Published in final edited form as:

Pharmacogenet Genomics. 2007 August ; 17(8): 647–656. doi:10.1097/FPC.0b013e3280ef698f.

Effect of drug transporter genotypes on pravastatin disposition in European- and African-American participants

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Abstract

Objective—Our aims were to evaluate the effects of polymorphisms in the hepatic drug uptake transporter organic anion transporting polypeptide 1B1 (OATP1B1, *SLCO1B1*) and efflux transporters multidrug resistance-associated protein 2 (MRP2, *ABCC2*), bile salt export pump (BSEP, *ABCB11*), and breast cancer-related protein (BCRP, *ABCG2*) on single-dose pravastatin pharmacokinetics in healthy European- and African-American participants.

Methods—The pharmacokinetics of a single oral 40mg dose of pravastatin was determined in 107 participants (69 European-Americans and 38 African-Americans). Participants were genotyped for known OATP1B1, MRP2, BSEP, and BCRP polymorphisms. Baseline serum total and unconjugated plasma bilirubin concentrations were also determined.

Results—OATP1B1 genotypes were ethnicity-dependent with a 521C allele frequency of ~15% in European-Americans and ~1% in African-Americans. *SLCO1B1* 521TC genotype was associated with significantly higher pravastatin area under the curve [$AUC_{(0-5)}$] ($P=0.01$) and C_{max} values ($P<0.05$). When analyzed by diplotype, *SLCO1B1**1a/*15 ($N=8$) participants exhibited 45 and 80% higher AUC values than *SLCO1B1**1a/*1a ($N=29$) ($P=0.013$) and *SLCO1B1**1b/*1b ($N=34$) ($P=0.001$) carriers, respectively. *SLCO1B1**15/*15 ($N=2$) participants exhibited 92 and 149% higher AUC values than *SLCO1B1**1a/*1a ($P=0.017$) and

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The authors have no conflicts of interest to disclose.

SLCO1B1*1b/*1b ($P=0.011$) carriers, respectively. European-Americans had significantly higher plasma pravastatin $AUC_{(0-5)}$ ($P=0.01$) and C_{max} values ($P=0.009$) than African-Americans. Neither ABCC2, ABCB11, nor ABCG2 genotypes were associated with differences in pravastatin pharmacokinetics. We did not observe an effect of *SLCO1B1* genotype on baseline total or unconjugated bilirubin levels.

Conclusion—*SLCO1B1* genotype, in particular the 521C allele, had a significant effect on the pharmacokinetics of pravastatin. Even when adjusted for the presence of the *SLCO1B1* 521C or 388G variant allele, European-Americans demonstrated significantly higher pravastatin AUC and C_{max} values than African-Americans.

Keywords

ABCB11; ABCC2; ABCG2; BCRP; bilirubin; BSEP; MRP2; OATP1B1; pharmacokinetics; pravastatin; *SLCO1B1*; transporter

Introduction

In the liver, efficient extraction of drugs from the portal blood into hepatocytes is often mediated by uptake transporters expressed on the sinusoidal (basolateral) membrane. For many drugs, carrier-mediated uptake into hepatocytes may represent a critical rate-limiting step to the overall disposition of such drugs and thus be an important component of hepatic first-pass elimination. The organic anion transporting polypeptides (OATPs) represent an important class of drug uptake transporters that mediate the sodium-independent transport of a diverse range of amphipathic organic compounds including bile salts, steroid conjugates, thyroid hormones, anionic peptides, numerous drugs and other xenobiotics [1]. Recently, the HUGO Gene Nomenclature Committee approved a new species-independent classification and nomenclature system based on divergent evolution that defines the *SLCO*-gene superfamily through stratification into families, subfamilies and individual genes according to evolutionary relationships and the degree of amino acid sequence identities [2] (<http://www.bioparadigms.org/slc/intro.asp>).

Many OATP family members exhibit widespread tissue expression, but certain OATPs, such as OATP1B1 (also known as OATP-C, LST1, OATP2) and OATP1B3 (also known as OATP8, LST2), appear to exhibit an organ-selective pattern of expression, most notably in the liver [3]. OATP1B1 is primarily expressed at the basolateral membrane of hepatocytes and plays a key role in the hepatic disposition of structurally diverse substrates, including bile salts, conjugated and unconjugated bilirubin, BSP, steroid conjugates, the thyroid hormones T4 and T3, eicosanoids, cyclic peptides, and drugs such as benzylpenicillin, methotrexate, pravastatin and rifampin [1,4].

We now know that there are a number of synonymous and nonsynonymous single nucleotide polymorphisms (SNPs) in the coding region of the *SLCO1B1* gene [5–8]. Moreover, the presence or frequency of these SNPs appears to be ethnicity-dependent. In-vitro experiments in a number of cell-based assays demonstrated that certain *SLCO1B1* variants exhibit significantly impaired transport activity for prototypical substrates such as

estrone sulfate and estradiol-17 β -glucuronide [5]. Decreased activity, in part, could be attributed to decreased cell surface expression.

Pravastatin, an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (statins), is a drug widely used to treat hypercholesterolemia [9]. Owing to its hydrophilicity, active transport mechanisms are thought to be important for its hepatic disposition. Subsequently, pravastatin was shown to be a substrate for OATP1B1, and transport by this mechanism is thought to be the rate-limiting step in pravastatin hepatic clearance [10,11]. Previous studies have demonstrated substantial interindividual variation (6-fold) in pravastatin pharmacokinetic parameters, including area under the curve (AUC) and the maximum concentration (C_{max}) but not half-life, in healthy participants [9, 12]. More recently, clinical studies have confirmed that variation in pravastatin pharmacokinetics can be attributed in part to the presence of SLCO1B1 polymorphisms [7, 13, 14]. Specifically, studies have demonstrated that individuals carrying the 521C allele either in combination with the 388A allele (SLCO1B1*5) or the 388G allele (SLCO1B1*15) exhibited significantly higher mean pravastatin AUC values compared with individuals carrying reference alleles SLCO1B1*1a or *1b, consistent with in-vitro data indicating markedly decreased transport function for the 521C variant.

In addition to OATP1B1, statins are substrates for efflux transporters including multidrug resistance-associated protein 2 (MRP2; *ABCC2*) [15], breast cancer resistance protein (BCRP; *ABCG2*) [16] and the bile salt export pump (BSEP; *ABCB11*) [17], all of which have been localized to the canalicular membrane of hepatocytes [18]. Furthermore, MRP2 and BCRP expression has also been localized to the apical membrane of intestinal enterocytes [18]. Therefore genetic variation in efflux transporters may be important determinants of the oral bioavailability of statins. Polymorphisms have been identified in the *ABCC2*, *ABCG2* and *ABCB11* genes, but there is a paucity of data regarding their functional significance [19–23] (<http://www.pharmgkb.org>). Therefore, in addition to the contribution of polymorphisms in SLCO1B1, it is plausible to hypothesize that functionally relevant polymorphisms in these efflux transporters are also important determinants of pravastatin disposition.

In this study, we assessed the relationship between genotype and phenotype of pravastatin pharmacokinetics in a large number of European- and African-American participants to effectively evaluate the functional significance of drug transporter polymorphisms on the in-vivo disposition of pravastatin. We also describe the influence of ethnicity on pravastatin pharmacokinetics among European- and African-Americans. Our findings suggest that genetic variation in SLCO1B1 results in higher systemic exposure to pravastatin, whereas known SNPs in the efflux transporters studied do not have a major influence. Importantly, we report that plasma concentrations of pravastatin differ significantly between European- versus African-American participants, suggesting that as yet undefined environmental or genetic factors play additional roles in determining the interethnic variation in the disposition of pravastatin.

Methods

Participants

One hundred and seven healthy, unrelated nonsmoking adult participants (69 European-Americans and 38 African-Americans; age, 19–55 years; weight, 47.7–109 kg) were enrolled in the study. Mean body surface area (BSA), weight and height was 1.96 m², 77.3 kg and 1.81m for European-American men ($n = 37$) and 2.05 m², 84.6 kg and 1.81m for African-American men ($n = 19$), respectively. Mean BSA, weight and height was 1.70 m², 63.3 kg and 1.64m for European-American women ($n = 32$) and 1.75 m², 67 kg and 1.65m for African-American women ($n = 19$), respectively. The health status of each individual was confirmed by history and physical examination, including heart rate and blood pressure measurements, and laboratory tests including a complete blood count and blood chemistry screening including direct (conjugated) and total bilirubin values. After confirmation of medical history and normal physical examination, participants were instructed to ingest no drugs for at least 1 week before their assigned study day. The study protocol was approved by the Institutional Review Board and General Clinical Research Center at Vanderbilt University Medical Center, and participants provided written informed consent before study enrollment.

Study design

The study was performed in the General Clinical Research Center at Vanderbilt University Medical Center. After an overnight fast, each study participant received a single oral dose of 40 mg pravastatin (Bristol-Myers-Squibb, New York, USA) with water. An indwelling venous cannula was placed and serial heparinized blood samples (10 ml) were obtained immediately before and at 1, 2, 3, 4, 5, 6, 8, and 12 h after pravastatin administration. Plasma was separated by centrifugation and immediately stored at –20°C until quantitative analysis was performed.

Genotyping

During the screening visit, ~30 ml venous blood was obtained for pharmacogenetic analyses. Genomic DNA was obtained from peripheral blood mononuclear cells via a standard DNA extraction protocol (QIAmp DNA Mini Kit, Qiagen, Valencia, California, USA) and samples were stored at –20°C until genotyping was performed. Samples were genotyped for previously reported SLCO1B1 polymorphisms (*1b – 388G, *5 – 521C, and *9 – 1463C) as well as commonly occurring nonsynonymous polymorphisms in the efflux transporters ABCC2 (1249A and 3563A), ABCG2 (34A and 421A) and ABCB11 (1331C and 2029G). Genotyping was performed using a validated 5' nuclease PCR-based assay with allele specific fluorescent probes (TaqMan SNP Genotyping Assays, Applied Biosystems, Foster City, California, USA) [24]. Fluorescent probes for each SNP were designed through Applied Biosystems from reference sequences (Gen-Bank) for SLCO1B1, ABCC2, ABCG2, and ABCB11 and are as follows: SLCO1B1 388G, SLCO1B1 521C, SLCO1B1 1463C, ABCC2 1249A, ABCC2 3563A, ABCG2 34A, ABCG2 421A, ABCB11 1331C, and ABCB11 2029G. PCRs were performed on an Applied Biosystems Gene-Amp 9700 PCR System (ABI, Foster City, California). Each 50µl reaction contained 100 ng genomic DNA, 10mmol/l Tris-HCl (pH 8.3), 50mmol/l KCl, 4mmol/l MgCl₂, 200 µmol/l each dNTP, 200

nmol/l each primer, 50 nmol/l each fluorescent-tagged probe, 0.5 U AmpErase uracil *N*-glycosylase (Perkin-Elmer, Waltham, Massachusetts), and 1.25U AmpliTaq DNA polymerase (Perkin-Elmer). Cycling conditions were 50°C, 2 min; 95°C, 10 min; 40 cycles of 95°C, 15 s; 64°C, 1 min. Each individual TaqMan probe was validated by assaying a sequenced-verified DNA standard for each wild-type and variant allele tested. Following amplification, 40 µl of each reaction was transferred to a microtiter plate (Perkin-Elmer) and fluorescence measured using the ABI Prism 7900HT Sequence Detection System. SNPs were noted and grouped into cluster plots with software package SDS version 2.2 Enterprise Edition Software Suite. OATP1B1 haplotypes were inferred using an algorithm based on Bayesian inference with the statistical software package PHASE (<http://www.stat.washington.edu/stephens/software.html>) [25,26] and named according to current nomenclature conventions for SLCO1B1 haplotype [5,7].

Drug analysis and pharmacokinetics

A 100 mg C₁₈ 1ml capacity preparatory solid-phase extraction column (Bakerbond, JT Baker, Phillipsburg, New Jersey, USA) was initially conditioned with 2 ml double-distilled water. Aliquots of 1000 µl of plasma and 50 µl of internal standard 5-methoxypsoralen (Indofine Chemical Company Inc., Somerville, New Jersey, USA) at 1000 ng/ml in methanol/water (50: 50, v/v) were added and the column was washed with 3 ml of methanol/water (10: 90, v/v) containing 0.05% acetic acid and then 3 ml of methanol/water (10: 90, v/v) containing 0.05% ammonium hydroxide. The sample was eluted with 1 ml methanol and was evaporated to dryness at 40°C under a gentle stream of nitrogen. The recovery of pravastatin and 5-methoxypsoralen was complete. The residue was reconstituted with an aliquot (50 µl) of methanol/water (50: 50, v/v) and centrifuged at 8000g for 3 min. An aliquot (20 µl) of the supernatant was injected onto a Zorbax SB C₁₈, 5µm particle size, 3.0 × 150 mm² column (Agilent, VWR International Ltd, Mississauga, Ontario, Canada) that was attached to a Hewlett Packard 1090 HPLC (high-pressure liquid chromatography) system equipped with a Hewlett Packard 1050 variable wavelength ultraviolet detector. The effluent was monitored at an absorbance wavelength of 238 nm. The HPLC mobile phase in Pump A was acetonitrile/water (28: 72, v/v) containing 0.08% triethylamine at pH 3.0 with 10 mmol/l potassium phosphate buffer and in Pump B was acetonitrile/water (70: 30, v/v) containing 0.08% triethylamine at pH 3.0 with 10mmol/l potassium phosphate buffer at a flow rate of 0.5 ml/min. The timetable was 100% of mobile phase in Pump A from 0 to 18 min and 100% mobile phase in Pump B from 18 to 21 min. The retention times of pravastatin and 5-methoxypsoralin were 10.4 and 17.4 min, respectively. The standard curve of pravastatin was linear over the range tested. The intra-assay coefficient of variation (CV) was 5% at 5 ng/ml (*n* = 5) and the limit of detection was 1 ng/ml. The inter-assay CV was 18% at 50 ng/ml (*n* = 27) and 11% at 100 ng/ml (*n* = 19). The sensitivity of the assay was improved from a limit of detection 5–1 ng/ml after 73 participants had been studied. Assay sensitivity accounted for 8% of variability in pravastatin AUC and was accounted for in the statistical analysis but did not affect the overall results of the study.

Pharmacokinetic parameters of pravastatin were calculated using the time points 0 h before pravastatin administration and 1, 2, 3, 4, and 5 h after pravastatin intake as the majority of pravastatin concentrations at 6, 8, and 12 h were below the detection limits of our assay.

Pharmacokinetic analysis of pravastatin included peak concentration in plasma ($C_{\max}$) and area under the plasma concentration vs. time curve from 0 to 5 h (AUC_{0-5}). AUC_{0-5} values were calculated by linear trapezoidal method.

Statistical analysis

All data are presented as mean $\pm$ SD for continuous variables and the number of participants or proportion for categorical variables. The associations between each gene and ethnicity were examined using Pearson χ^2 test. Multiple regression models were used to examine the association between outcomes and genotypes for each gene after adjustment for potential confounding factors. The major outcomes of interest were AUC and the $C_{\max}$ as pharmacokinetic (PK) parameters, and total bilirubin and unconjugated bilirubin. Although total bilirubin concentrations were measured for all participants, the conjugated bilirubin concentrations were available for only 23 participants while 72 participants had partial information only (conjugated bilirubin concentration < 0.1 owing to lower limits of assay sensitivity). As a supplementary measure, the conjugated bilirubin concentrations were imputed for the 12 missing values and the 72 values having partial information.

Theoretically, the fraction of the conjugated bilirubin is known to be 5% of the total bilirubin [27]. To take into account uncertainty and measurement errors, the imputed values were generated from log normal distribution with mean of 5% of the total bilirubin and SD of 0.3 (assuming that the coefficient of variance for measurements is approximately 30%). Then, the unconjugated bilirubin concentrations were calculated as the total bilirubin concentration subtracted by either the measured or the imputed conjugated bilirubin concentrations.

The potential confounding factors adjusted for included gender, [BSA = height (cm) $\times$ weight (kg)/3600], ethnicity and assay sensitivity indicator in the models for pharmacokinetic parameters as outcomes, and sex, BSA, interaction between sex and BSA, and ethnicity in the models for the total and unconjugated bilirubin as outcomes. These covariates were selected based on potentially important factors affecting pravastatin pharmacokinetics in humans as well as the considerations of the effect sample size rule where the number of covariates should not exceed more than 1/10th–1/15th of the sample size [28]. The overall gene effects on the outcomes and the interaction between covariates were tested using the likelihood-ratio test. As the distributions of AUC, $C_{\max}$ and the total and unconjugated bilirubin were skewed to the right, they were transformed to square root of each value to satisfy the normality assumption for regression models. All significant findings were reanalyzed using the bootstrap method (1000 bootstraps) to reduce a chance of false-positive finding and the bootstrapped P -values and 95% confidence intervals (CI) were reported, as appropriate. All tests were two-tailed, and a P -value of < 0.05 was considered significant. Hardy–Weinberg equilibrium was tested for each gene stratified by race using a STATA user-written program, Hardy–Weinberg equilibrium tests and allele frequency estimation [29]. All other analyses were performed with the software STATA 9.1 (StataCorp, College Station, Texas, USA) and R (www.r-project.org).

Results

Comparison of transporter polymorphism allele frequencies between European-Americans and African-Americans

The allele frequencies of *SLCO1B1*, *ABCC2*, *ABCG2*, and *ABCB11* variants are summarized in Table 1. The *SLCO1B1* 388G (*1b) variant was more common in African-Americans than in European-American participants (77 vs. 38% allele frequency, $P < 0.001$). On the other hand, the *SLCO1B1* 521C (*5) variant had a relatively high allele frequency in European-American participants than African-American participants (15 vs. 1%, $P = 0.008$), in line with our previous work [5]. Haplotype analysis revealed the 521C variant was most likely to occur in conjunction with the 388G allele to form *SLCO1B1**15 (combination of *1b and *5), consistent with several recently published observations in Japanese and European Caucasian populations (Table 3) [7, 13]. The 1463C (*9) variant, initially identified with a relatively high allele frequency in African-Americans [5], was only identified with a 1% allele frequency in the current study, indicating this variant may be less common in African-Americans than previously thought.

Participants were also genotyped for commonly occurring polymorphisms in the efflux transporters *ABCC2*, *ABCG2* and *ABCB11*. The frequency of *ABCC2* 4544A and *ABCB11* 1331C and 2029G variants differed significantly among European-American and African-American participants, whereas there was a lack of difference in the prevalence of *ABCG2* polymorphisms between ethnic groups (Table 1). Overall, these results reveal that allele frequencies of commonly occurring *SLCO1B1*, *ABCC2* and *ABCB11* variants appear to be dependent on race, consistent with previously published reports [5] (www.pharmgkb.org). All genes were in Hardy–Weinberg equilibrium for both European-Americans and African-Americans.

Pravastatin pharmacokinetics in relation to *SLCO1B1* genotypes and ethnicity

There was wide interindividual and interethnic variability in pravastatin disposition. In our model, the combination of gender, BSA, assay sensitivity, race and *SLCO1B1* 521TC genotype accounted for 31% of the interindividual variability in pravastatin AUC, whereas *SLCO1B1* 521TC genotype and race individually explained 6.5 and 6.2% of variability, respectively (Table 2).

SLCO1B1 521TC genotype was significantly associated with higher AUC ($P=0.01$) and $C_{\max}$ values ($P<0.05$) after adjusting for gender, BSA and assay sensitivity (Fig. 1a). *SLCO1B1* wild-type T/T carriers had significantly lower AUC values compared with T/C carriers (adjusted mean difference 31.3 ng · h/ml, 95% CI 6.2–56.4 ng · h/ml, $P = 0.015$) and C/C carriers (adjusted mean difference 58.2 ng · h/ml, 95% CI 14.6–101.9 ng · h/ml, $P = 0.009$). T/T carriers also demonstrated significantly lower $C_{\max}$ values compared with T/C carriers (adjusted mean difference 14.6 ng · h/ml, 95% CI 0.5–28.7 ng · h/ml, $P = 0.042$) and tended toward lower $C_{\max}$ values compared with homozygous C/C carriers (adjusted mean difference 17.5 ng · h/ml, 95% CI –4.3 to 39.4 ng · h/ml, $P = 0.116$).

When considering overall *SLCO1B1* gene effects, *SLCO1B1* diplotype (521C and 388G alleles) was significantly associated with increase in AUC ($P = 0.048$), but not $C_{\max}$ ($P =$

0.153) (Table 3), after adjusting for gender, BSA, and assay sensitivity. *SLCO1B1**1a/*15 participants had 45 and 80% higher AUC values than *SLCO1B1**1a/*1a (adjusted mean difference 42.9 ng · h/ml, 95% CI 8.9–76.8 ng · h/ml; $P = 0.013$) and *SLCO1B1**1b/*1b (adjusted mean difference 59.7 ng · h/ml, 95% CI 24.9–94.4 ng · h/ml; $P = 0.001$) carriers, respectively (Fig. 2). AUC was 92 and 149% higher in *SLCO1B1**15/*15 than *SLCO1B1**1a/*1a (adjusted mean difference 55.1 ng · h/ml, 95% CI 9.7–100.6 ng · h/ml; $P = 0.017$) and *SLCO1B1**1b/*1b (adjusted mean difference 71.9 ng · h/ml, 95% CI 16.7–127.1 ng · h/ml; $P = 0.011$) carriers, respectively. $C_{\max}$ was 46% and 78% higher in *SLCO1B1**1a/*15 than *1a/*1a (adjusted mean difference 24.7 ng/ml, 95% CI 3.1–46.3 ng/ml; $P = 0.025$) or *1b/*1b (adjusted mean difference 29.8 ng/ml, 95% CI 8.0–51.6 ng/ml; $P = 0.007$) carriers.

Overall, pravastatin AUC (98.3 ± 60.7 vs. 69.5 ± 56.3 ng · h/ml; $P = 0.010$) and $C_{\max}$ values (53.3 ± 33.3 vs. 37.4 ± 29.2 ng/ml; $P = 0.009$) were significantly higher in European-Americans compared with African-Americans (Fig. 1b). After adjustment for *SLCO1B1* 521TC genotype, gender, BSA and assay sensitivity, European-Americans still demonstrated significantly higher AUC ($P = 0.028$) and $C_{\max}$ values ($P = 0.036$) than African-Americans. Likewise, after adjustment for *SLCO1B1* 388AG genotype, gender, BSA and assay sensitivity, European-Americans consistently had higher AUC ($P = 0.004$) and $C_{\max}$ values ($P = 0.006$). A subgroup analysis for European-Americans demonstrated pravastatin AUC was significantly greater for those participants who carried the 521C allele vs. wild-type individuals (adjusted mean difference 28.7 ng · h/ml, 95% CI 3.4–54.1 ng · h/ml; $P = 0.026$). Furthermore, *SLCO1B1**1a/*15 participants demonstrated significantly higher pravastatin AUC values than *1a/*1a participants (adjusted mean difference 46.0 ng · h/ml, 95% CI 11.2–80.7 ng · h/ml; $P = 0.011$), but not *1b/*1b participants (adjusted mean difference 33.9 ng · h/ml, 95% CI 37.5–105.4 ng · h/ml; $P = 0.35$). After adjusting for all covariates, sex was not associated with significant differences in pravastatin pharmacokinetics.

Pravastatin pharmacokinetics in relation to ABCC2, ABCG2, and ABCB11 genotypes

Commonly occurring ABCC2, ABCG2, or ABCB11 genotypes did not associate with differences in pravastatin AUC or $C_{\max}$ (Table 4) when adjusted for covariates. Although some of these variants have demonstrated altered function for other substrates such as estradiol-17 β -glucuronide and methotrexate [19,20], it appears that they are not relevant to the observed interindividual variability in pravastatin disposition.

Bilirubin concentrations and *SLCO1B1* genotypes

Recent studies in Asian participants have noted a correlation between *SLCO1B1* genotype and unconjugated bilirubin concentrations and risk for hyperbilirubinemia [30–32]. Total and direct bilirubin concentrations were measured in each subject as part of routine laboratory screening for entry into the study. Unconjugated bilirubin was calculated by subtracting the direct fraction from the total bilirubin concentration. Regarding *SLCO1B1* genotype, there were no significant differences in total or unconjugated bilirubin concentrations for individuals when grouped by the 521TC (data not shown) or 388AG (Fig.

3) genotype. Moreover, there were no significant differences in total or unconjugated bilirubin concentrations amongst *SLCO1B1**1a/*1a, *1a/*1b, or *1b/*1b carriers (Table 3).

Discussion

There is an increasing appreciation of the important contribution of the OATP superfamily of uptake transporters to the disposition of numerous endogenous and xenobiotic substrates. Recently, polymorphisms have been identified in the *SLCO1B1* gene and both in-vitro and in-vivo studies have demonstrated that a number of these variants result in impaired function compared with wild-type *SLCO1B1*. Previous studies using pravastatin as an in-vivo probe for *SLCO1B1* activity have demonstrated altered disposition profiles among Japanese or European Caucasian participants who possess variant *SLCO1B1* alleles *1b, *5 and *15 [7,13,14]. This report extends these findings by confirming higher AUC of the *SLCO1B1* substrate pravastatin in individuals carrying the 521C allele (*5 or *15). Furthermore, we observed significant ethnic differences in plasma exposure between European-Americans and African-Americans after adjustment for *SLCO1B1* genotype, suggesting additional genetic factors may be of importance to the interethnic and interindividual variability in pravastatin disposition.

Studies from our laboratory and others have demonstrated a number of *SLCO1B1* variants, most notably 521C, are associated with impaired transporter activity *in vitro*. Reduced function of this variant appears to be due, in part, to mistrafficking of the protein to the cell surface [5]. Importantly the 521C variant has a relatively high allele frequency of ~15–18% in Asian and Caucasian populations [5,6]. Studies in healthy Asian and European Caucasian participants utilizing single-dose pravastatin as a probe for *SLCO1B1* activity demonstrated that carriers of 521C polymorphism (*5), regardless of whether this was in linkage equilibrium with 388A (*15), had significantly higher pravastatin AUC values [7,13,14], consistent with in-vitro functional data.

In this report, we also used pravastatin as an in-vivo probe of transporter activity in a large population of healthy European-American and African-American individuals. We genotyped for commonly occurring polymorphisms in transporter genes of potential importance to pravastatin disposition, including the uptake transporter OATP1B1 and the efflux transporters MRP2, BSEP, and BCRP. Overall, the *SLCO1B1* 521C variant was significantly associated with higher AUC and $C_{\max}$ values, consistent with previous reports. Individuals homozygous for the variant allele (C/C) had the highest pravastatin AUC, whereas individuals heterozygous for the variant allele (T/C) or wild-type (T/T) exhibited intermediate, and lowest AUC values respectively, suggesting a gene–dose effect. Similar gene–dose effects were noted for $C_{\max}$ values.

Previous reports have suggested there may be phenotypic differences between the two reference wild-type alleles 388A (*1a) and 388G (*1b), as pravastatin AUC values for individuals carrying the *1b allele tended to be lower than for the *1a allele, although this has not been consistently statistically significant [7,14,33]. Likewise, in our study, we found that individuals homozygous for the *1b allele (388GG) did have a tendency, albeit not

statistically significant, to have lower AUC values than those homozygous for the *1a allele (388AA).

When analyzed by haplotype, individuals carrying the SLCO1B1*1a/*15 or *15/*15 diplotype had significantly higher AUC values than carriers of either of the wild-type diplotypes SLCO1B1*1a/*1a or *1b/*1b. Individuals carrying the SLCO1B1*1b/*15 diplotype tended to have higher AUC values than *1a/*1a or *1b/*1b carriers, but this did not reach statistical significance, possibly related to the inverse effects of the *1b and *15 haplotypes. Individuals carrying the SLCO1B1*1a/*15 diplotype exhibited significantly higher $C_{\max}$ values than either *1a/*1a or *1b/*1b carriers. Participants carrying the SLCO1B1*1b/*15 and *15/*15 diplotypes also tended to have higher $C_{\max}$ values than *1a/*1a or *1b/*1b carriers. Overall, these results are consistent with previously published studies and confirm that individuals carrying the SLCO1B1 521C variant attain significantly higher plasma pravastatin AUC and $C_{\max}$ values than those carrying the wild-type reference allele after single-dose administration of pravastatin.

The clinical implications of this observed genotypemediated variation in pravastatin disposition are unclear. A recent prospective clinical study evaluating the effect of SLCO1B1 genotype on pharmacokinetics and lipidlowering efficacy of multiple-dose pravastatin confirmed significantly higher plasma AUC and $C_{\max}$ values in individuals carrying the 521C allele but did not observe a significant genotype dependence on lipid-lowering effect [34]. As the number of participants in that study was, however, small, potential subtle differences in lipidlowering efficacy among individuals with various genotypes may not have been detected. Furthermore, SLCO1B1 genotype only explained ~7% of pravastatin AUC variability in our study. Niemi *et al.* [13] noted in their study that characteristics such as body weight did not explain variability in pravastatin AUC. In contrast, we noted that gender and BSA contributed 11 and 12% of pravastatin variability, respectively. Therefore, consideration of other factors such as gender, BSA and race, in addition to genotype may provide a more accurate prediction of the interindividual variability in pravastatin pharmacokinetic and pharmacodynamic parameters.

Recently, interethnic differences in statin disposition have been noted. Rosuvastatin, with similar physicochemical properties to pravastatin, had ~2-fold higher systemic exposure in Asian participants compared with Caucasian participants in Western Europe or the United States [35,36]. A recent study of rosuvastatin disposition in Asian and Caucasian participants living in the same geographic area, Singapore, confirmed ~2-fold higher plasma exposure in the Asian group [37]. Interestingly, neither the SLCO1B1 388G nor 521C allele accounted for the observed pharmacokinetic differences between Asian and Caucasian participants. In this study, we report ethnic differences in pravastatin pharmacokinetics between European-Americans and African-Americans. Overall, European-American individuals demonstrated significantly higher plasma pravastatin AUC and $C_{\max}$ values than African-American participants. As SLCO1B1 521C genotype had the most significant effect on pravastatin pharmacokinetics and the allele frequency of this variant is very low (~1%) in African-Americans, we adjusted the analysis for SLCO1B1 521TC genotype. Even after adjustment for genotype, European-Americans had significantly higher AUC and $C_{\max}$ values than African-Americans, suggesting interethnic differences exist in pravastatin

disposition between these two groups and that other genetic factors, possibly polymorphisms in other transporter genes, may contribute to the observed interethnic variation in disposition.

Recent studies have indicated that statins are substrates for efflux transporters, including MRP2, BCRP and BSEP [15–17]. Polymorphisms have been identified in these transporters and some have been shown to have functional consequences *in vitro* or *in vivo* [19,20,38–40]. We genotyped individuals for commonly occurring polymorphisms in these transporters. Although we noted significant interethnic differences in the frequencies of some of these variants, significant associations between polymorphisms in these efflux transporters and pravastatin AUC or $C_{\max}$ were not found. The likely explanation is that hepatic uptake mediated by OATP1B1 is thought to be the rate-limiting step in pravastatin hepatic clearance [10]. Therefore, although efflux transporter polymorphisms influence the pharmacokinetics of drugs such as diflomotecan [39], these do not clearly impact pravastatin disposition.

Recent in-vitro studies have suggested that unconjugated bilirubin may be a substrate for OATP1B1, although controversy exists as to whether OATP1B1 alone is sufficient to mediate hepatic extraction of bilirubin from the systemic circulation [41,42]. Several clinical studies have suggested that SLCO1B1 genotype, in particular the 521C and 388G variant alleles, may predict risk for severe hyperbilirubinemia in Asian infants, influence serum bilirubin concentrations, and be a risk for unconjugated hyperbilirubinemia in healthy Asian adults [30–32]. Our clinical study enrolled healthy adults with normal complete blood counts and comprehensive metabolic panels, including hepatic function panels. Therefore, individuals with mild hyperbilirubinemia would not have been eligible for this study. Our analysis, nevertheless, indicates that SLCO1B1 genotype alone, in particular the 521C and 388G variant alleles, was not associated with significant differences in total or unconjugated bilirubin concentrations. Moreover, when analyzed by diplotype, there were no significant differences in total or unconjugated bilirubin concentrations among *SLCO1B1**1a/*1a, *1a/*1b, *1b/*1b, *1a/*15, *1b/*15, or *15/*15 carriers.

In conclusion, our findings confirm SLCO1B1 variants, in particular SLCO1B1*15, are important contributing factor to the observed interindividual variability in pravastatin pharmacokinetics after single-dose oral administration. In addition, there appear to be significant interethnic differences in pravastatin disposition between European-Americans and African-American participants which are not attributable to SLCO1B1 521TC or 388AG genotype, suggesting other genetic factors may be of importance when considering differences in pravastatin disposition between these two groups. The consequences of SLCO1B1 polymorphisms on the pharmacokinetic variation of other statins and the pharmacodynamic effects of SLCO1B1 polymorphisms to lipid response in patients with dyslipidemia remain unclear and warrant further study.

Acknowledgments

We thank Katherine Lee, Rachel McCumsey, Santiago Vilanova and Scott Bailey for their technical assistance on this project. We also thank Dr David Freeman for thoughtful advice relating to pravastatin HPLC analysis. This work was funded in part by United States Public Health Service grants GM54724 (R.B.K.), M01 RR-00095 from

the National Center for Research Resources, and a Vanderbilt University Physician Scientist Development Award (R.H.H.).

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Fig. 1. Mean $\pm$ SD plasma concentrations after a single 40 mg dose of pravastatin in relation to (a) SLCO1B1 521TC genotype and (b) ethnicity.

Fig. 2.

Mean ( ) $\pm$ SD (arrows) pravastatin AUC in relation to SLCO1B1 diplotype. Box plots represent 25th–75th percentiles, horizontal line within plots represent the median, horizontal lines upper and lower quartiles represent minimum and maximum values except outliers that are represented by a single horizontal line beyond 1.5 interquartile range. AUC, area under the curve.

Fig. 3. Mean ( ) $\pm$ SD (arrows) serum (a) total and (b) unconjugated bilirubin concentrations in relation to SLCO1B1 388AG genotype. Box plots represent 25th–75th percentiles, horizontal line within plots represent the median, horizontal lines upper and lower quartiles represent minimum and maximum values except outliers, which are represented by a single horizontal line beyond 1.5 interquartile range.

Table 1

Allele frequencies for transporter polymorphisms

Transporter gene	Genotype (<i>P</i> value) ^a	Allele frequency		
		European-American	African-American	Total
<i>SLCO1B1</i>	388AG	A 0.62	A 0.23	A 0.52
	(<i>P</i> < 0.001)	G 0.38	G 0.77	G 0.48
	521TC	T 0.85	T 0.99	T 0.90
	(<i>P</i> =0.008)	C 0.15	C 0.01	C 0.10
	1463GC	G 1.0	G 0.99	G 0.995
	(<i>P</i> = 0.181)	C 0.0	C 0.01	C 0.005
<i>ABCC2</i>	1249GA	G 0.85	G 0.86	G 0.855
	(<i>P</i> = 0.740)	A 0.15	A 0.14	A 0.145
	3563GA	T 0.95	T 0.96	G 0.95
	(<i>P</i> =0.685)	A 0.05	A 0.04	A 0.05
	4544GA	G 0.95	G 0.83	G 0.91
	(<i>P</i> = 0.021)	A 0.05	A 0.17	A 0.09
<i>ABCB11</i>	1331TC	T 0.28	T 0.54	T 0.37
	(<i>P</i> = 0.001)	C 0.72	C 0.46	C 0.63
	2029AG	A 1.0	A 0.79	A 0.93
	(<i>P</i> < 0.001)	G 0.0	G 0.21	G 0.07
<i>ABCG2</i>	421CA	C 0.08	C 0.04	C 0.065
	(<i>P</i> = 0.210)	A 0.92	A 0.96	A 0.935
	34GA	G 0.02	G 0.09	G 0.05
	(<i>P</i> = 0.110)	A 0.98	A 0.91	A 0.95

^a*P*-values for the association between genotype and race.

Table 2

Factors contributing to variability in pravastatin pharmacokinetics

Model covariates	<i>P</i> -value	<i>R</i> ² for model (%)
Gender	< 0.001	11.1
BSA	< 0.001	12.2
Assay sensitivity	0.004	7.8
521TC	0.031	6.5
Race	0.010	6.2
521TC and race	0.012	10.0
Gender/BSA/assay sensitivity/521TC/race	< 0.001	30.7

BSA, body surface area.

Table 3

SLCO1B1 diplotype and pravastatin pharmacokinetic variables

Number	Diplotype	*1a/*1a	*1a/*1b	*1b/*1b	*1a/*5	*1a/*15	*1b/*15	*15/*15	Total
69	European-American	26	23	2	1	8	7	2	
38	African-Am	3	11	23	0	0	1	0	
107(1.00) ^{††}	Total	29 (0.27)	343 (0.32)	25 (0.24)	1 (0.01)	8 (0.07)	8 (0.07)	2 (0.02)	
AUC ₀₋₅ (ng h/ml)	European-American	83.4 ± 66.3	103.2 ± 63.7	71.5 ± 58.7	58.9	120.9 ± 37.0	105.1 ± 48.8	167.0 ± 31.4	98.3 ± 60.7 [#]
	African-American	119.4 ± 82.7	62.3 ± 46.6	66.7 ± 57.8			61.8		69.5 ± 56.3
	Total	87.1 ± 67.4	90.0 ± 61.1	67.1 ± 56.7	58.9	120.9 ± 37.0 [*]	99.7 ± 47.7	1 67.0 ± 31.4 ^{**}	
C _{max} (ng/ml)	European-American	45.1 ± 35.1	58.0 ± 37.6	41.5 ± 16.3	29.3	66.4 ± 24.6	54.1 ± 24.7	75.6 ± 2.1	53.3 ± 33.3 [#]
	African-American	49.6 ± 29.3	35.3 ± 27.1	36.9 ± 31.6			35.1		37.4 ± 29.2
	Total	45.6 ± 34.1	50.7 ± 35.8	37.3 ± 30.5	29.3	66.4 ± 24.6 [†]	51.7 ± 23.9	75.6 ± 2.1	
Total bilirubin (mg/dl)	European-American	0.62 ± 0.23	0.50 ± 0.25	0.75 ± 0.49	0.50	0.61 ± 0.19	0.59 ± 0.29	0.4 ± 0.14	
	African-American	0.37 ± 0.12	0.56 ± 0.30	0.53 ± 0.33			0.40 ±		
	Total	0.60 ± 0.23	0.52 ± 0.27	0.55 ± 0.33	0.50	0.61 ± 0.19	0.56 ± 0.28	0.4 ± 0.14	

AUC, area under the curve.

* $P < 0.05$ vs. *1a/*1a and $P = 0.001$ vs. *1b/*1b.

** $P < 0.05$ vs. *1a/*1a and $P < 0.05$ vs. *1b/*1b.

# $P < 0.05$ European-American vs. African-American.

[†] $P < 0.05$ vs. *1a/*1a and $P < 0.01$ vs. *1b/*1b.

^{††} $P < 0.001$ for the association between haplotype and race.

Table 4

Efflux transporter polymorphisms and pravastatin pharmacokinetics

		MRP2			BCRP			BSEP	
		1249GA	3563TA	4544GA	421CA	34GA	1331TC	2029AG	
Total	AUC	Wt/Wt	89.9 ± 61.6	87.8 ± 60.4	87.8 ± 60.9	89.8 ± 61.3	88.1 ± 61.8	92.2 ± 64.9	91.9 ± 62.1
		Wt/Var	80.0 ± 56.4	98.0 ± 67.4	83.3 ± 56.0	77.4 ± 58.4	85.0 ± 53.7	86.3 ± 64.8	62.5 ± 47.3
		Var/Var	198.1		211.2		128.4	88.8 ± 55.9	80
C _{max}	Wt/Wt	47.5 ± 32.0	48.1 ± 33.0	48.8 ± 33.5	48.8 ± 33.7	48.2 ± 33.4	47.7 ± 30.8	49.9 ± 33.4	
	Wt/Var	46.8 ± 34.3	48.9 ± 33.2	41.3 ± 29.2	41.8 ± 26.5	40.7 ± 26.2	46.3 ± 34.1	34.7 ± 26.5	
	Var/Var	104		80		70.4	49.6 ± 32.8	32.6	
European-American	AUC	Wt/Wt	95.1 ± 62.9	97.3 ± 60.1	97.3 ± 60.1	100.8 ± 61.2	97.0 ± 61.2	92.3 ± 56.8	98.7 ± 61.1
		Wt/Var	102.9 ± 53.6	110.6 ± 73.2	110.6 ± 73.2	87.7 ± 62.3	134.4 ± 53.6	107.2 ± 67.6	
		Var/Var	198.1					93.6 ± 57.6	
C _{max}	Wt/Wt	50.7 ± 32.9	53.7 ± 33.5	53.7 ± 33.5	55.1 ± 34.4	53.2 ± 33.7	55.8 ± 32.0	53.7 ± 33.5	
	Wt/Var	59.0 ± 33.8	53.2 ± 35.9	53.2 ± 35.9	46.1 ± 28.2	63.7 ± 30.4	56.3 ± 34.0		
	Var/Var	104					51.4 ± 34.1		
African-American	AUC	Wt/Wt	80.4 ± 59.1	70.7 ± 57.9	65.5 ± 57.9	72.0 ± 58.0	69.8 ± 59.6	92.1 ± 72.3	72.8 ± 62.4
		Wt/Var	42.5 ± 39.0	68.6 ± 50.4	66.0 ± 35.7	39.9 ± 10.3	55.4 ± 26.1	60.5 ± 51.8	62.5 ± 47.3
		Var/Var			211.2		128.4	63.9 ± 40.0	80
C _{max}	Wt/Wt	41.8 ± 29.9	37.9 ± 29.8	37.4 ± 31.2	38.4 ± 30.1	38.0 ± 30.8	42.8 ± 30.8	39.1 ± 31.5	
	Wt/Var	26.8 ± 25.4	38.9 ± 29.7	33.7 ± 22.7	26.2 ± 10.84	27.0 ± 10.5	33.9 ± 30.6	34.7 ± 26.5	
	Var/Var			80		70.4	40.2 ± 24.8	32.6	

All *P* values > 0.05.

Wt, wild-type; Var, variant.