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Individual and combined associations of genetic variants in *CYP3A4, CYP3A5*, and *SLCO1B1* with simvastatin and simvastatin acid plasma concentrations

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Abstract

Our objective was to evaluate the associations of genetic variants affecting simvastatin (SV) and simvastatin acid (SVA) metabolism (*CYP3A4**22 and *CYP3A5**3) and transport (*SLCO1B1* T521C) with 12-hour plasma SV and SVA concentrations. The variants were genotyped, and concentrations were quantified by HPLC-MS/MS in 646 participants of the Cholesterol and Pharmacogenetics clinical trial of 40 mg/day SV for 6 weeks. The genetic variants were tested for association with 12-hour plasma SV, SVA, or the SVA/SV ratio using general linear models. *CYP3A5**3 was not significantly associated with 12-hour plasma SV or SVA concentration. *CYP3A4**1/*22 participants had 58% higher 12-hour plasma SV concentration compared to *CYP3A4**1/*1 participants (p=0.006). *SLCO1B1* 521T/C and 521C/C participants had 71% (p<0.001) and 248% (p<0.001) higher 12-hour plasma SVA compared to *SLCO1B1* 521T/T participants, respectively. *CYP3A4* and *SLCO1B1* genotypes combined categorized participants into low (<1), intermediate (≈1), and high (>1) SVA/SV ratio groups (p=0.001). In conclusion, *CYP3A4**22 and *SLCO1B1* 521C were significantly associated with increased plasma 12-hour concentrations of SV and SVA, respectively. *CYP3A5**3 was not significantly associated with 12-hour plasma SV or SVA concentrations. The combination of *CYP3A4**22 and *SLCO1B1* 521C was significantly associated with SVA/SV ratio, which may translate into different clinical SV risk/benefit profiles.

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Keywords

statins; pharmacokinetics; pharmacology; genetic; pharmacogenetics

Introduction

Simvastatin (SV) is a pro-drug that undergoes reversible metabolism *in vivo* to the active simvastatin acid (SVA), which is capable of inhibiting cholesterol synthesis and reducing cardiovascular disease risk. Plasma concentrations of SV and SVA are highly variable among patients,[1] which can translate into inter-patient variability in efficacy and toxicity.[2,3] SV is primarily metabolized by CYP3A4 and to a lesser extent CYP3A5,[4,5] whereas SVA is a poor substrate for CYP enzymes.[5,6] SVA is a substrate for the organic anion transporting polypeptide 1B1 (gene: *SLCO1B1*), while SV is a poor substrate.[1,7] The activities of these three proteins, CYP3A4, CYP3A5, and SLCO1B1, are strongly affected by genetic variants, which have been associated with *in vivo* concentrations of SV and SVA. In one study, the bioavailability of SV in *CYP3A4**1/*22 individuals was 49% higher than in *CYP3A4**1/*1 individuals.[8] In another study, the areas under the concentration-time curves (AUC) for SV in *CYP3A5**1/*3 and *CYP3A5**3/*3 healthy volunteers were 2.3- and 3.3-fold higher, respectively, than in *CYP3A5**1/*1 healthy volunteers.[9] A third study reported that the AUC for SVA in *SLCO1B1* 521C/C healthy volunteers was 3.2-fold higher than in *SLCO1B1* 521T/T healthy volunteers.[1]

However, these observations were made in relatively small studies (n = 74;[8] n = 22;[9] n = 32[1]), with mostly healthy volunteers (only 40 subjects had hyperlipidemia in all three studies), and few African-Americans (n = 5 combined). Therefore, these findings must be evaluated in a larger cohort to confirm clinical validity. Moreover, these genetic variants are not uncommon (minor allele frequency[10] of *CYP3A4**22 = 2-6%, *CYP3A5**3 = 15-97%, and *SLCO1B1* 521C = 1-22%); therefore some patients may possess multiple variants affecting SV and SVA metabolism and/or transport. Previous research has not characterized the consequences of such multi-variant combinations. Our preliminary research has shown potential associations of *CYP3A4**22 and *CYP3A5**3 with plasma concentrations of SV and SVA,[11] but a necessary extension is the evaluation of those variants in the context of *SLCO1B1* T521C. The goal of this study was to evaluate the association of the *CYP3A4**22, *CYP3A5**3, and *SLCO1B1* 521C alleles (alone and in combination) with *in vivo* plasma concentrations of SV and SVA in a large sample that included a substantial proportion of African-Americans.

Methods

Participants

Participants for this study came from the Cholesterol and Pharmacogenetics (CAP) Study (n = 944; clinicaltrials.gov identifier NCT00451828), which has been described in detail previously.[12] Briefly, the CAP Study enrolled self-identified black (African-American; n = 335) and white (Caucasian-American; n = 609) men and women, aged ≥30 years, with a baseline total serum cholesterol level of 160 to 400 mg/dl between March 2002 and October

2004. Participants were recruited and enrolled at San Francisco General Hospital and the University of California, Los Angeles, School of Medicine. Baseline data collected during the screening and enrollment visits included demographics, medical history, risk factors for coronary heart disease, physical examination, and laboratory data. Participants were followed for a total of six weeks on SV therapy (40 mg at bedtime) and were seen at clinic visits conducted at two-week intervals. Participants were considered African-American if ≥3 of their grandparents were reported to be African-American and were considered Caucasian-American if ≥3 of their grandparents were reported to be Caucasian-American. Several exclusion criteria were applied, including concomitant use of lipid-lowering drugs or interacting prescription or over-the-counter drugs; known liver disease or elevated transaminase levels more than twice the upper limit of normal; uncontrolled hypertriglyceridemia, blood pressure, or diabetes mellitus; abnormal renal or thyroid function; current alcohol or drug abuse; and known statin intolerance. Compliance was determined by pill counts performed at each two-week clinic visit. The institutional review board at the clinical centers, laboratory centers, and coordinating center approved the CAP Study, and all participants provided informed consent before enrollment.

SV and SVA plasma concentrations

Plasma samples for measurement of SV and SVA concentrations were obtained 12 hours post-dose at the six week visit. SV and SVA were quantified by liquid-liquid cartridge extraction and liquid chromatography/tandem mass spectrometry as previously described.[13] The method has a linear calibration range of 0.05-50 ng/mL for both SV and SVA, and only concentrations within this range were considered for further analysis. Eighty percent of participants had both SV and SVA concentrations within this range (n = 759). The allele frequencies of *CYP3A4**22, *CYP3A5**3, and *SLCO1B1* 521C and all other characteristics were similar among participants missing SV and SVA concentration data (6%) and with SV and SVA concentrations within, above, and below the quantitative range (supplementary tables I & II).

Genotyping

*CYP3A4*22* (rs35599367) was determined using a TaqMan genotyping assay (C_59013445_10; Life Technologies, NY, USA). For the Caucasian-American participants, *CYP3A5*3* (rs776746) was determined using the Illumina HumanHap300 or HumanHap610-Quad genotyping platforms (Illumina, San Diego, CA). For the African-American participants, *CYP3A5*3* was determined using a TaqMan genotyping assay (C_26201809_30; Life Technologies, NY, USA). *SLCO1B1* T521C (rs4149056) was determined using the Illumina Cardio-MetaboChip genotyping platform[14] (Illumina, San Diego, CA) in both African-Americans and Caucasian-Americans.

Ancestry/relatedness analysis

To assess relatedness between all of the participants, the Cardio-MetaboChip[14] data (n = 839) was used to calculate pairwise identity-by-state (IBS) distances. To meet the assumption of independent observations, we tested whether any participants were first cousins or more closely related (pi_hat > 0.125), and we excluded one member of a pair of

African-American participants with pi_hat = 0.1653. To assess genetic ancestry of the participants, the resulting matrix of IBS distances was used to perform multidimensional scaling (MDS) analysis in Plink.[15] The first three MDS components were used as covariates in the multivariable models to adjust for background genetic ancestry (in addition to adjusting for self-reported race).

Statistical analysis

Weighted and balanced contrasts within general linear models were used to assess the independent associations of *CYP3A4**22, *CYP3A5**3, *SLCO1B1* 521C with SV and SVA 12-hour post-dose plasma concentration. The contrasts were weighted by the sample size of each genotype group and balanced by comparing equal numbers of genotype groups (supplementary table III). The contrasts were adjusted for the following covariates: age, gender, self-reported race, smoking status, body mass index (kg/m^2), compliance (percentage of doses taken during the two weeks leading up to when the blood sample was drawn), and genetic ancestry. Covariate-adjusted means of logSV and logSVA within the genotype comparison groups were calculated using the method of least squares means. SV and SVA were log-transformed to meet the assumption of normality. No assumptions were made as to the genetic inheritance mode (*e.g.*, dominant, recessive, or additive).

Only participants with complete data for all genotypes and covariates were included in the analysis, yielding an analytical sample size of n = 646. The most common reasons participants were excluded from the analysis were for SV or SVA below the limit of quantitation (n = 124), missing Cardio-MetaboChip data to adjust for ancestry (n = 105), or missing SV or SVA (n = 52). *A priori* evidence demonstrating the expected directions of association for each of the genetic variants supports the use of one-sided statistical testing ($\alpha = 0.1$), but we adjusted the level of statistical significance for making multiple comparisons (5 contrasts) with the Bonferroni correction, yielding $\alpha = 0.1/5 = 0.02$. Based on $\alpha = 0.02$, we estimated ≥ 80% power to detect a 25% difference in SV and SVA for all contrasts.

Variants with statistically significant independent associations with SV or SVA were combined into three levels according to whether participants were expected to have low, intermediate, or high SVA/SV ratio. The same variants were also tested for gene-gene interactions by incorporating a multiplicative interaction term within the multivariable model. Differences in genetic effects by self-reported race were assessed by stratification and also testing the multiplicative interaction between genotype and self-reported race within the multivariable model. Monte Carlo estimates of the exact p-values for Hardy-Weinberg equilibrium within the self-reported racial groups were calculated using 10,000 permutations. (Statistical analyses were performed using SAS Power and Sample Size 3.1 and SAS version 9.3 (Cary, NC).

Results

*CYP3A4**22, *CYP3A5**3, and *SLCO1B1* 521C allele frequencies and baseline characteristics stratified by self-reported race in the 646 CAP participants used in our analyses are presented in Table 1. Genotypes at all three loci were within Hardy-Weinberg equilibrium within the self-reported racial groups ($p > 0.05$). The represented genotypes for the three loci

with sample sizes are presented in Table 2, and these were used to calculate the balanced and weighted contrasts (supplementary table III). The results for the contrasts are presented in Table 3.

Only *CYP3A4**22 was significantly associated with plasma logSV concentration at 12 hours (estimate = 0.20; p = 0.006). *CYP3A5**3 and *SLCO1B1* 521C were not significantly associated with plasma SV concentration at 12 hours. Participants with the *CYP3A4**1/*22 genotype had a 58% higher plasma SV concentration at 12 hours compared to participants with the *CYP3A4**1/*1 genotype (Figure 1A). Only *SLCO1B1* 521C was significantly associated with plasma logSVA concentration at 12 hours (for T/T vs T/C, estimate = 0.23 and p < 0.001; for T/T vs C/C, estimate = 0.54 and p < 0.001). *CYP3A4**22 and *CYP3A5**3 were not significantly associated with plasma SVA concentration at 12 hours. Participants with the *SLCO1B1* 521T/C and 521C/C genotypes had 71% and 248% higher plasma SVA at 12 hours, respectively, compared to the participants with the *SLCO1B1* 521T/T genotype (Figure 1B). The tests for interaction of genotype with self-reported race were not statistically significant (all p > 0.08; supplementary tables IV & V), indicating the pharmacogenetic effects were not significantly different between the self-reported races.

Because *CYP3A4**22 and *SLCO1B1* 521C had independent associations with SV and SVA respectively, we categorized participants into three genotype groups according to whether they were expected to have a low, intermediate, or high SVA/SV ratio from their *CYP3A4**22 and *SLCO1B1* 521C genotypes (low ratio genotype group = *CYP3A4**1/*22 + *SLCO1B1* 521T/T [n = 24]; intermediate ratio genotype group = *CYP3A4**1/*1 + *SLCO1B1* 521T/T or *CYP3A4**1/*22 + *SLCO1B1* 521C carrier [n = 509]; high ratio genotype group = *CYP3A4**1/*1 + *SLCO1B1* 521C carrier [n = 113]). This categorization scheme was significantly associated with logSVA/SV ratio after adjusting for all covariates (estimate = 0.15; p = 0.001). Participants in the intermediate ratio genotype group had approximately equal plasma concentrations of SVA and SV at 12 hours (ratio = 1.1), but the SVA/SV ratio was greater than 1 in the high ratio genotype group (ratio = 1.5) and lower than 1 in the low ratio genotype group (ratio = 0.73) (Figure 1C). The multiplicative test for gene-gene interaction between *CYP3A4**22 and *SLCO1B1* 521C within the multivariable model for SVA/SV ratio was not statistically significant (p = 0.22), supporting a linear association between genotype group category and SVA/SV ratio.

Discussion

Oral administration of simvastatin (SV) results in variable *in vivo* plasma concentrations of SV and SVA,[1] which can translate into inter-patient variability in the efficacy and toxicity of SV treatment.[2,3] Previous studies[1,8,9] assessing the association between *CYP3A4**22, *CYP3A5**3, and *SLCO1B1* 521C alleles and SV and SVA concentrations had small sample sizes of almost exclusively participants of European ancestry, and they did not examine multi-variant combinations. The analysis presented herein of the CAP trial data provides several advantages over previous studies: large sample size, a substantial proportion of African-Americans, availability of genotyping chip data for MDS analysis to adjust for genetic ancestry, and multi-variant analysis of SVA/SV ratio. SV pharmacology and its connection to clinical outcomes are extremely complex and many knowledge gaps remain.

Our preliminary research demonstrated potential associations of *CYP3A4**22 and *CYP3A5**3 with plasma concentrations of SV and SVA,[11] but other research supports strong effects of *SLCO1B1* T521C.[1] Thus this study was a necessary extension of our preliminary research by evaluating *CYP3A4**22 and *CYP3A5**3 in the context of and in combination with *SLCO1B1* T521C. Notably, many other genes are also involved in SV and SVA pharmacology (*e.g., CYP2D6* and *LDLR*),[16] and a limitation of our study is that we only evaluated three. Moreover, the inter-conversion that occurs between SV and SVA *in vivo* is reversible but not in equilibrium,[17] and it is mediated by both enzymatic and non-enzymatic processes. The interpretation of our findings is further complicated by SV- and SVA-specific pharmacogenetic effects.

We report 58% higher 12-hour plasma SV concentration in participants with *CYP3A4**1/*22 genotype compared to participants with *CYP3A4**1/*1 genotype. *CYP3A4**22 was not associated with 12-hour plasma SVA concentration. To our knowledge, the relationship between *CYP3A4**22 and SV and SVA concentrations has only been evaluated in one previous study.[8] Our finding is consistent with the *CYP3A4**22 finding in the previous study,[8] in which participants with *CYP3A4**1/*22 genotype had 49% higher SV bioavailability compared to participants with *CYP3A4**1/*1 genotype. SV demonstrates linear pharmacokinetics in the dose range of 5 mg to 120 mg.[18] Therefore our data indicate that participants with the *CYP3A4**1/*22 genotype treated with 10mg of SV would have approximately the same 12-hour plasma concentration of SV as participants with the *CYP3A4**1/*1 genotype treated with 20mg of SV. Previous studies have not demonstrated an association between *CYP3A4* variants and SV-induced myopathy,[19-22] but *CYP3A4**22 has been associated with SV dose requirements in patients titrated to cholesterol-lowering targets.[23]

We also found that participants with the *SLCO1B1* 521T/C and 521C/C genotypes had 71% and 248% higher plasma SVA at 12 hours, respectively, compared to the participants with the *SLCO1B1* 521T/T genotype. Notably, our sample did not have any African-American participants with the *SLCO1B1* 521C/C genotype; therefore our results for this genotype cannot be extrapolated specifically to African-Americans and is a limitation of our study. Previous studies on the relationship between *SLCO1B1* T521C and SV or SVA concentrations did not report the 12-hour plasma concentration,[1,8] but our finding is consistent with the previously published findings in that individuals with the 521T/C and 521C/C genotypes have higher plasma exposure to SVA. Strong evidence supports the association between *SLCO1B1* T521C and SV-induced myopathy;[22] prompting the Clinical Pharmacogenetics Implementation Consortium (CPIC) to write guideline recommendations for the use of *SLCO1B1* T521C genotype information in SV treatment when genotype information is available.[24] CPIC recommends to lower doses of SV (or choose an alternative statin) in patients with the *SLCO1B1* 521T/C and 521C/C genotypes. Our findings are consistent with the CPIC recommendation, but our findings may also provide further insight into the magnitude of the dose reduction. Because SV demonstrates linear pharmacokinetics, our data indicates that participants with the 521T/C genotype treated with 10mg of SV would have approximately the same 12-hour plasma concentration of SVA compared to participants with the 521T/T genotype treated with 40mg of SV.

Previous studies have shown large effects of the *CYP3A5**3 allele on CYP3A5 expression and SV pharmacokinetics. *CYP3A5**3 explains the absence of CYP3A5 protein in the livers of some individuals.[25] Kim et al reported a significantly decreased clearance and increased AUC of SV with *CYP3A5**3 (n = 22).[9] Tsamandouras et al replicated the association of *CYP3A5**3 with significantly reduced clearance of SV (n = 74).[8] In our much larger study (n = 646), we did not find a significant association between *CYP3A5**3 and 12-hour plasma concentration of SV (p = 0.119) or SVA (p = 0.534). Our negative finding is consistent with a pharmacokinetic study by Zhou et al (n = 17),[26] but the discordance between our finding and the findings by Kim et al and Tsamandouras et al could be due to the difference in pharmacokinetic measure (a single 12-hour time point versus clearance and AUC). It is possible that *CYP3A5**3 has significant effects on SV clearance and AUC that are not detectable at the 12-hour time point measured in our study. The elimination half-life of SV is 3 to 4 hours; therefore the majority of SV was cleared from the plasma by the 12-hour time point, which is a limitation of our study. Another possibility is that the effects of *CYP3A5**3 could be masked by the large effects of *SLCO1B1* 521C. Indeed, our preliminary research demonstrated a marginal association of *CYP3A5**3 with SV in African-Americans,[11] but when tested in the context of *SLCO1B1* 521C, the association was no longer significant.

Because *CYP3A4**22 and *SLCO1B1* 521C had distinct effects on SV and SVA 12-hour plasma concentration in the CAP study respectively, we evaluated the effect of the combination of those variants on the ratio of SVA/SV. Based on those two genotypes, participants fell into one of three categories: SVA/SV < 1 (low ratio; 4% of CAP participants), SVA/SV ≈ 1 (intermediate ratio; 79% of CAP participants), and SVA/SV > 1 (high ratio; 17% of CAP participants). *CYP3A4**22 has been associated with increased efficacy[23] but not toxicity,[19-22]and *SLCO1B1* 521C has been associated with increased myopathy[22] but not reliably with increased efficacy.[27-32] Therefore patients with SVA/SV > 1 (high ratio group; *CYP3A4**1/*1 and *SLCO1B1* 521C carrier) may have the least favorable risk/benefit profile with SV treatment and require closer clinical monitoring or an alternative statin. According to the study by Wang et al,[23] patients with the *CYP3A4**1/*1 genotype required higher statin doses than patients with the *CYP3A4**1/*22 genotype to achieve cholesterol-lowering goals. However compensating for the increased dose requirements in the high ratio group by increasing the SV dose would be risky because this group already has increased risk for myopathy due to their *SLCO1B1* 521C-carrier genotype.[22] Alternatively, patients with plasma SVA/SV < 1 (low ratio group; *CYP3A4**1/*22 and *SLCO1B1* 521T homozygous) may have the optimal risk/benefit profile because they may have the lowest dose requirements for efficacy[23] and also lowest risk for myopathy.[22] The intermediate ratio genotype group, in which the majority of patients will fall, will have an intermediate risk/benefit profile. Current guidelines for the treatment of blood cholesterol to reduce the risk of atherosclerotic cardiovascular disease (ASCVD) risk in adults recommend low, moderate, or high intensity statin therapy depending on the patient's ASCVD risk.[33] Our findings suggest that SVA/SV ratio is related to *CYP3A4**22 and *SLCO1B1* 521C genotypes. Therefore it is possible that the optimal intensity of SV therapy is dependent on an individual patient's *CYP3A4**22 and *SLCO1B1* 521C genotypes, but this hypothesis needs to be tested in a prospective clinical trial with clinical outcomes endpoints. Research

is currently ongoing to determine the association of these combined genotypes with lipid-lowering and creatine kinase elevation in response to SV treatment.

Conclusions

We report significant associations of *CYP3A4**22 and *SLCO1B1* 521C with plasma 12-hour concentrations of SV and SVA, respectively, in a large sample of participants of European and African ancestry. *CYP3A5**3 was not significantly associated with 12-hour plasma SV or SVA. The pharmacogenetic associations were not significantly different between participants of European and African ancestry. The combination of *CYP3A4**22 and *SLCO1B1* 521C was significantly associated with SVA/SV ratio, which may translate into different SV treatment risk/benefit profiles in patients and requires further study. Research on the effects of this genotype combination on low-density lipoprotein (LDL) response is currently ongoing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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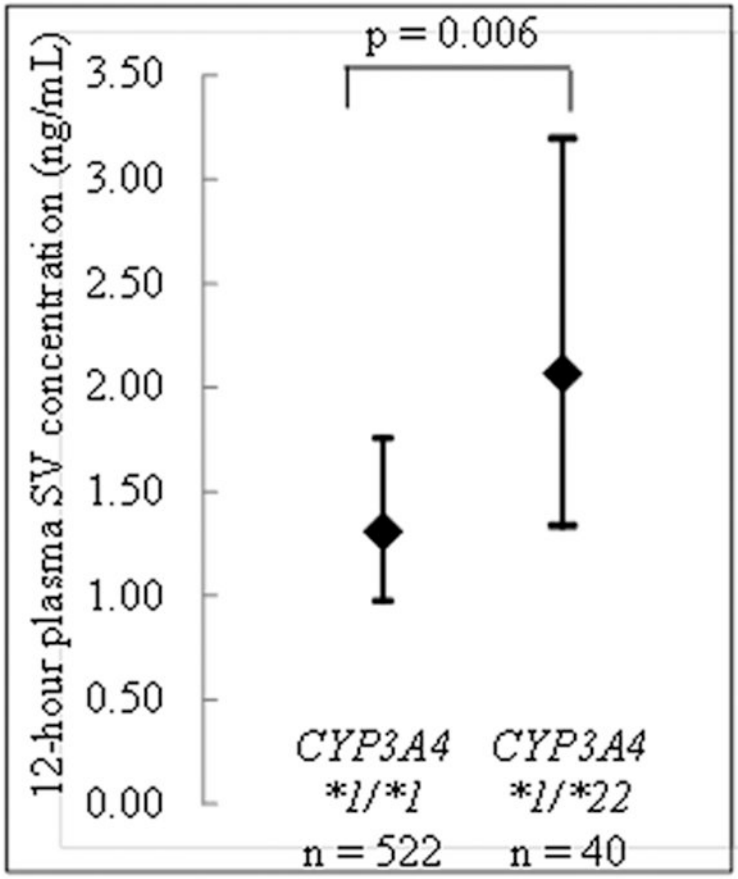

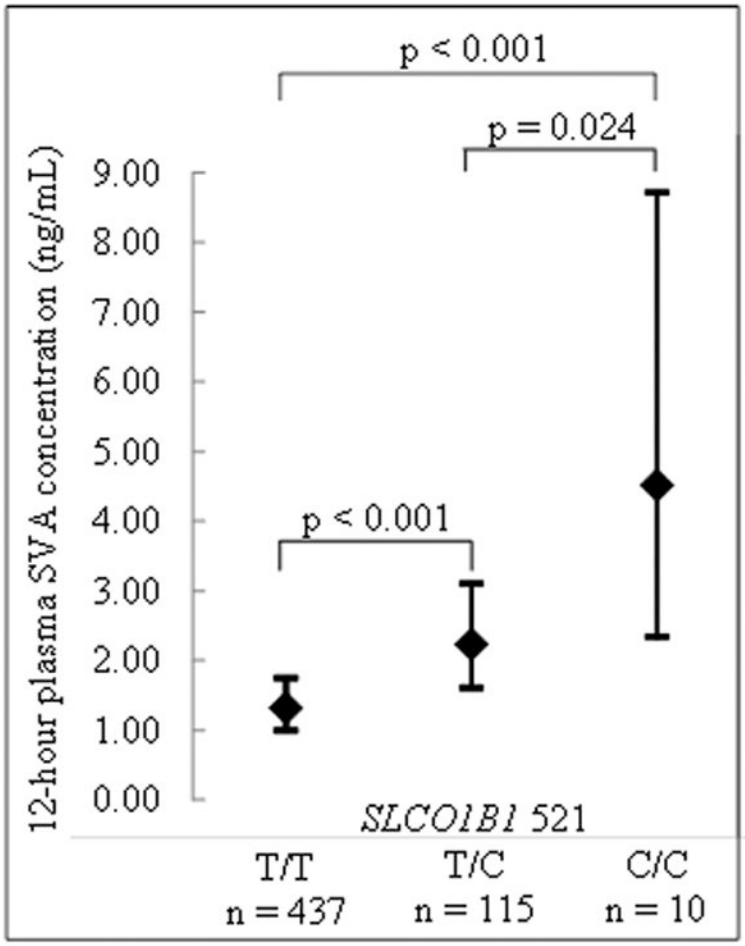

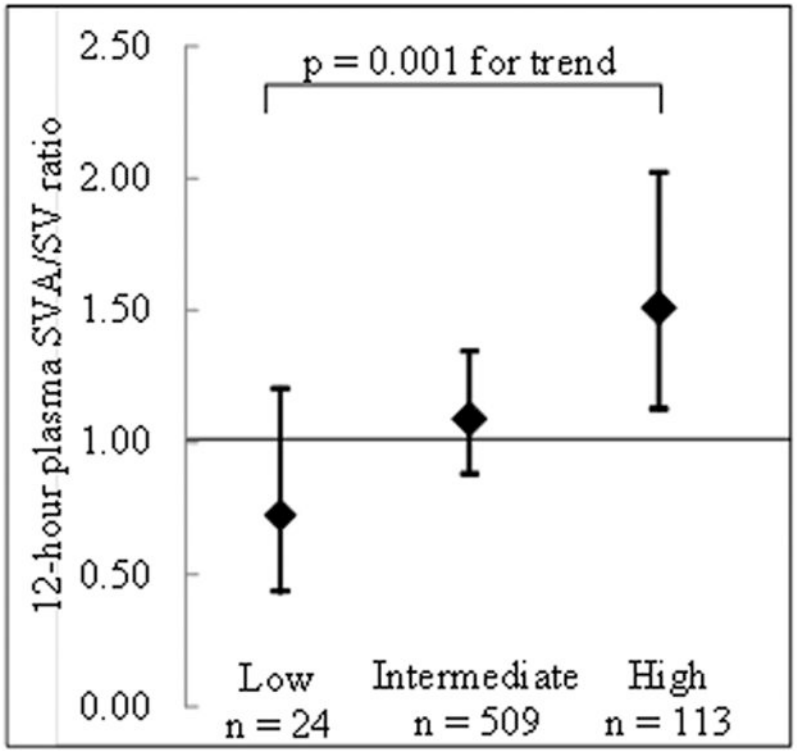

Figure 1.

A-C. Least squared mean and 95% confidence intervals for (A) SV 12-hour plasma concentration by *CYP3A4* genotype, (B) SVA 12-hour plasma concentration by *SLCO1B1* genotype, and (C) 12-hour plasma SVA/SV concentration ratio by low (*CYP3A4**1/*22 + *SLCO1B1* 521T/T), intermediate (*CYP3A4**1/*1 + *SLCO1B1* 521T/T or *CYP3A4**1/*22 + *SLCO1B1* 521C carrier), and high (*CYP3A4**1/*1 + *SLCO1B1* 521C carrier) genotype categories. Values have been adjusted for age, gender, self-reported race, smoking status, body mass index (kg/m2), compliance (percentage of doses taken two weeks leading up to the blood sample being drawn), and ancestry (the first three MDS components).

Table 1
Baseline characteristics compared by self-reported race

Baseline characteristic	Self-reported Caucasian-Americans (n = 447; 69%)	Self-reported African-Americans (n = 199; 31%)	* p-value
SV (ng/mL)	1.12 (1.51)	1.09 (1.19)	0.607
SVA (ng/mL)	1.57 (1.88)	1.45 (1.98)	0.172
Age (years)	55 (17)	53 (20)	0.963
BMI (kg/m^2)	27 (7)	29 (7)	<.001
Compliance (%)	100 (7)	100 (7)	0.026
Male gender	237 (53%)	103 (52%)	0.767
Smoker	60 (13%)	46 (23%)	0.002
*CYP3A4**22 allele frequency	0.04	0.02	0.076
*CYP3A5**3 allele frequency	0.93	0.35	<.001
SLCO1B1 521C allele frequency	0.14	0.03	<.001

* Continuous variables are represented as median (interquartile range) and compared using the Wilcoxon rank sum test. Categorical variables are represented as count (%) and compared using the chi-square test or Fisher's exact where necessary.

BMI indicates 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; SV, inactive parent simvastatin lactone; SVA, active metabolite simvastatin acid

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Table 2

Median (interquartile range) of SV and SVA 12-hour plasma concentration in genotype groups represented in the CAP study

CYP3A4 genotype	*CYP3A5* genotype	*SLCO1B1* genotype	† Label in contrast	n	Unadjusted * SV (ng/mL)	Unadjusted * SVA (ng/mL)
*1/*1	*1/*1	T/T	A	80	1.29 (1.23)	1.21 (1.84)
		T/C	B	4	0.90 (0.59)	0.22 (3.04)
		C/C	C	0	-	-
	*1/*3	T/T	D	132	0.96 (1.00)	1.45 (1.46)
		T/C	E	20	1.75 (2.26)	2.26 (2.37)
		C/C	F	0	-	-
	*3/*3	T/T	G	281	1.12 (1.56)	1.40 (1.69)
		T/C	H	80	1.08 (1.17)	1.94 (2.83)
		C/C	I	9	1.26 (2.65)	3.52 (9.88)
*1/*22	*1/*1	T/T	J	0	-	-
		T/C	K	0	-	-
		C/C	L	0	-	-
	*1/*3	T/T	M	4	2.68 (5.15)	0.94 (2.00)
		T/C	N	2	0.64 (0.17)	1.54 (2.01)
		C/C	O	0	-	-
	*3/*3	T/T	P	20	1.34 (0.98)	1.54 (2.01)
		T/C	Q	13	1.92 (3.47)	5.58 (6.30)
		C/C	R	1	10.70 (0)	21.13 (0)
Total				646	1.11 (1.44)	1.52 (1.84)

* SV and SVA are presented as median (interquartile range) and they have not been adjusted for covariates.

† Contrasts can be found in the supplemental table III.

CYP3A4 indicates 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; SV, inactive parent simvastatin lactone; SVA, active metabolite simvastatin acid

Table 3

Results of contrasts for the difference in least squared means between genotype groups

Genotype comparison	Estimate	Standard Error	p-value
logSV			
CYP3A4 *1/*22 vs *1/*1	0.200	0.072	*__0.006__
CYP3A5 *3/*3 vs *1/*3	0.084	0.054	0.119
SLCO1B1 521T/C vs C/C	0.212	0.146	0.147
SLCO1B1 521T/T vs T/C	0.063	0.047	0.179
SLCO1B1 521T/T vs C/C	0.232	0.141	0.099
logSVA			
CYP3A4 *1/*22 vs *1/*1	0.138	0.071	0.051
CYP3A5 3/*3 vs *1/*3	0.033	0.053	0.534
SLCO1B1 521T/C vs C/C	0.326	0.144	0.024
SLCO1B1 521T/T vs T/C	0.232	0.046	*__<.001__
SLCO1B1 521T/T vs C/C	0.541	0.139	*__<.001__

*Below Bonferroni-corrected alpha level of one-sided statistical significance = 0.1/5 = 0.02.

CYP3A4 indicates 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; SV, inactive parent simvastatin lactone; SVA, active metabolite simvastatin acid