy at baseline, were prescribed atorvastatin, and had both baseline and follow-up lipid level measurements were enrolled in the study. Subjects with a history of stroke, renal diseases, and diabetes mellitus were excluded from the study. All the patients were started on atorvastatin therapy (20 mg/day) and overnight fasting blood sample was taken at baseline and at 6 weeks after starting therapy. Patients who met the aforementioned inclusion criteria and gave an informed consent were enrolled into the study. This study was approved by the Institute Research Ethics Committee, Post Graduate Institute of Medical Education and Research, Chandigarh. All patients on atorvastatin were followed for up to 1 year for occurrence of MI and side effects such as myopathy and liver toxicity. None of the patients reported any side effect. We followed patients for only a year and this may be a limitation of our study.

Biochemical investigations

TC, triglycerides (TG), LDL-C, and high-density lipoprotein-cholesterol (HDL-C) levels were measured in overnight fasting blood samples, using standard enzymatic kits (Accurex Pvt. Ltd.). Body mass index was calculated as weight (kg) divided by height squared (m^2). Genomic DNA was isolated from the whole blood as described previously (Lahiri and Nurnberger, 1991).

Selection of single-nucleotide polymorphisms

The selected single-nucleotide polymorphisms (SNPs) in this study were from promoter, exonic, and intronic regions of different chosen genes. They either have been studied in other ethnic populations for association with statin response or were chosen because of their functional significance in the gene.

Genotyping

Eighteen SNPs were selected from exonic, promoter, and intronic splice site regions of six genes involved in statin metabolism pathway and also in pathways influenced by statins (*ABCB1*, *CYP3A4*, *APOAI*, *HMGCR*, *CYP7A1*, and *CETP*). Genotyping of the *CETP* gene polymorphisms: -629C/A (Eiriksdottir *et al*., 2001), *Taq*IB (Padmaja *et al*., 2007), and 405I/V (Wu *et al*., 2001); *APOAI* gene polymorphisms: -2500C/T (Masana *et al*., 2001), *Pst*I (Masana *et al*., 2001), and -75G/A (Dallinga-Thie *et al*., 1996); *CYP7A1* gene polymorphisms: -278A/C (Wang *et al*., 1998) and -204A/C (Couture *et al*., 1999); *CYP3A4* gene polymorphisms: -290A/G (Cavalli *et al*., 2001) and F189S (Lee *et al*., 2005); *ABCB1*: 3435C/T (Tanabe *et al*., 2001), G2677T/A (Tanabe *et al*., 2001), and -41A/G (Tanabe *et al*., 2001) were performed by polymerase chain reaction–restriction fragment length polymorphism assays. The genotyping of *HMGCR* gene polymorphisms (rs5908A/G, rs12916C/T, and SNP29G/T) and *CYP7A1* rs8192871A/G was carried out by allelic discrimination assays using Taqman technology (Applied Biosystems). All the genotypes were confirmed and validated by random DNA sequencing.

Statistical analysis

All the study variables are given as mean ± standard deviation. Paired *t*-test was performed to see the effect of atorvastatin therapy on biochemical parameters. For association of SNPs with lipid-lowering response, the dominant model (wild-allele homozygote genotype vs. variant allele carrying heterozygote and homozygote genotypes) was used. Pearson's χ^2 test was used for statistical comparisons between the groups wild-type versus variants, MI versus MI-free, and responders versus nonresponders. The dominant model was also used to assess association of genetic variants with response to atorvastatin therapy. The gene–gene interactions between various genes were analyzed using multiple dimensionality reduction software with permutation and combination testing. The Hardy–Weinberg equilibrium was calculated separately for controls and patients by gene counting and χ^2 analysis. Power of the study was calculated using the Power for Association with Errors (PAWE) software (http://linkage.rockefeller.edu/pawe) (Gordon *et al*., 2002, 2003). Power assuming $\alpha = 0.05$ and relative risk of >1 was 89%, 90%, 91%, 85%, and 75% for *CETP*, *APOAI*, *ABCB1*, *CYP7A1*, and *HMGCR* gene polymorphisms, respectively ($p < 0.05$) (Poduri *et al*., 2009). Multiple regression analysis was carried out to determine the genotypic association with mean changes in lipid levels. Age, sex, smoking status, alcohol intake, waist–hip ratio (WHR), and diet were included in each model as covariates. All the statistical analyses were performed using

Table 1. Demographic Profile of Patients on Atorvastatin Treatment

	Patients (n = *265*)	*MI-free* (n = *196*)	*MI*[a] (n = *69*)
Age (years)	47.52 ± 7.7	47.58 ± 7.58	47.36 ± 8.35
Male/Female	222/43	167/29	55/14
BMI (kg/m^2)	28.18 ± 3.03	27.32 ± 2.62	28.49 ± 3.11[b]
SBP (mmHg)	138.02 ± 14.07	137.96 ± 14.00	138.18 ± 14.34
DBP (mmHg)	87.53 ± 8.81	86.43 ± 10.09	87.91 ± 8.31
Waist-hip ratio	0.91 ± 0.08	0.90 ± 0.08	0.93 ± 0.09[b]
Lipid levels	*Baseline*	*After treatment*	*After treatment*
TC (mg/dL)	203.55 ± 34.75	141.31 ± 37.84	150.41 ± 36.14[b]
HDL (mg/dL)	35.51 ± 9.12	36.83 ± 9.54	36.98 ± 8.74
TG (mg/dL)	189.25 ± 72.02	126.88 ± 48.32	137.64 ± 62.20[b]
LDL-C (md/dL)	130.18 ± 32.33	79.10 ± 35.19	86.89 ± 32.67[b]

Values given are mean ± standard deviation.

[a]Patients with MI after starting atorvastatin treatment in 1-year follow-up.

[b]*p*-value <0.05; baseline versus after treatment in MI and MI-free patients.

MI, myocardial infarction; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein-cholesterol; HDL, high-density lipoprotein.

the SPSS (version 13.0) software. A p-value of <0.05 was considered significant.

Results

The baseline characteristics of CAD patients and patients stratified on the basis of cardiovascular outcome as occurrence of MI in a 1-year follow-up after atorvastatin therapy are described in Table 1. Body mass index, WHR, TC, TG, and LDL-C levels were found to be significantly higher in MI group when compared with MI-free subjects ($p < 0.05$; Table 1). All genotypes except *ABCB1*-41A/G and *HMGCR* SNP29G/T were in Hardy–Weinberg equilibrium.

Association of genotypes with lipid concentrations

Genotypes showing significant association with lipid levels (baseline and after atorvastatin) are shown in Table 2

Table 2. Genotypes and Lipid Levels (Baseline and After Atorvastatin Therapy)

	Baseline			*After treatment*		
Genes/genotypes (N)	*TC (mg/dL)*	*HDL-C (mg/dL)*	*LDL-C (mg/dL)*	*TC (mg/dL)*	*HDL-C (mg/dL)*	*LDL-C (mg/dL)*
		APOAI			*APOAI*	
*Pst*I						
WT (175)	200 ± 32	35 ± 9	128 ± 30	148 ± 35	36 ± 8	83 ± 33
Variants (90)	209 ± 38[a]	35 ± 9	132 ± 35	146 ± 41	37 ± 9	82 ± 34
-75G/A						
WT (133)	203 ± 32	36 ± 9[a]	131 ± 30	147 ± 35	36 ± 9	84 ± 32
Variants (132)	203 ± 37	34 ± 8	128 ± 34	148 ± 39	37 ± 8	83 ± 34
		CETP			*CETP*	
*Taq*I						
WT (117)	202 ± 33	34 ± 9	129 ± 30	142 ± 40	35 ± 8	81 ± 37
Variants (148)	204 ± 36	36 ± 8[a]	130 ± 33	152 ± 34[a]	37 ± 6[a]	86 ± 30
405I/V						
WT (116)	200 ± 35	36 ± 8[a]	129 ± 30	149 ± 38	37 ± 7	83 ± 34
Variants (149)	205 ± 34	34 ± 9	134 ± 32[a]	146 ± 35	36 ± 8	84 ± 32
-629C/A						
WT (127)	200 ± 35	34 ± 8	126 ± 31	145 ± 33	38 ± 9[a]	81 ± 29
Variants (138)	206 ± 34	35 ± 9	134 ± 32[a]	150 ± 40	35 ± 8	86 ± 36
		ABCB1			*ABCB1*	
-41A/G						
WT (250)	203 ± 34	35 ± 9	130 ± 32	148 ± 37	36 ± 8	84 ± 33[a]
Variants (15)	200 ± 35	41 ± 9[a]	120 ± 31	145 ± 33	40 ± 8	76 ± 31
		CYP7A1			*CYP7A1*	
-278A/C						
WT (116)	202 ± 32	36 ± 9[a]	129 ± 29	145 ± 36	38 ± 8[a]	83 ± 34
Variants (149)	204 ± 36	34 ± 9	130 ± 34	150 ± 37	35 ± 8	84 ± 32
-204A/C						
WT (131)	204 ± 30	35 ± 8	131 ± 28	142 ± 37	36 ± 8	80 ± 32
Variants (134)	202 ± 38	35 ± 9	128 ± 35	153 ± 36[a]	37 ± 9	87 ± 33
		HMGCR			*HMGCR*	
rs5908A/G						
WT (233)	203 ± 35	35 ± 8	129 ± 32	149 ± 37	37 ± 8	85 ± 33[a]
Variants (32)	205 ± 33	34 ± 11	133 ± 31	138 ± 35	35 ± 11	76 ± 32
SNP29 G/T						
WT (12)	215 ± 49[a]	36 ± 10	139 ± 48[a]	140 ± 50	37 ± 8	76 ± 48
Variants (253)	202 ± 33	35 ± 9	129 ± 31	152 ± 36[a]	36 ± 8	84 ± 32[a]
rs12916C/T						
WT (115)	204 ± 37	36 ± 9	127 ± 33	142 ± 34	37 ± 9	77 ± 31
Variants (150)	202 ± 32	35 ± 8	131 ± 31	154 ± 38[a]	36 ± 8	89 ± 33[a]

Values given are mean ± standard deviation.
WT: wild-type genotype; variants: variant allele-carrying genotypes (heterozygous and homozygous).
[a]$p < 0.05$, independent sample t-test performed between WT versus variant genotypes.
HDL-C, HDL-cholesterol; SNP, single-nucleotide polymorphism.

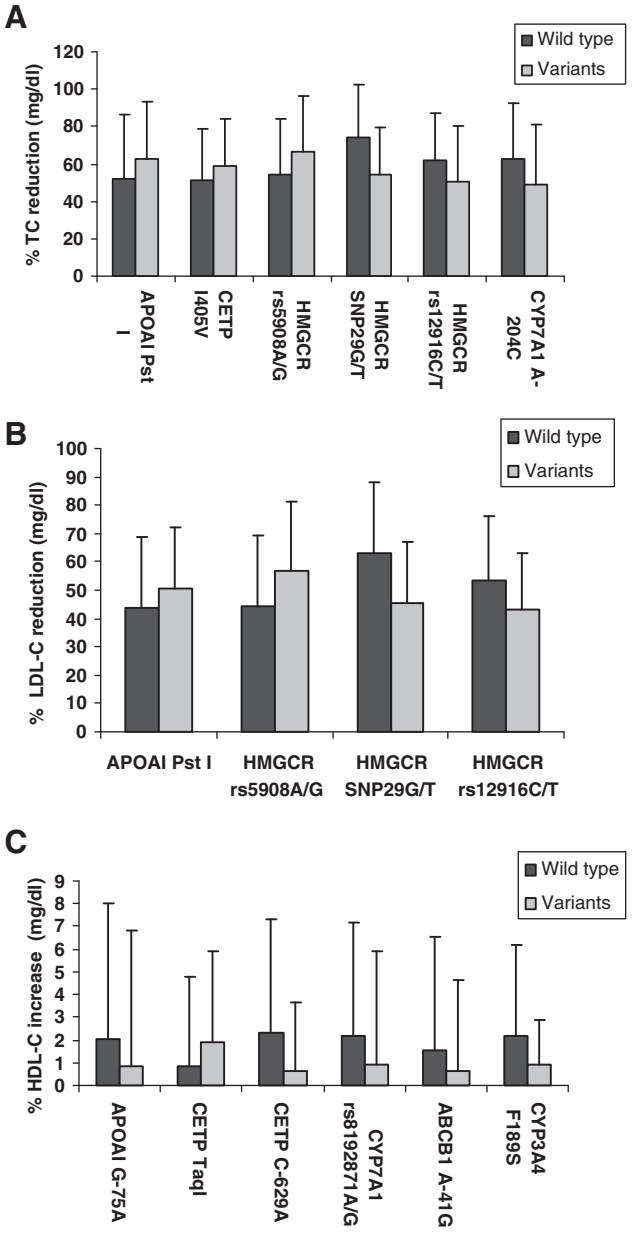

FIG. 1. Gene variants and changes in lipid levels after atorvastatin therapy: Comparison between wild and variant genotypes. (**A**) Percent TC reduction; (**B**) percent LDL-C reduction; (**C**) percent HDL-C increase. Values are given in mean ± standard deviation. Only genotypes showing significant association ($p < 0.05$) are given. LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; TC, total cholesterol.

(details in Supplemental Table 1, available online at www.liebertonline.com). Variant genotype is represented as heterozygous and homozygous variant allele-carrying genotypes. Significantly higher baseline TC levels were observed in variant genotype of *APOAI Pst*I ($p < 0.05$); higher baseline LDL-C levels were observed in *CETP* 405I/V and -629C/A variant genotypes ($p < 0.05$), and lower baseline HDL-C levels were observed among *APOAI*-75G/A, *CETP* 405I/V, and *CYP7A1*-278A/C variant genotypes ($p < 0.05$). Patients carrying *HMGCR* SNP29 G/T wild genotype had significantly higher baseline TC and LDL-C levels and those with *ABCB1*-41A/G wild genotype had lower baseline HDL-C concentrations ($p < 0.05$). Patients with variant genotypes of *CETP Taq*I, *CYP7A1*-204A/C, and *HMGCR* rs12916C/T showed higher TC levels, and carriers of *HMGCR* SNP29 G/T and rs12916C/T variant genotypes had higher LDL-C levels ($p < 0.05$) after atorvastatin therapy. Variant genotypes of *CYP7A1*-278A/C were associated with lower HDL-C levels after atorvastatin therapy ($p < 0.05$; Table 2). *HMGCR* rs5908A/G wild-type genotype carriers had higher TC levels and patients carrying *ABCB1*-41A/G wild genotype had higher LDL-C levels after atorvastatin therapy ($p < 0.05$).

Lipid-lowering response and genotypes

Genotypes showing significant association with changes in TC, LDL-C, and HDL-C levels following atorvastatin therapy are shown in Figure 1 (full details in Supplemental Table 2, available online at www.liebertonline.com). Variant allele-carrying genotypes of *APOAI Pst*I, *CETP* 405I/V, and *HMGCR* rs5908A/G and variant genotypes of *HMGCR* SNP29G/T, rs12916C/T, and *CYP7A1*-204A/C showed significantly lower reduction of TC levels after therapy ($p < 0.05$; Fig. 1A). The variant allele of *APOAI Pst*I and *HMGCR* rs5908A/G and wild type alleles of *HMGCR* SNP29G/T and 12916C/T exhibited significantly lower reduction of LDL-C levels after atorvastatin therapy ($p < 0.05$; Fig. 1B). Patients with wild-type genotypes of *APOAI* -75G/A, *CETP* -629C/A, *CYP7A1* rs8192871A/G, and *CYP3A4* 189F/S and variant allele-carrying genotype of *CETP Taq*I showed greater increase in HDL-C concentrations following atorvastatin therapy ($p < 0.05$; Fig. 1C). Seventy-five percent of patients ($n = 200$) were observed to be responders (who achieved LDL-C concentrations ≤ 100 mg/dL) and 25% ($n = 65$) were nonresponders (in whom LDL-C concentrations remained >100 mg/dL after atorvastatin therapy) in our cohort. No significant difference in genotype frequencies of various SNPs was observed between responders and nonresponders ($p > 0.05$) (data not shown).

Gene–gene interactions with lipid levels

Three-loci interaction comprising of *APOAI*(*Pst*IP1P1)/*CYP7A1* (rs8192871AA)/*HMGCR*(rs12916CT) was found to be associated with maximum decrease in LDL-C levels in responders when compared with loci interaction in nonresponders ($p < 0.05$; Fig. 2). Multiple regression analysis performed with lipid response as the dependent variable and age, sex, smoking, alcohol intake, CAD history, and genotypes as independent variables did not reveal any significant association of smoking, alcohol intake, age, sex, WHR, and CAD history with response to atorvastatin therapy (data not shown).

SNP interactions with MI

Sixty-nine patients suffered MI in a 1-year follow-up after atorvastatin therapy. Significant association between variant genotypes of *APOAI*-2500C/T, *CETP* 405I/V, and *ABCB1* 3435C/T SNPs and incidence of MI was observed in a 1-year follow-up of patients on atorvastatin therapy ($p < 0.05$; Fig. 3).

Discussion

Despite the fact that statin treatment efficacy is very high, there are substantial differences in treatment effectiveness

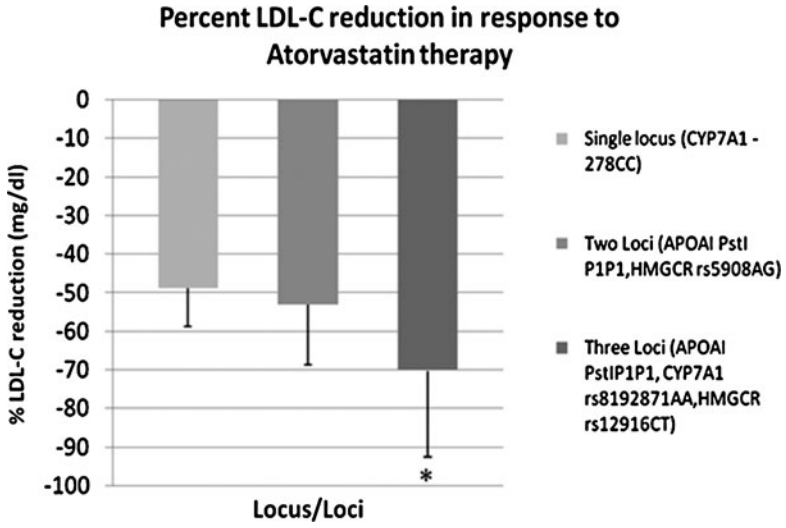

FIG. 2. Gene–gene interactions and response to atorvastatin therapy: Comparison between the number of interacting locus/loci and percent LDL-C reduction in response to atorvastatin ($*p < 0.05$).

among individuals. It is thought that genetic predisposition plays an important role in these differences, but the contribution of individual and combination of polymorphisms is poorly understood. In this study, we examined the association between genetic variants in some of the key genes involved in lipid and statin metabolism (Table 3) with the changes in TC, LDL-C, and HDL-C levels in response to atorvastatin. Our chief observations were that (a) the *ABCB1* (-41A/G), *HMGCR* SNP29 G/T, rs5908A/G, rs12916C/T, and *CYP7A1*-204A/C polymorphisms were significantly, independently associated with a poor response to atorvastatin in terms of LDL-C lowering; (b) *CETP Taq*I, -629C/A, and *APOAI Pst*I polymorphisms were associated with higher increase in HDL-C; and (c) there appeared to be a synergistic effect among three loci, *CYP7A1*rs892871AA/ *APOAIPst*IP1P1/ *HMGCR* rs12916CT, on statin response and was a better predictor for achievement of a target LDL-C, when compared with single polymorphisms analysis, and there was an SNP–statin interaction between *CETP* 405I/V and MI as cardiovascular event in patients on statin treatment.

The lack of association between the *ABCB1* polymorphisms (*ABCB1* 3435C/T and G2677T/A) and statin response shown in this study is in contrast to those of previous population studies, which examined the effects of these polymorphisms on baseline LDL-C levels in Caucasians (Table 4) (Kajinami *et al*., 2004c; Becker *et al*., 2009). These differences may be due to ethnic differences in the genotype frequencies between Asian Indians and Caucasians. However, we observed another polymorphism, *ABCB1* (−41A/G), which is in the promoter region of the region and thus affects its transcriptional activity, to be associated with greater increase in HDL-C concentrations following atorvastatin therapy. This is the first study to show the association of this polymorphism with lipid response to atorvastatin therapy and warrants examination in

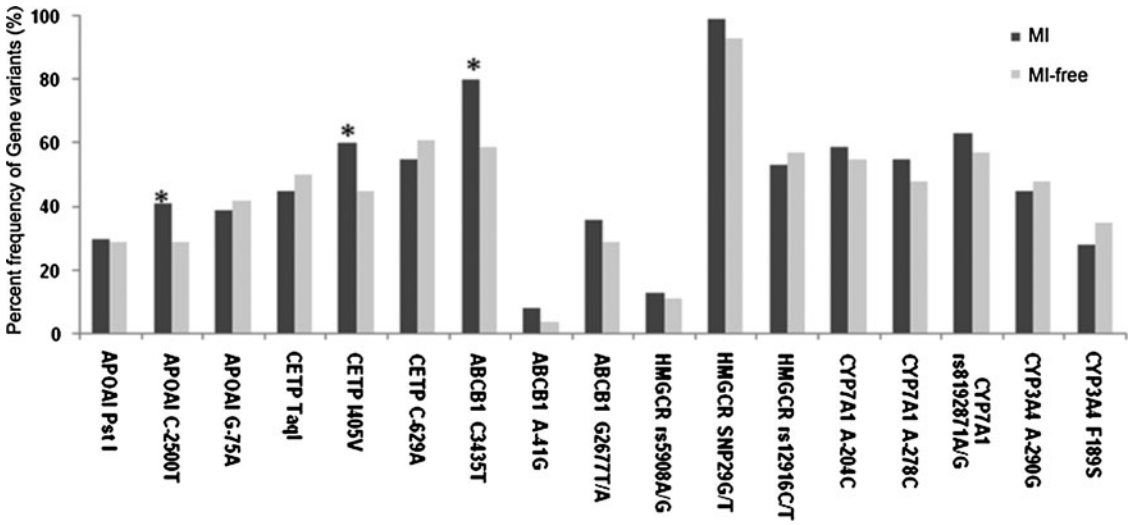

FIG. 3. Percent frequency of gene variants and incidence of myocardial infarction (MI) in a 1-year follow-up of patients on atorvastatin therapy ($*p < 0.05$). Independent sample *t*-test was performed to compare the frequency of gene variants between MI and MI-free groups.

Table 3. Gene Variants Associated with Lipid-Lowering Response to Atorvastatin Therapy in This Study

Gene	*Variant*	*Location*	*Association*
CETP	*Taq*I	Intron	Variants associated with higher baseline HDL-C and elevated HDL-C and TC after atorvastatin therapy
	-629C/A	Promoter	Variants associated with higher baseline and elevated LDL-C after atorvastatin therapy
ABCB1	-41A/G	Promoter	WT associated with higher LDL-C in response to therapy
CYP7A1	-204A/C	Promoter	Variants associated with higher TC in atorvastatin therapy
HMGCR	Rs5908A/G	Exon	Variants associated with increased TC after therapy
	SNP29G/T	Intron	Variants associated with enhanced TC and LDL-C in response to atorvastatin therapy
	Rs12916C/T	3′ UTR	Variants associated with elevated with TC and LDL-C in response to atorvastatin therapy

other populations. *ABCB1* encodes P-glycoprotein, which has been implicated in the transport of many drugs. *ABCB1* gene variants, which alter protein functional activity, could alter hepatic exposure of different statins to site of action and to other metabolizing enzymes and its efficacy (Table 4) (Fiegenbaum *et al*., 2005; Rodrigues *et al*., 2005).

The CYP3A4 enzyme is involved in the metabolism of atorvastatin; hence, variations in its activity will affect the efficacy of this drug. Genetic polymorphisms in *CYP3A4* gene have been found to influence its enzymatic activity and thus could modulate lipid response to this drug (Kajinami *et al*., 2004a). Pharmacogenetics studies of this gene have yielded conflicting results; Kajinami *et al*. reported *CYP3A4* (-290A/G) to be associated with higher posttreatment LDL-C after atorvastatin therapy (Kajinami *et al*., 2004a), whereas another trial ACCESS (Table 4) (Thompson *et al*., 2005), did not find significant association between *CYP3A4* variants and lipid response to statins (Table 4). We too did not observe any significant association between −290A/G SNP and lipid response to atorvastatin; however, we found another *CYP3A4* variant (189F/S) located in exon 7 associated with posttreatment increased HDL-C concentrations in response to atorvastatin. This variant is involved in conformational stability of the protein and thus may affect structure and activity of protein (Yano *et al*., 2004). This is the first study demonstrating the association of this variant with statin response in this study population and needs to be validated in other populations.

HMGCR is direct target of statin therapy and there are conflicting reports of association of allelic variations of this

Table 4. A Meta-Analysis of Association of Studied Genes with Response to Statins

Gene	*Variant*	*Drug*	*Association*	*Reference*
ABCB1	C3435T, G2677T/A	Atorva	Smaller reductions in LDL-C and greater increase in HDL-C in sex-specific manner	Kajinami *et al*., 2004c
		Atorva, Simva	Variants alleles showed association with greater reduction of cholesterol levels	Becker *et al*., 2009
		Atorva	High baseline serum total and LDL-C associated with ABCB1 Haplotypes	Rodrigues *et al*., 2005
	C3435T, G2677T/A, C1236T	Simva	Increased TC LDLC reduction also associated with increased adverse drug reactions	Fiegenbaum *et al*., 2005
CYP3A4	A-290G, F189S	Atorva	A-290G was associated with higher LDL-C after therapy	Kajinami *et al*., 2004a
	A-290G		No significant association	Wilke *et al*., 2005
	A-290G	Atorva	Gene dose-dependent effect on percentage reduction in serum TC	Gao *et al*., 2008
CETP	*Taq*IB	Prava	More atherosclerotic progression with B2B2	Kuivenhoven *et al*., 1998
		Prava	NS	Klerkx *et al*., 2004
	*Taq*IB	Atorva	B2 variants were associated with increase in apoAI levels in response to therapy	Goulas *et al*., 2008
	I405V	Simva	Wild type associated with higher TG reduction and HDL-C elevation	Anagnostopolu *et al*., 2007
APOAI	G-75A	Atorva	Reduction in TG and VLDL in women in response to therapy	Sorkin *et al*., 2005
			Higher baseline HDL-C and smaller increase in HDL-C	Lahoz *et al*., 2003
HMGCR	SNP 29 G/T	Prava	Associated with reduction in TC and LDL-C levels after therapy	Chasman *et al*., 2004
CYP7A1	A-204C	Atorva	Variants associated with reduction of LDL-C	Kajinami *et al*., 2004b

gene with statin response. We observed *HMGCR* SNP29GG to be associated with greater reduction in LDL-C levels after atorvastatin therapy. A similar association between *HMGCR* SNP29GG and LDL-C response has been reported in the Pravastatin Inflammation/CRP Evaluation study (Chasman *et al*., 2004), but not in the ACCESS cohort (Thompson *et al*., 2005) (Table 4). We also observed association of two *HMGCR* variants, rs5908A/G and rs12916C/T, with higher LDL-C concentrations in our cohort. SNPs rs5908A/G and rs12916C/T are located in exonic and 3′-UTR region of *HMGCR* gene, respectively, which affect its transcriptional activity and could thus affect the intracellular cholesterol metabolism and response to statins.

CYP7A1 plays an important role in maintaining the cholesterol homeostasis. An allelic variant of *CYP7A1* (-204A/C) has been earlier reported to be associated with higher plasma LDL-C concentrations and poor LDL-C reduction following atorvastatin therapy (Table 4) (Kajinami *et al*., 2005). We too observed lower reduction in LDL-C and TC in *CYP7A1*-204CC genotype carriers in response to atorvastatin, further suggesting that this variant may be an important determinant of atorvastatin response. It has been speculated that this variant nucleotide substitution may reduce expression of CYP7A1, resulting in increased LDL-C (Wang *et al*., 1998).

Statins are known to result in variable increases in plasma HDL-C levels. There have been conflicting results on the role of genetic variants of *CETP* and *APOAI* in modulating statin response (Isaacs *et al*., 2007). *CETP* 405I allele was shown to be associated with higher TG reduction and HDL-C elevation in response to simvastatin (Table 4) (Anagnostopoulou *et al*., 2007); however, this was not replicated in our study. These observed differences could be due to ethnic differences in the population's genotyped or patient categories, or due to the statin used in two studies.

There are conflicting reports on the pharmacogenetic association of *CETP Taq*I polymorphism with statin in Caucasians (Table 4) (Mohrschladt *et al*., 2005). We observed that *CETP* TaqI was associated with greater increase in HDL-C levels in response to atorvastatin. These observed differences could be due to different ethnic groups genotyped or patient categories, or due to the statin used in two studies. Thus, further studies are needed to confirm this association. We also found *CETP* -629A allele to be associated with higher HDL-C posttreatment. This variant allele results in decreased promoter activity and reduced CETP mass activity (Kondo *et al*., 1989). However, decreasing CETP activity may also lower reverse cholesterol transport, the mechanism by which HDL-C exerts its beneficial effects and may influence LDL-C levels.

Polymorphisms in *APOAI* gene are known to be associated with variations in HDL-C levels (Table 4) (Kamboh *et al*., 1996; Sorkin *et al*., 2005). Lahoz *et al*. have reported that *APOAI* (-75G/A) polymorphism modulated HDL-C response to pravastatin (Lahoz *et al*., 2003); however, this was not confirmed in our study. But we observed that another polymorphism, *APOAI Pst*I, was associated with greater reduction of TC and LDL-C levels in response to atorvastatin therapy (Fig. 1). *APOAI Pst*I SNP is in the 3′ UTR region of the gene and the variant allele P2 is associated with lower HDL-C (Vavatsi *et al*., 1995).

As multiple pathways are involved in lipid and statin metabolism, combined effect of several gene variants are expected to influence changes in LDL-C levels after atorvastatin therapy. We found that three-loci interaction, comprising of *CYP7A1* rs892871AA/*APOAIPst*IP1P1/*HMGCR*rs12916CT, was a better predictor of decrease in LDL-C than individual polymorphisms, indicating that co-occurrence of multiple gene variants could influence the changes in LDL-C in response to atorvastatin therapy. As the prime target of statin treatment is to reduce secondary cardiovascular events such as MI and stroke, we followed up our cohort for any incidence of MI for 1 year after starting atorvastatin therapy. We observed higher frequency of *ABCB1* 3435TT *CETP* 405VV and *APOAI*-2500TT genotypes in patients who had an MI event within a year of starting statins. Similar SNP–statin interaction between *CETP* 405I/V and stroke as cardiovascular outcome has been reported recently (Hindroff *et al*., 2008). These observations suggest that *CETP* 405I/V could be used clinically as a potential biomarker for cardiovascular events in patients on statin treatment.

Thus, our results suggest that polymorphisms in *CETP*, *ABCB1*, *CYP3A4*, *CYP7A1*, *HMGCR*, and *APOAI* could significantly modulate the TC and LDL-C lowering efficacy of atorvastatin in Indian patients with CAD. Further, the co-occurrence of specific loci appears to be a better predictor of reduction of LDL-C than did single locus analysis. These observed associations between various genetic variants and lipid levels are biologically plausible, because all studied genes are involved in intracellular cholesterol homeostasis and could modulate lipid levels. However, the relatively small sample size of our cohort could be a limitation of our study. Further, in contrast to some earlier studies, we did not find any significant differences in lipid-lowering response between male and female patients, which could be due to loss of power of the study on sex-based stratification.

To conclude, contribution of individual genetic determinants to overall response variation is small, but the combined effects of multiple genotypes may be more substantial. Initial observations must be replicated across multiple statin clinical trials and in different population groups of differing race and ethnicity before they can be assessed for clinical utility.

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Disclosure Statement

No competing financial interests exist.

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