ATP binding cassette transporter G5 and G8 genotypes and plasma lipoprotein levels before and after treatment with atorvastatin

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Abstract The mechanisms responsible for interindividual variation in response to statin therapy remain uncertain. It has been shown that hepatic cholesterol synthesis is associated with ATP binding cassette transporter G5 and G8 (ABCG5/8) activities. To test the hypothesis that genetic variation in ABCG5/8 might influence the plasma lipid response to statin therapy, we examined five nonsynonymous polymorphisms at the ABCG5/8 loci (Q604E, D19H, Y54C, T400K, and A632V) in 338 hypercholesterolemic patients treated with 10 mg atorvastatin. In carriers of the D19H variant, means of posttreatment values and adjusted percent reductions in LDL cholesterol (LDLC) were significantly lower ($P = 0.028$) and greater ($P = 0.036$) (112 mg/dl, 39.7%) than those of noncarriers (119 mg/dl, 36.2%), respectively, while no significant difference was observed in percent reductions in total cholesterol. Stepwise multiple regression analysis revealed significant and independent associations with absolute or percent reduction between D19H genotype and posttreatment LDL cholesterol levels. The other polymorphisms were not significantly associated with treatment effects. These results suggest that, in patients with hypercholesterolemia, the ABCG8 D19H variant is associated with greater LDLC-lowering response to atorvastatin therapy.—Kajinami, K., M. E. Brousseau, C. Nartsupha, J. M. Ordovas, and E. J. Schaefer. **ATP binding cassette transporter G5 and G8 genotypes and plasma lipoprotein levels before and after treatment with atorvastatin. *J. Lipid Res.* 2004.** 45: **653–656.**

Supplementary key words 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor • pharmacogenetics • low density lipoprotein cholesterol • adenosine 5′-triphosphate binding cassette transporter

HMG-CoA reductase inhibitors, statins (1), are effective in the primary (2, 3) and secondary (4–8) prevention of coronary artery disease. Statins competitively inhibit cholesterol biosynthesis, thereby decreasing intracellular cholesterol content. In hepatocytes, the reduction in cholesterol content, in turn, causes a decrease in the secretion of apolipoprotein B (apoB)-containing lipoproteins and an upregulation of LDL receptor activity, both of which contribute to the reduction in plasma LDL cholesterol (LDLC) levels observed with statin therapy. ATP binding cassette transporters G5 and G8 (ABCG5/8) are unique proteins located in the plasma membrane, as well as in the intracellular membranes of enterocytes and hepatocytes. Mutations in the genes encoding for ABCG5/8 have been identified as the cause of sitosterolemia (9–11), a rare inborn error of metabolism characterized by elevated plasma levels of plant sterols due to hyperabsorption and decreased biliary sterol secretion. Impairment of these pathways results in a decrease of cholesterol biosynthesis in hepatocytes (12, 13), raising the possibility that interindividual variation in plasma cholesterol concentrations (14, 15), and possibly the response to statin treatment, is, in part, due to variation in the ABCG5/8 genes.

We, and others, have previously shown that apoE genotype is associated with the plasma lipid response to statin therapy (16, 17). The present study was undertaken to test the hypothesis that polymorphisms at the ABCG5/8 gene loci are associated with plasma lipid response to statin treatment.

SUBJECTS AND METHODS

Study subjects were the same patient group, in which we found the gender-specific effects of apoE genotype on plasma

Manuscript received 24 June 2003 and in revised form 14 October 2003.

Published, JLR Papers in Press, January 1, 2004.
DOI 10.1194/jlr.M300278-JLR200

Abbreviations: ABCG8, ATP binding cassette transporter G8; apoB, apolipoprotein B; BMI, body mass index; TC, total cholesterol; LDLC, LDL cholesterol; HDLC, HDL cholesterol; TG, triglyceride.

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lipid response to atorvastatin therapy (17, 18). Patients were recruited from 31 community- and university-based research centers in the USA. Identical protocols were reviewed and approved by an institutional review board at each site, and written informed consent was obtained prior to the entry of the study from each participant. Men and women with primary hypercholesterolemia, 18–80 years old, with a body mass index (BMI) ⩽32 kg/m^2 were screened, and all patients began the NCEP step 1 diet during baseline and followed it throughout the study (18). Fasting plasma lipoprotein level entry criteria were LDLC values ⩾160 mg/dl at week 4 and >145 mg/dl at week 2, with the lower value being within 20% of the higher value, and triglyceride (TG) <400 mg/dl. Patients with impaired hepatic or renal function, diabetes mellitus type I, or uncontrolled diabetes mellitus type II were excluded. Additional exclusion criteria included all unstable medical conditions that might interfere with the evaluation and <80% compliance with study medication during the placebo baseline phase. Patients with elevated plasma aspartate aminotransferase or alanine aminotransferase values >1.5× the upper limit of normal, creatine phosphokinase >3× the upper limit of normal, or unexplained elevations in creatine phosphokinase during the placebo baseline phase were excluded. Patients were excluded if they were taking: any immunosuppressive agent; drugs known to affect lipid levels, possibly interact with study medications, or possibly affect clinical laboratory parameters; and drugs known to be associated with rhabdomyolysis in combination with statins (e.g., cyclosporine and erythromycin); or other lipid-regulating drugs. Patients currently taking a lipid-altering drug could be screened after a 4-week washout period, except for probucol, which required at least 6 months for washout. At the start of the double-blind treatment phase, qualified patients were randomized to one of four parallel treatment groups with once-daily dosing: atorvastatin (10 mg) or lovastatin (20 mg) for 52 weeks, placebo for 16 weeks, and then atorvastatin or lovastatin for 36 weeks. The ABCG5/8 genotypes were determined in 338 subjects from the atorvastatin (10 mg per day) arm of the study. The mean (±SD) age was 58 ± 11 years and BMI 27.0 ± 3.0 kg/m^2. Two hundred and three were males (mean age 56 ± 11 years, BMI 27.3 ± 2.7 kg/m^2) and 135 females (mean age 61 ± 11 years, BMI 26.5 ± 3.3 kg/m^2). Plasma lipid responses were calculated based on both baseline values on placebo and after the 52-week treatment for each subject.

Laboratory methods for lipid and lipoprotein measurements, as well as those for DNA analyses, were also described elsewhere (17). In the present study, five prevalent DNA polymorphisms, one in ABCG5 (Q604E) and four in ABCG8 (D19H, Y54C, T400K, and A632V), were studied using a PCR-restriction length polymorphism method. For the D19H polymorphism, a 131 bp fragment, including a G to C substitution site at codon 19 of the ABCG8 gene, was amplified using an oligonucleotide primer set (5′-GCTGGGTCTAAGAGAGCTGC-3′ and 5′-CTTCCCATTGCTCACTCACC-3′) with 35 cycles of amplification (95°C for 30 s, 60°C for 30 s, and 72°C for 30 s). PCR products were digested with 10 units of BstNI (New England BioLabs, Inc., MA), and fragments were separated by 3% agarose gel electrophoresis and visualized by UV after ethidium bromide staining. The remaining four polymorphisms were examined, as described in previous reports (11, 15).

All statistical analyses were carried out using the SPSS version 11.0.1. A logarithmic transformation was applied to plasma TG before analysis because of its skewed distribution. To test the effects of genotype on the variables with minimal risk for false positive association, we made comparisons by two stages. First, one-way ANOVA (ANOVA) was applied to evaluate the difference across three genotypes with post hoc Bonferroni correction for multiple comparisons. Second, subjects who carried one or two less common (variant) allele(s) were compared with those who were homozygotes for the common (wild-type) allele by Student's *t*-test for unpaired groups. In stepwise multiple regression analysis to find independent predictor(s), male and female gender was assigned 1 and 2, and wild-type homozygotes and subjects having at least one variant allele were assigned 1 and 2, respectively. Haplotype frequency was estimated using the EH program under the condition of allowing for linkage disequilibrium. Normalized linkage disequilibrium coefficients (D') were calculated as described elsewhere (15). All data are given as

TABLE 1. Lipid and lipoprotein concentrations before and after atorvastatin treatment according to D19H polymorphism of ABCG8 gene

		All Subjects	DD	DH/HH[a]	*P*
No. of subjects		338	294	44	
Age (years)		58 ± 11	58 ± 11	56 ± 11	0.306
BMI (kg/m^2)		27.0 ± 3.0	27.0 ± 3.0	26.8 ± 2.6	0.685
Baseline (mg/dl)	TC	272 ± 26	273 ± 25	264 ± 28	0.030
	LDLC	188 ± 22	189 ± 22	184 ± 22	0.161
	HDLC	50 ± 11	50 ± 11	47 ± 9	0.088
	TG	172 ± 71	173 ± 71	168 ± 66	0.666
Treatment (mg/dl)	TC	199 ± 26	200 ± 26	190 ± 22	0.012
	LDLC	118 ± 19	119 ± 19	112 ± 18	0.028
	HDLC	53 ± 12	54 ± 12	51 ± 11	0.102
	TG	137 ± 58	137 ± 59	136 ± 57	0.957
% Change (adjusted)	TC	−27 ± 8[b]	−26.6 (−25.7, −27.5)	−28.8 (−26.4, −31.2)	0.092
	LDLC	−37 ± 10[b]	−36.2 (−35.4, −37.6)	−39.7 (−36.9, −42.4)	0.036
	HDLC	+8 ± 13[b]	+7.7 (+6.3, +9.1)	+6.9 (+3.2, +10.6)	0.696
	TG	−17 ± 30[b]	−17.3 (−14.0, −20.6)	−16.2 (−7.7, −24.7)	0.811

ABCG8, ATP binding cassette transporter G8; BMI, body mass index; TC, total cholesterol; LDLC, LDL cholesterol; HDLC, HDL cholesterol; TG, triglyceride. All data are presented as mean ± SD.

[a] Genotype DH and HH were combined because HH was found only in one case, whose cholesterol, LDLC, HDLC, and TG levels at baseline were 235, 168, 39, and 141, levels after treatment were 178, 111, 46, and 105, and the percent changes were −24, −34, +18, and −26, respectively. Two types of comparison were originally performed: an additive model (DD, DH, and HH) and a dominant model (DD vs. DH/HH). Only results of the latter were shown.

[b] The mean values of percent changes were adjusted for age and pretreatment values of each variable and were shown with 95% confidence interval, except for the values of all subjects.

mean ± SD, and all reported *P* values are based on two-sided tests of significance.

RESULTS

Genotype, allele, haplotype frequencies, linkage disequilibrium

Genotype distributions (wild-type allele homozygote, variant heterozygote, variant homozygote) in each polymorphism were as follows; Q604E (212, 112, 14), D19H (294, 43, 1), Y54C (138, 146, 54), T400K (196, 130, 12), and A632V (225, 96, 17). All were consistent with the Hardy-Weinberg equilibrium. Allele frequencies (wild-type, variant) were Q604E (0.79, 0.21), D19H (0.93, 0.07), Y54C (0.62, 0.38), T400K (0.77, 0.23), and A632V (0.81, 0.19).

Twenty possible haplotypes were estimated by combination of five examined polymorphisms (data not shown). The three most common haplotypes were observed in exactly the same order as previously reported (15), and their estimated frequencies were 0.242, 0.214, and 0.145, respectively. Significant linkage disequilibrium was found in 5 out of 10 pairs of five polymorphisms: Q604E/D19H (D' = 0.6503, $P < 0.0001$), Q604E/Y54C (−0.3461, 0.0244), D19H/Y54C (−0.9986, 0.0003), Y54C/T400K (−0.4957, 0.0003), and T400K/A632V (−0.5484, 0.0456).

Effects of genotype on lipid levels

Among the five examined polymorphisms, only the D19H variant was significantly associated with pre- and post-treatment lipid levels. Posttreatment LDLC values of subjects having at least one variant allele were significantly lower than those of wild-type allele homozygotes. In addition, percent reductions of LDLC in carriers of D19H variant were significantly greater than that of noncarriers after adjustment for age and pretreatment LDLC levels, both of which are well-known as major determinants for statin response (**Table 1**). The difference was also significant when only pretreatment LDLC levels were adjusted (data not shown). In contrast, no significant difference was observed in percent reductions of total cholesterol. To identify independent variables associated with atorvastatin effects, we performed stepwise multiple regression analysis, including age, BMI, gender, pretreatment lipid levels [LDLC, TG, and HDL cholesterol (HDLC)], and each genotype as independent variables (**Table 2**). This analysis revealed that D19H genotype, in addition to age and pretreatment LDLC, was significantly and independently associated with posttreatment LDLC level. D19H genotype was also associated significantly and independently with the absolute and percent reduction of LDLC (data not shown). None of the remaining polymorphisms were significantly associated with treatment effects (data not shown).

TABLE 2. Stepwise multiple regression coefficients and adjusted R square values for the posttreatment LDLC level by atorvastatin[a]

Variables	Coefficient	SE	*P*	Cumulative Adjusted R Square
Pretreatment LDLC	0.363	0.042	<0.001	0.183
Age	−0.282	0.082	0.001	0.204
D19H variant	−5.529	2.774	0.047	0.211

[a] Stepwise multiple regression analysis was performed using seven variables (gender, age, BMI, pretreatment LDLC, pretreatment HDLC, log-transformed pretreatment TG, and D19H genotype). Male gender and homozygotes for wild-type allele of D19H polymorphism were assigned 1, and female gender and subjects carrying variant allele of D19H polymorphism were assigned 2, respectively.

DISCUSSION

The results of our study demonstrated that the ABCG8 D19H variant is significantly and independently associated with a greater LDL-lowering response to atorvastatin, while none of the remaining polymorphisms was associated with the response.

Our results are consistent with a novel genetic association, rather than a chance observation, based on the following evidence. First, recent findings in patients with sitosterolemia and genetically-engineered animal models suggest that impairment of ABCG5/8 activities significantly reduces cholesterol synthesis in hepatocytes (12, 13, 19). Our observation that common genetic variation in ABCG8 was associated with LDLC level, especially after statin therapy, is consistent with these observations due to the fact that higher cholesterol synthesis rate would be predicted to cause a greater reduction in LDLC with statin therapy. Second, a previous study reported that the plasma cholestanol/cholesterol ratio in carriers of the D19H variant was significantly lower than that of noncarriers, while the difference in plasma cholesterol levels failed to reach statistical significance (15). In the 4S study (20), an increased plasma cholestanol/cholesterol ratio was associated with a poor response to simvastatin therapy and, consequently, a lack of treatment benefit; however, the mechanism responsible for this observation was uncertain. Our findings may provide a possible explanation for this observation. Third, among the five polymorphisms examined, only the D19H variant was significantly associated with statin response. This strongly suggests that the D19H variant itself, or another as yet undefined linked variant, appears to have functional significance. An aspartic acid at amino acid 19 in ABCG8 is highly conserved from plant to vertebrates, and its substitution to histidine results in the loss of negative charge. In addition, the corresponding amino acid in ABCG5, glycine, is also conserved among vertebrates. In this regard, it is possible to speculate that the D19H variant may result in conformational changes, which may, ultimately, alter such functions as dimerization or ATP binding.

Estimated haplotype frequencies and the results of linkage disequilibrium tests in the present study were consistent with those of a previous report (15). Our increased statistical power was likely due to our larger number of subjects. However, we failed to find a specific ABCG5/8 haplotype that was more significantly associated with the response to atorvastatin therapy than D19H variant alone. This could be, at least in part, explained by the fact that

only a very small proportion of individuals carry any given allele that encodes two or more amino acid substitutions.

We previously reported that subjects with the apoE3/E4 genotype showed smaller LDLC reductions after atorvastatin treatment than those with the apoE3/E3 genotype, especially in men (17). In the present study, E3/E4 subjects who also carried the D19H variant allele showed similar LDLC reductions (−39 ± 8%) and a lower posttreatment level of LDLC (111 ± 18 mg/dl), when compared with E3/E3 subjects who also were D19H wild allele homozygotes (−38 ± 10%, 116 ± 20 mg/dl). This finding suggests that the D19H variant allele may compensate for the presence of the apoE4 allele in terms of LDLC lowering.

In conclusion, in patients with hypercholesterolemia, the ABCG8 D19H variant is significantly and independently associated with greater LDLC response, but not with total cholesterol response, to atorvastatin therapy.

This work was supported in part by Grant No. HL54776 from NHLBI and by Park-Davis-Pfizer Pharmaceutical Company, Ann Arbor, MI (E.J.S.), and was also supported by the Rotary Foundation, Evanston, IL (K.K.). The authors thank Dr. Noboru Takekoshi, Professor and Chairman, Department of Cardiology, Kanazawa Medical University, Japan, for his generous support of this research.

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