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ARTICLE

Candidate-Gene Study of Functional Polymorphisms in *SLCO1B1* and *CYP3A4/5* and the Cholesterol-Lowering Response to Simvastatin

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Cholesterol-lowering response to 40 mg simvastatin daily for 6 weeks was examined for associations with common genetic polymorphisms in key genes affecting simvastatin metabolism (*CYP3A4* and *CYP3A5*) and transport (*SLCO1B1*). In white people ($n = 608$), *SLCO1B1* 521C was associated with lesser reductions of total and low-density lipoprotein cholesterol. Associations between *SLCO1B1* 521C and cholesterol response were not detected in African Americans ($n = 333$). Associations between *CYP3A422 or *CYP3A5**3 and cholesterol response were not detected in either race, and no significant race-gene or gene-gene interactions were detected. As several of the analyses may have been underpowered (especially the analyses in the African American cohort), the findings not suggesting an association should not be considered conclusive and warrant further investigation. The finding regarding *SLCO1B1* 521C in whites was consistent with several previous reports. *SLCO1B1* 521C resulted in a diminished cholesterol-lowering response, but a marginal effect size limits utility for predicting simvastatin response.**
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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
✔Common functional variants in key genes involved in simvastatin metabolism (*CYP3A4**22 and *CYP3A5**3) and transport (*SLCO1B1* 521C) have been associated with altered simvastatin concentrations in several studies. A marginal effect (diminished cholesterol reduction) of *SLCO1B1* 521C on simvastatin response has been reported in several studies, but results from studies of *CYP3A4**22 or *CYP3A5**3 and simvastatin response have had inconsistent results.

WHAT QUESTION DID THIS STUDY ADDRESS?
✔This candidate-gene analysis examined the association of common genetic polymorphisms affecting simvastatin metabolism (*CYP3A4**22 and *CYP3A5**3) and transport (*SLCO1B1* T521C) with the cholesterol-lowering effect of simvastatin in 333 African Americans and 608 whites who received 40 mg simvastatin daily for 6 weeks.

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE
✔This analysis provided a unique opportunity to examine the relationships among simvastatin response and these polymorphisms (*CYP3A4**22, *CYP3A5**3, and *SLCO1*B1 T521C) in a sizeable African American population. Furthermore, we previously reported that these polymorphisms significantly affected simvastatin concentrations in this cohort, providing an opportunity to examine the influence of these polymorphisms on both the pharmacokinetics and pharmacodynamics of simvastatin.

HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE
✔Individually, common genetic polymorphisms affecting simvastatin metabolism (*CYP3A4**22 and *CYP3A5**3) and transport (*SLCO1B1 T521C*) seem to have negligible/marginal influence on cholesterol response to simvastatin. Collectively, however, effect may be clinically relevant, and future studies involving genetic score approaches may be more successful.

Statins remain among the most prescribed classes of medication in the United States and are indicated for the prevention of cardiovascular disease.[1] By inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A reductase, statins decrease intrahepatic cholesterol synthesis, upregulate hepatocyte surface low-density lipoprotein cholesterol (LDL-C) receptors, increase hepatic LDL-C uptake, and ultimately decrease blood concentrations of LDL-C. Reduction in LDL-C concentration is a common proxy measure of statin efficacy, with an estimated reduction in risk of major cardiovascular events of nearly 20% per mmol/L (38 mg/dL) reduction in LDL-C.[2] However, not all patients respond favorably to statins. A substantial proportion of patients do not achieve cholesterol goals, and some experience

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adverse effects, such as statin-induced myopathy.[3] Clinical outcomes and cholesterol-lowering response to simvastatin have demonstrated associations with clinical factors including age, race, gender, smoking, diet, comorbidities, and use of concomitant medications.[4] Genetic variation also seems to contribute to interindividual variability in response to statins.[5] In recent decades, a plethora of candidate-gene studies and genomewide association studies have focused on genetic polymorphisms and patient response to statins. Polymorphisms in several genes (e.g., *ABCB1, ABCG2, SLCO1B1, UGT1A1, UGT1A3, UGT2B7, CYP2C9, CYP2C19, CYP2C8, CYP2D6, CYP3A4,* and *CYP3A5*) have been associated with statin blood concentrations, and polymorphisms in several additional genes (e.g., *APOE, CETP, CLMN, CYP7A1, HMGCR, LDLR,* and *LPA*) have been associated with response to statins or incidences of statin-induced myopathy.[6]

For simvastatin, one of the most commonly prescribed statins,[1] investigations have largely focused on *SLCO1B1* – the gene encoding the organic anion transporting polypeptide 1B1 that mediates intrahepatic transport of simvastatin.[7] Increased simvastatin concentrations and an increased risk of myopathy have been associated with *SLCO1B1* polymorphisms in several studies, prompting the Clinical Pharmacogenetics Implementation Consortium to establish formal prescribing recommendations for simvastatin based on *SLCO1B1* genotype-defined risk of statin-induced myopathy.[7–10] Although nearly 200 common variants in *SLCO1B1* have been described, *SLCO1B1* 521C (rs4149056) has the highest level of evidence. All of the haplotypes identified as having an increased risk of statin-induced myopathy (*5, *15, and *17) in the Clinical Pharmacogenetics Implementation Consortium's *Recommended Dosing of Simvastatin Based on SLCO1B1 Phenotype* contain the *SLCO1B1* 521C polymorphism.[10] Myopathy risk is increased because the *SLCO1B1* 521C polymorphism results in increased systemic concentrations of simvastatin (secondary to decreased transport of simvastatin into hepatocytes), ultimately increasing the exposure of muscle to simvastatin.[6,7,11] Because the primary action of simvastatin occurs within hepatocytes, *SLCO1B1* 521C also reduces the cholesterol-lowering response to simvastatin. This association has been demonstrated in several clinical studies. Sortica *et al.*[12] reported the percent reduction of LDL-C in a study of 216 Brazilian patients who received 20 mg simvastatin daily for 6 months was 38.6 $\pm$ 8.0, 39.9 $\pm$ 8.6, and 42.1 $\pm$ 15.8 for homozygous *SLCO1B1* 521C carriers, heterozygous *SLCO1B1* 521C carriers, and wildtype, respectively. Fu *et al.*[13] reported the percent reduction of LDL-C in a study of 174 patients of Chinese ancestry who received 20 mg simvastatin daily for 4 months was 27.2 $\pm$ 5.4, 28.9 $\pm$ 5.9, and 30.8 $\pm$ 5.4 for homozygous *SLCO1B1* 521C carriers, heterozygous *SLCO1B1* 521C carriers, and wildtype, respectively. As these reported findings were only suggestive trends, Dou *et al.*[14] performed a meta-analysis of these studies and determined that the association between *SLCO1B1* 521C and cholesterol reductions was statistically significant. Hopewell *et al.*[15] reported similar findings in the Heart Protection Study of 18,705 patients who received 40 mg simvastatin daily for 4–6 weeks: the percent reduction of LDL-C was 42.4 $\pm$ 0.2, 43.6 $\pm$ 0.4, and 44.7$\pm$ 0.4 for homozygous *SLCO1B1* 521C carriers, heterozygous *SLCO1B1* 521C carriers, and wildtype, respectively. Although the relationship between *SLCO1B1* 521C and cholesterol response has been reported in several independent studies, its marginal effect size substantially reduces its potential for clinical utility.

As simvastatin is metabolized primarily by the cytochrome P450 (CYP), family 3, subfamily A, polypeptides 4 and 5 enzymes (CYP3A4 and CYP3A5, respectively), associations have been reported between simvastatin pharmacokinetics/dynamics and functional polymorphisms in *CYP3A4* and *CYP3A5*. Specifically, the decrease-of-function *CYP3A4*22* (rs35599367) and the loss-of-function *CYP3A5*3* (rs776746) polymorphisms have been associated with increased simvastatin concentrations[6,16–18] and increased cholesterol-lowering response.[19–22] In fact, we reported significant associations between these polymorphisms and simvastatin concentrations in this cohort.[17,18] Reports of associations between these polymorphisms and simvastatin response have also been published. Kivistö *et al.*[19] reported that the mean percent reduction in total cholesterol was higher (31% vs. 17%; $P = 0.026$) in *CYP3A5*3* homozygotes compared with *CYP3A5*1/*3* or *CYP3A5*1* homozygotes in a study of 69 white patients who received lovastatin, simvastatin, or atorvastatin (all primarily metabolized by CYP3A). However, the percent reduction in LDL-C was not statistically different in *CYP3A5*3* homozygotes compared with *CYP3A5*1/*3* or *CYP3A5*1* homozygotes (31% vs. 46%; $P = 0.083$). No associations between *CYP3A5*3* and LDL-C lowering were detected in the following reports: the Fiegenbaum *et al.*[23] analysis of 99 Europeans who received 20 mg simvastatin daily for 6 months, the Hu *et al.*[21] analysis of 229 Chinese patients who received 40 mg simvastatin daily for 6 weeks, the Bailey *et al.*[24] analysis of 291 Europeans of the Genetic Effects On STATins who received 40 mg simvastatin daily for 3 months, and the Hopewell *et al.*[15] analysis of 18,705 Europeans of the Heart Protection Study who received 40 mg simvastatin daily for 4–6 weeks. These findings collectively do not suggest that *CYP3A5*3* alters cholesterol-lowering response to simvastatin, at least not in the patient populations studied.

Studies of *CYP3A4*22* and simvastatin response have been rare, and although associations have been reported, findings have been inconsistent. Elens *et al.*[20] reported that the LDL-C reduction in *CYP3A4*22* carriers was 7% greater compared with noncarriers (41% vs. 48%; $P = 0.054$) in 80 incident simvastatin users of the Rotterdam Study after adjusting for age, gender, baseline cholesterol, and simvastatin dose and duration. The mean daily dose of simvastatin required for optimal cholesterol control was nearly 40% less for *CYP3A4*22* carriers compared with noncarriers in a study of 84 patients with hyperlipidemia ($P = 0.042$).[22] Conversely, no association was reported between *CYP3A4*22* and LDL-C lowering response in a study of 209 patients who received 10–40 mg simvastatin daily for 6 months.[25] It remains uncertain whether *CYP3A4*22* affects simvastatin cholesterol-lowering.

Despite several reports of associations between increased simvastatin concentrations and the decrease-of-function *CYP3A4*22* polymorphism or the loss-of-function *CYP3A5*

polymorphism, associations between these polymorphisms and simvastatin cholesterol-lowering response have seldom been detected. The lack of genetic investigation of simvastatin response in African Americans and our recent reports of associations between these *CYP3A* polymorphisms and 12-h simvastatin concentrations in this cohort provided the rationale for this follow-up candidate gene study. Herein, we present our study aimed at evaluating the associations of *CYP3A4**22, *CYP3A5**3, and *SLCO1B1* 521C alleles with the cholesterol response to 40 mg simvastatin daily for 6 weeks in white and African American participants of the Cholesterol and Pharmacogenetics (CAP) clinical trial.

METHODS

Study participants

This candidate-gene analysis included 609 self-reported white and 335 self-reported African American men and women aged ≥30 years who had a baseline total serum cholesterol level between 160 and 400 mg/dL. The participants of CAP (ClinicalTrials.gov identifier NCT00451828) were recruited and enrolled at San Francisco General Hospital and the University of California, Los Angeles, School of Medicine, and collected baseline data included demographic, medical history, risk factors for coronary heart disease, physical examination, and clinical laboratory test results. They received 40 mg simvastatin daily for 6 weeks, clinic visits occurred at 2-week intervals during the 6-week study, and exclusion criteria included the following: concomitant use of medications (prescription or over-the-counter) known to significantly alter patient cholesterol levels or simvastatin pharmacokinetics; known liver disease or elevated transaminase levels more than twice the upper limit of normal; uncontrolled hypertriglyceridemia, hypertension, or diabetes mellitus; abnormal renal or thyroid function; current alcohol or drug abuse; and known statin intolerance. Compliance was determined by pill counts, and the CAP clinical trial was approved by the institutional review boards at all clinical, laboratory, and coordinating centers. Additional details of the clinical trial methodology were provided in the initial report of the CAP clinical trial.[26]

Genotype analyses

SLCO1B1 T521C (rs4149056) was determined using the Cardio-MetaboChip (Illumina, San Diego, CA) genotyping platform, and *CYP3A4**22 (rs35599367) was determined using a TaqMan genotyping assay (C_59013445_10; Life Technologies, Grand Island, NY). *CYP3A5**3 (rs776746) was determined using the Illumina Human Hap 300 or Human Hap 610-Quad genotyping platform (Illumina) for whites and was determined using a TaqMan genotyping assay (C_26201809_30; Life Technologies) for African Americans.

Ancestry and relatedness analysis

To assess relatedness between participants, the Cardio-MetaboChip was used to calculate pairwise identity-by-state distances.[27] To meet the assumption of independent observations, tests were performed to determine whether any participants were first cousins or more closely related (pi_hat > 0.125). One member of a pair of African American participants with pi_hat = 0.1653 was excluded, and two additional subjects were excluded due to high degrees of relatedness (final $n = 941$). To assess genetic ancestry of the participants, the resulting matrix of identity-by-state distances was used to perform multidimensional scaling analysis in Plink.[28] In addition to self-reported race, the first three multidimensional scaling components were used as covariates in order to account for background genetic ancestry.

Statistical analysis

Baseline continuous variables were summarized by median and interquartile range and compared by race groups using the Wilcoxon rank sum test. Baseline categorical variables were summarized by counts and percentages and compared between race groups using the chi-square test (or Fisher's exact where necessary). Monte-Carlo estimates of the exact *P* values for Hardy-Weinberg equilibrium within race groups were calculated using 10,000 permutations. Changes in cholesterol were determined by comparing measured concentrations of cholesterol in serum samples collected at baseline and at the 6-week post-treatment visit. Regression analyses were used to detect associations among genotypes (*CYP3A4**22, *CYP3A5**3, and *SLCO1B1* T521C) and changes in LDL and total cholesterol. Regression analyses were also used to test whether changes in LDL and total cholesterol were associated with 12-h plasma concentrations of simvastatin. Based on previously reported findings regarding nongenetic influences on the cholesterol-lowering response to simvastatin in the CAP trial,[26] we adjusted our models for covariates, including race, smoking status, and age. Genetic ancestry (first three multidimensional scaling components) was also included as a covariate. Differences in the cholesterol-lowering response by genotypes and race group were assessed in the race-combined cohort using a multiplicative interaction term (genotype*race). Race-specific differences in cholesterol-lowering response by genotypes were assessed in analyses stratified by race group. To assess whether the effects of *SLCO1B1* T521C on cholesterol-lowering response to simvastatin were dependent on *CYP3A4* and/or *CYP3A5* status, the following gene-gene interactions were tested by incorporating a multiplicative interaction term within unadjusted and adjusted regression models in both the race-combined and race-stratified cohorts: *SLCO1B1*CYP3A4*, *SLCO1B1*CYP3A5*, *SLCO1B1*CYP3A4/5* (*CYP3A4* and *CYP3A5* combined by the number of decreased function alleles), and *SLCO1B1*CYP3A4/5* (*CYP3A4* and *CYP3A5* combined into extensive, intermediate, and poor metabolizers, as previously described[29]). Statistical analyses were performed using SAS version 9.3 (Cary, NC), and $P < 0.05$ was considered statistically significant. Adjustments for multiple comparisons were made using the Benjamini and Hochberg's linear step-up method,[30] limiting the false discovery rate to 5%. We estimated 80% power to detect a 14% difference in LDL-C reduction by *CYP3A4**22 genotype, a 5% difference by *CYP3A5**3 genotype and an 8% difference by *SLCO1B1* T521C genotype in unadjusted models.

Table 1 Baseline characteristics

	Overall ($n = 941$)	Whites ($n = 608$; 65%)	African Americans ($n = 333$; 35%)	*P* value
Age, y[a]	53 ± 17	54 ± 16	52 ± 18	0.843
BMI, kg/m^2[a]	28 ± 7	27 ± 7	29 ± 7	**<0.001**
Compliance, %[a]	98 ± 5	98 ± 5	98 ± 6	**0.027**
Male gender[b]	484 (51%)	322 (53%)	162 (49%)	0.206
Smoker[a]	187 (20%)	84 (14%)	103 (31%)	**<0.001**
*CYP3A4*22*				
allele frequency	0.03	0.04	0.01	**0.003**
*CYP3A5*3*				
allele frequency	0.73	0.93	0.35	**<0.001**
SLC01B1 521 C				
allele frequency	0.10	0.14	0.02	**<0.001**

BMI, body mass index; *CYP3A4*, the gene encoding cytochrome P450, family 3, subfamily A, polypeptide 4; *CYP3A5*, the gene encoding cytochrome P450, family 3, subfamily A, polypeptide 5; *SLCO1B1*, the gene encoding solute carrier organic anion transporter family member 1B1.
[a]Continuous variables are represented as median ± interquartile range and compared using the Wilcoxon rank sum test. [b]Categorical variables are represented as count (%) and compared using the chi-square test or Fisher's exact where necessary. Bolded *P* values were significantly associated with low-density lipoprotein cholesterol lowering response to simvastatin in the Cholesterol and Pharmacogenetics clinical trial.

Table 2 Associations of *CYP3A4*22*, *CYP3A5*3*, and *SLCO1B1* 521C with changes in cholesterol levels after 40 mg simvastatin daily for 6 weeks

	Overall			Whites			African Americans		
	No. of patients	Δ LDL-C (mg/dL)	Δ TC (mg/dL)	No. of patients	Δ LDL-C (mg/dL)	Δ TC (mg/dL)	No. of patients	Δ LDL-C (mg/dL)	Δ TC (mg/dL)
CYP3A4									
*1/*1	864	−54 ± 22	−57 ± 24	561	-56 ± 21	-59 ± 23	303	−51 ± 24	−53 ± 25
*1/*22	54	−56 ± 23	−60 ± 26	46	-58 ± 21	-62 ± 23	8	−44 ± 29	−50 ± 38
P value[a]		0.816	0.444		0.489	0.307		0.376	0.742
P value[b]		0.898	0.726		0.901	0.783		0.820	0.963
CYP3A5									
*1/*1	125	−49 ± 25	-51 ± 24	6	−56 ± 17	−57 ± 16	119	−48 ± 25	−51 ± 25
*1/*3	216	−53 ± 22	-55 ± 24	73	−56 ± 20	−58 ± 22	143	−51 ± 24	−53 ± 25
*3/*3	537	−57 ± 21	-59 ± 23	507	−57 ± 21	−59 ± 23	30	−53 ± 21	−57 ± 25
P value[a]		0.464	0.328		0.507	0.414		0.832	0.683
P value[b]		0.726	0.669		0.901	0.828		0.972	0.963
SLCO1B1									
C/C	14	−44 ± 25	−43 ± 27	14	−44 ± 25	−43 ± 27	0	–	–
C/T	151	−55 ± 19	−58 ± 21	137	−55 ± 19	−57 ± 21	14	−57 ± 20	−60 ± 23
T/T	718	−55 ± 22	−58 ± 24	441	−57 ± 22	−60 ± 24	277	−51 ± 23	−54 ± 25
P value[a]		0.103	**0.028**		0.038	**0.008**		0.310	0.305
P value[b]		0.495	0.204		0.366	0.107		0.783	0.783

Δ LDL-C, change in low-density lipoprotein cholesterol; Δ TC, change in total cholesterol; *CYP3A4*, the gene encoding cytochrome P450, family 3, subfamily A, polypeptide 4; *CYP3A5*, the gene encoding cytochrome P450, family 3, subfamily A, polypeptide 5; *SLCO1B1*, the gene encoding solute carrier organic anion transporter family member 1B1.
[a]Adjusted for age, smoking status, genetic ancestry (first three multidimensional scaling components) and self-reported race (when applicable). [b]Corrected for multiple comparisons with a false discovery rate of 5%. Values are presented as mean ± SD. Only *P* values <0.05 are bolded.

RESULTS

Baseline characteristics of the 941 CAP participants included in this analysis are presented in (**Table 1**). Genotypes at all three loci were within Hardy-Weinberg equilibrium for both race groups, and minor allele frequencies of *SLCO1B1* 521C, *CYP3A4*22*, and *CYP3A5*3* were consistent with those reported in other cohorts.[8,15,19,20,22–24] Statistical analyses of the associations between cholesterol-lowering response to simvastatin and *CYP3A4*22*, *CYP3A5*3*, and SLCO1*B1* 521C genotypes are presented in (**Table 2**).

Results from the race-combined and race-stratified analyses revealed that *SLCO1B1* 521C was the only genetic variable that was significantly associated with cholesterol-lowering response to simvastatin 40 mg daily for 6 weeks, and this relationship occurred only in white patients: reduction of LDL-C was 44 ± 25, 55 ± 19, and 57 ± 22 mg/dL in whites of C/C, T/C, and T/T genotype, respectively ($P = 0.038$ without correction for multiple hypotheses), and reduction of total cholesterol was 43 ± 27, 57 ± 21, and 60 ± 24 mg/dL in whites of C/C, T/C and T/T genotype, respectively ($P = 0.008$ without correction for multiple hypotheses). Although the race-combined analysis of

SLCO1B1 521 C and LDL-C reduction resulted in a significant P value, data in the African American cohort demonstrated a relationship (nonsignificant P value) with direction both opposing biological plausibility and opposite that in the white cohort and in the previously reported analyses. Neither *CYP3A4**22 nor *CYP3A5**3 were associated with differences in cholesterol-lowering responses in either race or in the combined-race cohort. Race-gene and gene-gene interactions for each genotype (*SLCO1B1* 521C, *CYP3A4**22, and CYP3A5**3) were not statistically significant (interaction terms and P values not shown). Simvastatin cholesterol-lowering response was not associated with 12-h post-dose simvastatin concentrations in the race-combined and race-stratified cohorts of CAP.

DISCUSSION

Our study findings regarding *SLCO1B1* 521C were consistent with those observed in Heart Protection Study[15] and in the meta-analysis reported by Duo *et al.*[14]: *SLCO1B1* 521C was associated with a diminished cholesterol-lowering response. The consistencies regarding the magnitude and direction of this effect not only support the validity of our study but also provide additional evidence supporting the central role of SLCO1B1 in the pharmacology of simvastatin. Although readily observed in clinical studies, the effect is marginal and unlikely to provide prescribing guidance beyond its established role in Clinical Pharmacogenetics Implementation Consortium's recommendations regarding simvastatin myopathy.[9,10]

Although associations between simvastatin pharmacokinetics and the functional polymorphisms *CYP3A4**22 and *CYP3A5**3 have been reported in several cohorts[6,16–18] including our recent reports of analyses conducted in this cohort[17,18] reports of their association with the cholesterol-lowering response to simvastatin have been rare. In fact, associations have been detected in only a single cohort each (Kivistö *et al*.[19] for *CYP3A5**3 and Elens *et al*.[20] for *CYP3A4**22). Those cohorts were relatively small ($n = 69$ and $n = 80$ for the reports by Kivistö *et al*.[19] and Elens *et al*.,[20] respectively), and the reported associations failed to replicated in subsequent analyses of other larger cohorts and in this analysis of CAP. Specifically, *CYP3A*4*22 was not associated with change in LDL-C in the report by Ragia *et al*.[25] ($n = 209$), and *CYP3A5**3 was not associated with changes in LDL-C in the studies reported by Hu *et al*.[21] ($n = 229$), Fiegenbaum *et al*.[23] ($n = 99$), Bailey *et al*.[24] ($n = 291$), and Hopewell *et al*.[15] ($n = 18{,}705$).

This study had several advantages. The cohort size was relatively large ($n = 941$) and included a significant number of African Americans ($n = 333$), providing novel opportunity for genetic investigation in an understudied patient population. The availability of genotyping chip data was another advantage, allowing genetic-ancestry adjustment and the exclusion of related subjects. Another advantage was comprehensive data collection, allowing adjustments for several clinical variables (age, gender, and smoking status). The eligibility criteria of the CAP clinical trial helped minimize confounding factors, including comorbidities, diet, and concomitant prescription and over-the-counter medications.

Nevertheless, there were several limitations to this study: other genes reported to play a role (albeit a minor role compared with *SLCO1B1* and *CYP3A4/5*) in simvastatin pharmacokinetics (e.g., *ABCB1, ABCG2, UGT1A1, UGT1A3, UGT2B7, CYP2C9, CYP2C19, CYP2C8,* and *CYP2D6*) or statin cholesterol-lowering response (e.g., *APOE, CETP, CLMN, CYP7A1, HMGCR, LDLR,* and *LPA*) were not included.[3–6] Likewise, not all polymorphisms of CYP3A4, CYP3A5, and SLCO1B1 were included in this candidate-gene study, and epistatic and epigenetic factors were not investigated. Despite the associations between 12-h post-dose simvastatin concentrations and *CYP3A4*22, CYP3A5*3, and SLCO1B1 521C* in CAP that we reported previously, regression analyses did not reveal associations (results not shown) between cholesterol-lowering response and simvastatin concentration in the race-combined and race-stratified cohorts of CAP. This was not entirely unexpected because the pharmacokinetic data were limited (i.e., significant discordance likely exists between 12-h concentrations in plasma and daily systemic exposure in hepatocytes).[17,18] Several of our analyses, especially the race-stratified analyses in the African American cohort, were underpowered. In fact, the analyses of *SLCO1B1* 521C in African Americans included only 14 heterozygotes and did not include any 521C homozygotes. The lack of observable associations in this population should not be considered definitive, and further investigation is warranted.

With proven efficacy and relatively few adverse effects, statins remain among the most commonly prescribed medication classes in the United States. Although most patients benefit, some are unable to attain cholesterol-reduction goals, some experience atherosclerotic events despite therapy, and some experience adverse events. Despite the completion of hundreds of candidate gene studies and numerous genomewide association studies, a clinically relevant pharmacogenetic test to predict statin efficacy has not yet emerged. Our finding regarding *SLCO1B1* 521C in white patients confirmed the findings from several other studies: *SLCO1B1* 521C results in a diminished cholesterol-lowering response, but its marginal effect size limits utility for predicting simvastatin response. Although these common variants result in altered simvastatin metabolism (*CYP3A4*22*, *CYP3A5**3) and transport (*SLCO1B1* 521C), their clinical utility for predicting interindividual cholesterol-lowering response is not well supported. Future approaches utilizing multigene genetic-score approaches may prove superior for investigating the potential utility of these polymorphisms collectively to predict the cholesterol-lowering response or the efficacy (prevention of cardiovascular events) of simvastatin.

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Conflict of Interest. The authors declared no conflict of interest.

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