

CYP3A4*22 and CYP3A5*3 are associated with increased levels of plasma simvastatin concentrations in the cholesterol and pharmacogenetics study cohort

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Objective Simvastatin is primarily metabolized by CYP3A4. A combined CYP3A4/5 genotype classification, combining the decrease-of-function CYP3A4*22 and the loss-of-function CYP3A5*3, has recently been reported. We aim to determine whether CYP3A4*22 and CYP3A5*3 alleles are associated with increased plasma concentrations of simvastatin lactone (SV) and simvastatin acid (SVA). This is the first report evaluating associations between in-vivo simvastatin concentrations and CYP3A4*22, alone or in a combined CYP3A4/5 genotype-defined classification.

Participants and methods Genotypes and simvastatin concentrations were determined for 830 participants (555 Whites and 275 African-Americans) in the Cholesterol and Pharmacogenomics clinical trial with 40 mg/day simvastatin for 6 weeks. Concentrations were determined in 12-h postdose samples. Associations between simvastatin concentrations and CYP3A4*22 and CYP3A5*3 alleles were tested separately and in a combined CYP3A4/5 genotype-defined classification system.

Results In Whites, CYP3A4*22 carriers ($n = 42$) had 14% higher SVA ($P = 0.04$) and 20% higher SV ($P = 0.06$) compared with noncarriers ($n = 513$). CYP3A5*3 allele status was not significantly associated with SV or SVA in Whites. In African-Americans, CYP3A4*22 carriers ($n = 8$) had 170% higher SV ($P < 0.01$) than noncarriers ($n = 267$), but no significant difference was detected for SVA.

Introduction

Simvastatin is one of the most widely used drugs for the treatment of hypercholesterolemia. Formulated as a prodrug, simvastatin lactone [simvastatin (SV)] undergoes metabolism to simvastatin hydroxyl acid [simvastatin acid (SVA)], the active form of the drug. SVA is a potent competitive inhibitor of the rate-limiting enzyme (HMG-CoA reductase) of intrahepatic cholesterol synthesis and thereby lowers plasma cholesterol concentrations. SV and SVA are primarily metabolized by CYP3A4/5 [1], and increased plasma simvastatin concentrations have been associated with the use of

African-American CYP3A5 nonexpressors ($n = 28$) had 33% higher SV ($P = 0.02$) than CYP3A5 expressors ($n = 247$), but no significant difference was detected for SVA. For both races, SV appeared to decrease across the rank-ordered combined CYP3A4/5 genotype-defined groups (poor, intermediate, and extensive metabolizers); however, similar trends were not observed for SVA.

Conclusion Genetic variation in CYP3A4 was associated with plasma simvastatin concentrations in self-reported Whites. Genetic variations in CYP3A4 and CYP3A5 were associated with plasma simvastatin concentrations in self-reported African-Americans. *Pharmacogenetics and Genomics* 24:486–491 © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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concomitant medications that inhibit CYP3A metabolism [2,3]. Likewise, polymorphisms that decrease CYP3A activity result in increased simvastatin concentration, exposure, and consequently pharmacodynamic effects. In a single-dose study of 20 mg SV, the 12-h area under the curve of the concentration-versus-time plot (AUC_{12}) for homozygous carriers of the loss-of-function (LOF) CYP3A5*3 allele was 3.3-fold higher than for homozygous wild type (*1/*1) [4]. Similarly, in a cohort of Whites receiving simvastatin, atorvastatin, or lovastatin (statins all primarily metabolized by CYP3A4/5) it was found that CYP3A5 nonexpressors (*3/*3) had greater reductions in total cholesterol compared with CYP3A5 expressors (*1/*1 or *1/*3) [5]. The CYP3A4*22 decrease-of-function (DOF) allele has been associated with attenuated statin metabolism and with increased statin efficacy. CYP3A4 enzyme activity in livers with the CYP3A4*1/*1 wild-type

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genotype was 2.5-fold higher than in livers with the *CYP3A4*22/*1* or *CYP3A4*22/*22* genotypes [6]. In addition, *CYP3A4*22* carriers have been reported to require a 40% lower dose of simvastatin for optimal lipid control compared with wild type [6]. Furthermore, *CYP3A4*22* carriers from the Rotterdam study had significantly increased reductions in total and low-density lipoprotein-cholesterol with simvastatin treatment compared with noncarriers [7]. Although relationships between *CYP3A* polymorphisms and clinical outcomes have been attributed to variation in simvastatin concentrations, the relationship between the *CYP3A4*22* allele and in-vivo simvastatin concentrations has not yet been examined.

Currently, the FDA mandates drug labeling of simvastatin to include warnings recommending against the concomitant use of CYP3A4/5 inhibitors [8]. Drug labels may evolve to include information regarding *CYP3A* genetic polymorphisms if sufficient evidence accrues establishing a clinically significant link with increased simvastatin exposure. The aim of this brief report is to determine the association between plasma simvastatin concentrations and the DOF *CYP3A4*22* allele, alone and in combination with the LOF *CYP3A5*3* allele.

Patients and methods

Study participants

Investigators enrolled 1007 patients in the Cholesterol and Pharmacogenomics (CAP) study (clinicaltrials.gov identifier NCT00451828). The study's primary aims were to assess the associations of DNA sequence variants with simvastatin pharmacodynamics [9]. In total, 944 participants in the CAP study were genotyped, but complete data (*CYP3A4/5* genotype and plasma simvastatin concentrations) was available for only 830 (555 self-reported Whites and 275 self-reported African-Americans) participants.

Genotype analyses

For White study participants, *CYP3A5*3* (rs776746) status was determined using the Illumina HumanHap300 or HumanHap610-Quad (San Diego, California, USA) genotyping platforms. *CYP3A5*3* status for African-American study participants was determined using TaqMan (Life Technologies, Grand Island, New York, USA) genotyping assay (C_26201809_30). *CYP3A4*22* (rs35599367) allelic status was determined using TaqMan genotyping assay (C_59013445_10). To validate the rs776746 TaqMan assay, individuals of known genotype of each genotype class were included on each qPCR plate. These positive controls consisted of self-reported Whites for whom genome-wide genotype information was available. The rs35599367 TaqMan assay was validated in a similar manner using imputed genotype information. However, no minor allele homozygotes were available in our population to use as positive controls.

No template controls were run on each plate as negative controls. A subset of samples ($\leq 10\%$) were re-run, and no evidence of sample switching or of false genotyping was found.

Quantification of simvastatin and simvastatin acid

Plasma concentrations of SV and SVA were measured in CAP study participants after 6 weeks of 40 mg/day simvastatin. Compliance was monitored by pill count at 4 and 6 weeks after the start of statin treatment. As samples were collected ~ 12 h after the final dose, only the compliance scores collected at the 6-week time-point were considered relevant for this analysis. Quantification was performed on a PE SCIEX API 365 (Framingham, Massachusetts, USA) triple-quadrupole mass spectrometer coupled to a liquid-chromatography column. Samples were evaluated on a Kromasil C18 (Saint Louis, Missouri, USA) column following the previously published protocol [10]. Purified SV and SVA were gifted by Merck (New Jersey, USA), and serial dilutions were used to generate standard curves.

Statistical analyses

Statistical analyses were performed using Stata/IC-12 (StataCorp., College Station, Texas, USA) for Windows. All analyses were performed using nonparametric tests as data distributions were not normal.

On the basis of a-priori knowledge from in-vitro metabolism studies [4,6], we hypothesized the following: (i) simvastatin concentrations are higher in participants carrying at least one DOF *CYP3A4*22* allele compared with wild type (**/1**); (ii) simvastatin concentrations are higher for *CYP3A5* nonexpressors (**3/*3*) compared with *CYP3A5* expressors (**/1** or **/1**); and (iii) simvastatin concentrations are higher in combined *CYP3A4/5* genotype-defined 'poor metabolizers' (PMs) compared with 'intermediate metabolizers' (IMs) and compared with 'extensive metabolizers' (EMs). Methodology regarding the combined *CYP3A4/5* genotype-defined classification was first described by Elens *et al.* [11] and has subsequently been utilized in the study of several *CYP3A* substrates [12,13].

All analyses were stratified by race. Potential confounding factors such as smoking status, sex, age, BMI, and compliance were tested for significant associations with simvastatin concentrations utilizing Mann-Whitney *U*-tests and Spearman's rank correlation tests. In addition, associations with the genotype-defined groups were tested (Table 1) using Mann-Whitney *U*-tests and Kruskal-Wallis *H*-tests to compare medians of numeric variables between genotype-defined groups and Pearson's χ^2 -tests to compare percentages between genotype-defined groups.

Median and interquartile ranges for SV and SVA were determined for genotype-defined groups (Figs 1 and 2).

Table 1 Participant characteristics by CYP3A genotype-defined groups

Parameters	All participants	CYP3A4 *22/*1 or *22/*22	CYP3A4 *1/*1	P-value	CYP3A5 *3/*3	CYP3A5 *1/*3 or *1/*1	P-value	CYP3A4/5 PMs	CYP3A4/5 IMs	CYP3A4/5 EMs	P-value
Number	830	50	780		509	321		42	475	313	
Age ^a	53.5 (45.1, 62.4)	53.7 (44.4, 62.0)	53.5 (45.2, 62.4)	0.82 ^d	53.7 (45.1, 62.1)	53.2 (45.5, 62.6)	0.86 ^d	54.9 (44.4, 66.9)	53.5 (45.1, 62.0)	53.4 (45.5, 62.9)	0.91 ^e
Compliance ^a	100 (92.9, 100)	100 (92.9, 100)	100 (92.3, 100)	0.85 ^d	100 (92.9, 100)	100 (92.9, 100)	0.76 ^d	100 (92.9, 100)	100 (92.9, 100)	100 (92.9, 100)	0.93 ^e
BMI ^a	27.8 (24.6, 31.3)	26.0 (24.3, 29.6)	27.8 (24.6, 31.4)	0.08 ^d	26.8 (24.2, 30.8)	28.5 (25.5, 32.0)	0.01 ^d	26.0 (24.3, 29.6)	26.7 (24.1, 30.9)	28.6 (25.6, 32.3)	0.01 ^e
White ^{b,c}	555 (67)	42 (84)	513 (66)	0.01 ^f	481 (94)	74 (23)	0.00 ^f	40 (95)	443 (93)	72 (23)	0.00 ^f
Male ^c	435 (52)	32 (64)	403 (52)	0.09 ^f	266 (52)	169 (53)	0.91 ^f	26 (62)	246 (52)	163 (52)	0.45 ^f
Smoker ^c	154 (19)	5 (10)	149 (19)	0.00 ^f	77 (15)	77 (24)	0.11 ^f	5 (12)	72 (15)	77 (24)	0.00 ^f

EM, CYP3A4/5 extensive metabolizer; IM, CYP3A4/5 intermediate metabolizer; PM, CYP3A4/5 poor metabolizer.

^aNumeric variables with non-normal distributions presented as median (1st quartile, 3rd quartile).

^bRace was self-reported as White or African-American.

^cCategorical variables represented as counts (%).

^dP-values for numeric variables were determined using Mann-Whitney *U*-tests to compare medians in two groups.

^eP-values for numeric variables were determined using Kruskal-Wallis *H*-tests to compare medians in three groups.

^fP-values for categorical variables were determined using Pearson's χ^2 -tests to compare frequencies in two or three groups.

One-sided Mann-Whitney *U*-tests were performed for single-gene analyses comparing median values of SV and SVA between two genotype-defined groups. Directional hypotheses based on a-priori knowledge predicated the use of one-sided statistical tests. As BMI was associated with SV and SVA ($P < 0.05$), both SV and SVA are lipophilic, and the average CAP participant was classified as overweight, all analyses were repeated with BMI-adjusted simvastatin concentrations [14].

Results

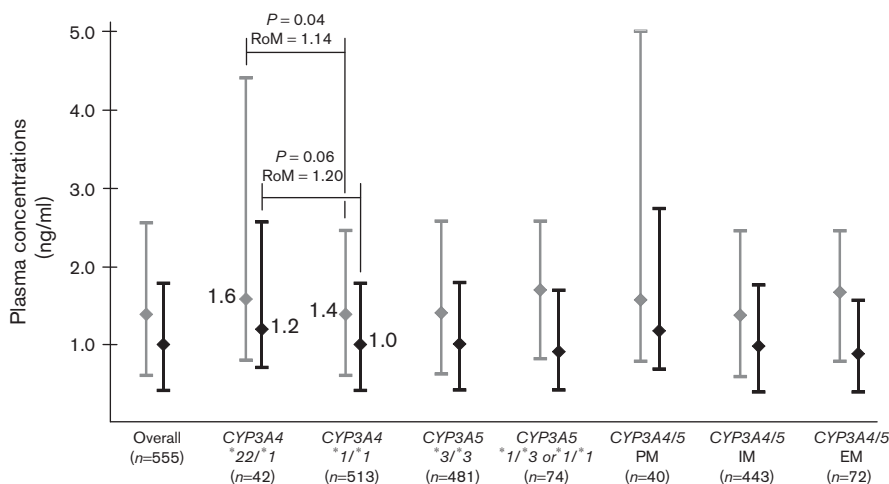
As shown in Table 2, the allele frequencies of *CYP3A4**22 and *CYP3A5**3 were significantly higher in Whites, and distributions in the various genotype-defined groups were significantly different between Whites and African-Americans. In both races, *CYP3A4**22 and *CYP3A5**3 distributions were in Hardy-Weinberg equilibrium. The alleles were determined to be in partial linkage disequilibrium (LD), and the degree of LD was different for Whites and African-Americans ($r^2 = 0.06$ and 0.14, respectively). As shown in Table 1, race, BMI, and smoking status had significant associations with genotype-defined groups. Race and BMI, however, were the only clinical characteristics with significant associations ($P < 0.05$) with simvastatin concentrations, predicated that analyses be stratified by race and simvastatin concentrations be adjusted for BMI. Analyses were also repeated with concentrations adjusted for body weight.

Single-gene and combined-gene analyses of SV and SVA are presented in Figs 1 and 2. In the single-gene analyses of self-reported Whites, *CYP3A5**3 allele status was not significantly associated with SV or SVA concentrations, and *CYP3A4**22 carriers had 14% higher concentrations of SVA ($P = 0.04$) and 20% higher concentrations of SV ($P = 0.06$) compared with noncarriers. In the single-gene analyses of self-reported African-Americans, *CYP3A4**22 carriers had 170% higher concentrations of SV than did noncarriers ($P < 0.01$), but no significant difference was detected for concentrations of SVA; and *CYP3A5* non-expressors (*CYP3A5**3/*3) had 33% higher concentrations of SV than *CYP3A5* expressors (*CYP3A5**1/*3 or *1/*1) ($P = 0.02$), but no significant difference was detected for concentrations of SVA.

In the analysis of *CYP3A4/5* genotype-defined groups (PMs, IMs, and EMs), SV concentrations appeared to decrease across the rank-ordered groups; however, no statistically significant associations were identified. Furthermore, similar trends were not observed for SVA concentrations. BMI adjustment had no appreciable effect in any analysis (results not shown).

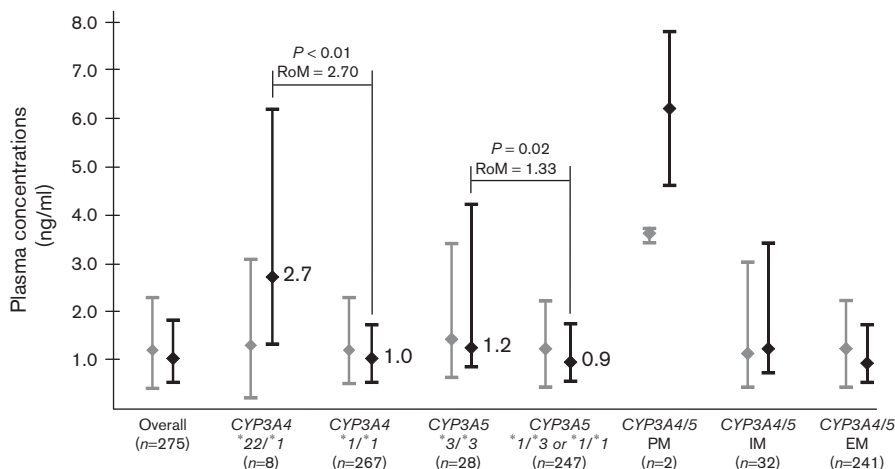
Both White and African American *CYP3A4**22 carriers had greater SVA than noncarriers ($P < 0.05$); however, this effect was much more remarkable in the African-Americans than Whites (170 vs. 14% increase, respectively). A nonstatistically significant trend of greater SV in

Fig. 1



Simvastatin concentrations for self-reported Whites. Data are presented as median and interquartile range. Plasma concentrations of simvastatin acid and simvastatin lactone are represented by light and dark, respectively. *P*-values determined using one-way Mann-Whitney *U*-tests, and only those less than 0.10 are displayed. EM, extensive metabolizer; IM, Intermediate metabolizers; PM, poor metabolizer; RoM, ratio of means.

Fig. 2



Simvastatin concentrations for self-reported African-Americans. Data are presented as median and interquartile range. Plasma concentrations of simvastatin acid and simvastatin lactone are represented by light and dark, respectively. *P*-values determined using one-way Mann-Whitney *U*-tests, and only those less than 0.10 are displayed. EM, extensive metabolizer; IM, Intermediate metabolizers; PM, poor metabolizer; RoM, ratio of means.

*CYP3A4*22* carriers was also observed. The effect of *CYP3A5* status was significant only for African-Americans; SV was 33% greater in *CYP3A5* nonexpressors (*3/*3) compared with *CYP3A5* expressors (*1/*3 or *1/*1) ($P=0.02$).

Discussion

This report describes associations between in-vivo simvastatin concentrations and *CYP3A5*3* and *CYP3A4*22* alleles, and the observed associations were consistent with the known functional effects of these alleles [4,6]. The DOF *CYP3A4*22* allele was associated with

increased simvastatin concentrations for both self-reported Whites and self-reported African-Americans. However, *CYP3A5* was influential in only the self-reported African-Americans. Although our results are not unexpected, to date this is the first report that has specifically tested the associations between plasma simvastatin concentration and *CYP3A4*22*, alone or in the combined *CYP3A4/5* genotype-defined classification.

Observed differences in *CYP3A* allele frequencies and LD between African-Americans and Whites are consistent with previously reported allele frequency analyses

Table 2 CYP3A genetic variables by race

Genetic variables	n (%)		P-value
	White (n = 555) (67%) ^a	African-American (n = 275) (33%) ^a	
CYP3A4*22 allele frequency (%)	4	1	<0.001
CYP3A4*1/*1	513 (92)	267 (97)	<0.001
CYP3A4*1/*22	42 (8)	8 (3)	
CYP3A4*22/*22	0 (0)	0 (0)	
CYP3A4*22 HWE	0.33	0.82	NA
P-value			
CYP3A5*3 allele frequency (%)	93	35	<0.001
CYP3A5*1/*1	6 (1)	112 (41)	<0.001
CYP3A5*1/*3	68 (12)	135 (49)	
CYP3A5*3/*3	481 (87)	28 (10)	
CYP3A5*3 HWE	0.08	0.17	NA
D'	1.0	1.0	NA
r ²	0.06	0.14	NA
CYP3A4/5 EMs ^b	72 (13)	241 (88)	<0.001
CYP3A4/5 IMs ^c	443 (80)	32 (12)	<0.001
CYP3A4/5 PMs ^d	40 (7)	2 (1)	<0.001

EM, extensive metabolizer; HWE, Hardy–Weinberg equilibrium; IM, intermediate metabolizer; PM, poor metabolizer.

^aRace was self-reported as White or African-American.

^bCYP3A4/5 EMs were CYP3A4*1/*1 and either CYP3A5*1/*1 or CYP3A5*1/*3.

^cCYP3A4/5 IMs were CYP3A*1/*1 and CYP3A5*3/*3, or CYP3A4*1/*22 and either CYP3A5*1/*1 or CYP3A5*1/*3.

^dCYP3A4/5 PMs were CYP3A4*1/*22 and CYP3A5*3/*3.

[15]. We observed that the majority (nearly 90%) of African-Americans were categorized as EMs in the combined CYP3A4/5 genotype-defined classification system (Fig. 2). We previously reported that simvastatin treatment has reduced efficacy for low-density lipoprotein-cholesterol lowering in African-Americans compared with Whites [9]. This difference may partially be attributed to the disproportionate number of EMs in African-Americans; however, further investigation is warranted.

Our analyses failed to identify a statistically significant relationship between CYP3A5 genotype and plasma SV or SVA concentrations in Whites. This is consistent with the analyses reported by Kim *et al.* [4] in which a statistically significant difference was determined for simvastatin concentration AUC₁₂ but not for 12-h postdose concentration. The LOF CYP3A5*3 allele is the major allele in Whites, and it is likely that variation in other similar enzymes, such as CYP3A4 or CYP3A7, may compensate for the loss of CYP3A5 function associated with the *3 allele.

Of the covariates tested, we only found evidence of a modest association between BMI and statin concentrations. This finding was not surprising because SV and SVA are lipophilic [16,17], the average BMI in the CAP study classifies as overweight (i.e. average BMI was 27.8), and BMI can alter the pharmacokinetic parameters of lipophilic substances. However, as the BMI range of the cohort was quite narrow and the effect size of BMI on SV and SVA was marginal, it was not unexpected that BMI adjustment did not impact the observed genotype–concentration associations. Similarly, all analyses were

repeated with adjustments for body weight and the observed genotype–concentration associations were not significantly altered (results not shown).

There are several limitations to the present report. It is based on a retrospective pharmacokinetic analysis in a study designed primarily for measuring pharmacodynamic endpoints and did not examine polymorphisms in other genes known to affect simvastatin pharmacokinetics (e.g. *SLC01B1*). In addition, racial categories were self-reported, and although race stratification does account for racial differences in LD, it does not account for unbalanced allele status among polymorphism-carrier groups. Finally, some genotype-determined groups were inadequately represented (e.g. only two of 275 African-American participants represented the CYP3A4/5 PM group).

Nevertheless, the study has several strengths for investigating influence of CYP3A: the overall sample size was large, the simplicity of the genotype groupings allowed for utilization of robust nonparametric statistical analyses, the sex profile was well balanced, the ranges of BMI and age were marginal, and simvastatin dose, strength, and duration were homogenous. Furthermore, the study design prohibited the use of concomitant medications and certain foods that could induce or inhibit CYP3A.

The genotype–concentration associations reported here are preliminary observations and are not intended to guide the dose selection of simvastatin. However, these results may be combined with additional data to account for ancestry, LD, and other polymorphic genes involved in the pharmacokinetics of simvastatin, possibly providing a more predictive model in the future.

Conclusion

Here, we presented the first report specifically testing the associations between plasma simvastatin concentration and CYP3A4*22, alone or in the combined CYP3A4/5 genotype-defined classification. Our analyses revealed associations between simvastatin concentrations and CYP3A4*22 in both racial categories and an association for CYP3A5 in only the self-reported African-Americans.

Guidance for statin pharmacotherapy is of increasing relevance given the dramatic expansion of statin-use recommendations outlined in the clinical guidelines recently issued by the American Heart Association [18]. Although CYP3A genotype is currently not considered when prescribing statins, future guidance and recommendations may be derived in part from models incorporating genotype status along with concomitant use of enzyme inhibitors or inducers.

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Conflicts of interest

There are no conflicts of interest.

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