

Clinical and Pharmacogenetic Predictors of Circulating Atorvastatin and Rosuvastatin Concentrations in Routine Clinical Care

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Background—A barrier to statin therapy is myopathy associated with elevated systemic drug exposure. Our objective was to examine the association between clinical and pharmacogenetic variables and statin concentrations in patients.

Methods and Results—In total, 299 patients taking atorvastatin or rosuvastatin were prospectively recruited at an outpatient referral center. The contribution of clinical variables and transporter gene polymorphisms to statin concentration was assessed using multiple linear regression. We observed 45-fold variation in statin concentration among patients taking the same dose. After adjustment for sex, age, body mass index, ethnicity, dose, and time from last dose, *SLCO1B1* c.521T>C ($P<0.001$) and *ABCG2* c.421C>A ($P<0.01$) were important to rosuvastatin concentration (adjusted $R^2=0.56$ for the final model). Atorvastatin concentration was associated with *SLCO1B1* c.388A>G ($P<0.01$) and c.521T>C ($P<0.05$) and 4 β -hydroxycholesterol, a CYP3A activity marker (adjusted $R^2=0.47$). A second cohort of 579 patients from primary and specialty care databases were retrospectively genotyped. In this cohort, genotypes associated with statin concentration were not differently distributed among dosing groups, implying providers had not yet optimized each patient's risk–benefit ratio. Nearly 50% of patients in routine practice taking the highest doses were predicted to have statin concentrations greater than the 90th percentile.

Conclusions—Interindividual variability in statin exposure in patients is associated with uptake and efflux transporter polymorphisms. An algorithm incorporating genomic and clinical variables to avoid high atorvastatin and rosuvastatin levels is described; further study will determine whether this approach reduces incidence of statin myopathy. (*Circ Cardiovasc Genet.* 2013;6:400-408.)

Key Words: ATP-binding cassette transporters ■ hydroxymethylglutaryl-CoA reductase inhibitors ■ organic anion transporters, sodium-independent ■ pharmacogenetics ■ pharmacokinetics

The 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, or statins, are commonly prescribed and proven to be effective in reducing cardiovascular event risk by lowering plasma concentration of low-density lipoprotein-cholesterol (LDL-C).¹ A recent report indicates that 25% of Americans aged >45 years takes a statin, and it is predicted that the number will grow as the populations of Westernized countries continue to age and maintain unhealthy lifestyles.^{2,3} A significant barrier to statin therapy is skeletal muscle toxicity associated with elevated systemic drug exposure.⁴ Up to 10% of statin-treated individuals will experience muscle pain or weakness, and in rare cases, life-threatening rhabdomyolysis occurs.⁵⁻⁷ Currently, we do not fully understand the drug exposure necessary for optimal statin therapy, making it difficult to predict an individual's dose requirement

to maximize LDL-C lowering while minimizing the risk for muscle injury.

Clinical Perspective on p 408

Remarkably few data are readily available on interpatient variability in plasma statin level, especially considering the number of large multicenter clinical trials of cardiovascular outcomes with statins performed to date. Until recently, drug-metabolizing enzymes, such as cytochrome P450 enzymes (CYPs), were considered to be the major determinants of statin disposition. However, studies from our laboratory and others suggest that statins, particularly the pharmacologically active acid forms of statins, are highly dependent on drug transporter proteins for their disposition and efficacy.^{8,9}

Received December 13, 2012; accepted July 17, 2013.

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The online-only Data Supplement is available at <http://circgenetics.ahajournals.org/lookup/suppl/doi:10.1161/CIRCGENETICS.113.000099/-/DC1>.

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Circ Cardiovasc Genet is available at <http://circgenetics.ahajournals.org>

DOI: 10.1161/CIRCGENETICS.113.000099

Our objective was to characterize the relationship between drug transporter polymorphisms and interindividual variability in plasma statin concentration, which, in the clinical situation, is not well understood. We measured 4 β -hydroxycholesterol concentration as a marker of CYP3A metabolic activity in vivo and lathosterol concentration to assess the efficacy of statin-mediated inhibition of endogenous cholesterol synthesis, as well as its relationship to statin concentration. Taken together, these data describe the relative contribution of transport genetics and metabolism to interindividual variability in statin pharmacokinetics and response.

Methods

Study Population

We prospectively invited adult outpatients at London Health Sciences Center (LHSC, London, Canada) taking atorvastatin or rosuvastatin daily to participate. Patients were excluded if they were taking atorvastatin or rosuvastatin in an alternate day dosing regimen, or if they had not taken their last atorvastatin or rosuvastatin dose within 24 hours of their clinic visit and blood draw. All patients had been taking atorvastatin or rosuvastatin at the same dose for ≥ 6 weeks before participation, with the exception of 1 patient who had been switched from 40 to 80 mg 1 week before blood sampling to achieve better cholesterol lowering. The study was conducted between August 2009 and May 2011. A detailed medical history was obtained, and the time of the last oral statin dose in relation to plasma level measurement was recorded. Ethnicity was self-reported. LDL-C response was defined by attainment of LDL-C target values according to the 2009 Canadian Lipid Guidelines¹ and by the clinical judgment of the treating physician. All subjects provided written informed consent. The study protocol was approved by the Research Ethics Board of the University of Western Ontario (London, Canada).

Sample Collection

A single venous 8-mL blood sample was drawn into EDTA-containing tubes and placed immediately on ice. Samples were centrifuged 2000g for 10 minutes; plasma was collected and stored at -80°C until further analysis. Genomic DNA was isolated from blood samples using the Genra Puregene extraction kit (Qiagen, Alameda, CA).

Retrospective Statin Dosing Analysis

We retrospectively examined genotype, clinical variables, and statin dose in a separate cohort comprised of outpatients from LHSC and Vanderbilt University Medical Center (BioVU, Nashville, TN).

BioVU

BioVU at Vanderbilt University is a large collection of DNA samples linked to comprehensive electronic medical records.¹⁰ BioVU is a dynamic clinical practice-based cohort nested within an even larger database containing a secure, deidentified copy of the entire electronic medical records used by Vanderbilt University Medical Center. Referred to as the synthetic derivative, this practice-derived database incorporates clinical information from multiple sources, including diagnostic and procedural codes, as well as provider progress notes, hospital admissions, discharge summaries, clinical laboratory data, and medication data.

Determination of Plasma Statin Concentration

All chemical and deuterated standards were obtained from Toronto Research Chemicals (North York, Canada). Plasma aliquots of 100 μL were precipitated in 300- μL acetonitrile containing internal standard d5-atorvastatin or d6-rosuvastatin and centrifuged at 14000 rpm for 20 minutes at 4°C . The supernatant was diluted 1:1 in 0.05% formic acid. Analytes were separated using mobile phases: 0.05%

formic acid in water and 0.05% formic acid in acetonitrile, starting at a ratio of 70:30, with a gradient to ratio of 10:90. Concentrations of rosuvastatin and atorvastatin were measured by liquid chromatography-mass spectrometry instrumentation and transitions as previously described.¹¹

Determination of Lathosterol and 4 β -Hydroxycholesterol Concentrations

Sterol concentrations were measured according to published methods for liquid chromatography-mass spectrometry.^{12,13}

Lathosterol, 4 β -hydroxycholesterol, and 4 β -hydroxycholesterol-d7 were obtained from Avanti Polar Lipids (Alabaster, AL), and lathosterol-d4 was obtained from CDN Isotopes (Pointe-Claire, Canada). All other chemicals were obtained from Sigma-Aldrich (St. Louis, MO). Standard curves ranging from 0- to 50- $\mu\text{g}/\text{mL}$ lathosterol were prepared in 1% fatty acid-free bovine serum albumin in phosphate-buffered saline. Aliquots of 50 μL of plasma or standard curve were saponified in 1 mL of 1 mol/L KOH in ethanol for 1 hour at 37°C . The samples were extracted twice in 750 μL of hexanes each time. After evaporation at 80°C to dryness, a mixture of the following derivatization reagents was added to each sample: 15-mg 2-methyl-6-nitrobenzoic anhydride, 4.5-mg 4-dimethylaminopyridine, 12-mg picolinic acid, 225- μL pyridine, and 30- μL triethylamine. Samples were incubated with the derivatization reagents at 80°C for 1 hour, extracted in 1 mL of hexanes, and evaporated at 80°C to dryness. Samples were reconstituted in 20- μL 0.9% NaCl and 80- μL water; 20 μL of sample was injected on an Eclipse Plus C18 column (1.8- μm pore size; 2.1×100 mm; Agilent Technologies, Mississauga, Canada) attached to an Agilent 1290 Infinity ultra-high pressure liquid chromatography system (Agilent Technologies) coupled with a TSQ Quantum triple-quadrupole mass spectrometer (Thermo Scientific). Analytes were separated and eluted with a gradient from 80% to 98% methanol:acetonitrile (1:1). The transition used for lathosterol was m/z 555.3 to 513.8. The transition used for 4 β -hydroxycholesterol was m/z 635.4 to 146.5. Interday variability was $<25\%$ for lathosterol and $<30\%$ for 4 β -hydroxycholesterol at relevant concentrations.

Determination of Total Cholesterol

Total cholesterol was measured by the enzymatic colorimetric method using the Cholesterol E kit from Wako (Richmond, VA). Samples were measured in triplicate using the microplate procedure, according to manufacturer's directions.

Genotyping

We identified single-nucleotide polymorphisms (SNPs) with a minor allelic frequency $>10\%$ in genes encoding drug transporters for which statins are known substrates and genotyped SNPs that have been demonstrated to have a functional effect on ≥ 1 substrates in vivo. Genotype was determined by TaqMan assay (Applied Biosystems, Foster City, CA) for uptake transporter polymorphisms *SLCO1B1* c.388A>G (rs2306283), *SLCO1B1* c.521T>C (rs4149056), *SLCO1B3* c.699G>A (rs7311358), *SLCO2B1* c.935G>A (rs12422149), and efflux transporter polymorphisms *ABCB1* c.3435C>T (rs1045642), *ABCC2* c.1249G>A (rs2273697), and *ABCG2* c.421C>A (rs2231142). For the atorvastatin group, polymorphisms in the drug-metabolizing enzymes *CYP3A4* (rs35599367) and *CYP3A5* (rs776746) were also assessed. Patients in the rosuvastatin group were also genotyped for *CYP2C9* *2 (rs1799853) and *CYP2C9* *3 (rs1057910). The SNPs assessed in the present study are summarized in Table I in the online-only Data Supplement. Missing genotypes ranged from 0% to 0.7% depending on the polymorphism. We repeated genotyping of 10% of the samples; 100% of replicated genotypes were concordant. Haplotypes were determined using the haplo.stats library in R using an indirect design matrix, and linear regression was conducted by comparing alternative haplotypes with the reference haplotype *SLCO1B1* c.388A-c521T.

Hardy-Weinberg equilibrium was tested using the χ^2 method of the genetics package of R. All genotypes tested were in Hardy-Weinberg

equilibrium with the exception of *ABCB1* c.3435C>T ($P=0.010$) and *SLCO1B1* c.521T>C ($P=0.041$). Genotypes associated with statin concentration were not differently distributed between whites and other ethnicities in our patient cohort by χ^2 test.

Statistical Analysis

Statistical analysis was performed using the statistical software R¹⁴ and GraphPad Prism 5 (La Jolla, CA). Differences in statin concentration with respect to each dose group were assessed by Tukey multiple comparisons tests. We defined the explainable variability as the variability attributed to characteristics other than dose and time from last dose. We calculated this by totaling the sum of squares for each final model and assessing the proportion contributed by the genetic variables.

For log-transformed rosuvastatin concentration, the effect sizes detectable with a power of ≥ 0.80 are 0.141, 0.145, and 0.187 for *SLCO1B1* c.521T>C, *SLCO1B1* c.388A>G, and *ABCG2* c.421C>A, respectively. For log-transformed atorvastatin concentration, the effect sizes detectable with a power of ≥ 0.80 are 0.274, 0.223, and 0.324 for *SLCO1B1* c.521T>C, *SLCO1B1* c.388A>G, and *ABCG2* c.421C>A, respectively.

Multiple Linear Regression Analysis

Statin concentration was log-transformed to adjust for right-skew. Only those patients with blood sampling times after the t_{\max} of the statin were included (1.5 and 4.0 hours for atorvastatin and rosuvastatin, respectively).¹¹ Different genetic models—dominant, co-dominant, recessive, and additive models—were considered for each transporter polymorphism, and the model that best described the fit with log-transformed statin concentration or lathosterol concentration was chosen. Each polymorphism was assessed for association with log statin concentration with a cut-off P value of 0.20 for further inclusion in the multiple linear regression model. *SLCO1B1* c.521T>C and c.388A>G, and *ABCG2* c.421C>A, were included in the model as additive models. In the additive model, homozygous wild-type genotypes were coded as 0, heterozygous genotypes were coded as 1, and homozygous variant genotypes were coded as 2. Regression analysis was performed by a step-wise search. All models were adjusted for the demographic and dosing variables: age, sex, body mass index, ethnicity, statin dose, and hours from last dose. Of these variables, age, dose, and time from last dose were statistically significant. Next, the number of concomitant medications or presence of the specific medications, ezetimibe, niacin, and fibrate, were assessed for their contribution to the model and retained if $P<0.20$. 4 β -Hydroxycholesterol values and transporter and drug-metabolizing enzyme genotypes were similarly introduced into the model. In the final model, only those variables with $P<0.05$ were retained, with the exception of the demographic and dosing variables listed above.

Dosing Algorithm

Maximum doses predicted to result in statin concentrations less than the 90th percentile were calculated on the basis of our linear regression models. The 90th percentile was determined by adjusting the atorvastatin or rosuvastatin concentrations measured in our population to the concentration predicted at the average time of the blood sampling across the population (11.5 hours for atorvastatin and 12.9 hours for rosuvastatin). For covariates that were not significant in the model, we substituted the average population value; predicted concentration was calculated for a hypothetical white patient of our average population height and weight (body mass index of 29.0 kg/m² for atorvastatin and 30.1 kg/m² rosuvastatin) and for atorvastatin, average 4 β -hydroxycholesterol concentration (22 ng/mL). Age was rounded to the nearest 5-year interval.

Results

Patient Characteristics

The patients' baseline characteristics are summarized in Table 1. In total, 299 patients were enrolled in the study: 134

taking atorvastatin and 165 patients on rosuvastatin therapy. Of these patients, 3 taking rosuvastatin and 6 taking atorvastatin had undetectable statin levels and were excluded from further analysis. Two patients taking rosuvastatin were excluded from lathosterol-related analysis because of inability to measure lathosterol or total cholesterol. A list of the concomitant medications observed in our population is provided in Table II in the online-only Data Supplement.

Rosuvastatin Concentration

We observed ≤ 45 -fold variability in plasma rosuvastatin concentration among individuals on the same dose (Figure 1B). In patients taking 5-, 10-, 20-, or 40-mg rosuvastatin daily, mean plasma concentration of rosuvastatin was 1.6 ng/mL (SD, 1.8), 3.5 ng/mL (SD, 2.9), 6.3 ng/mL (SD, 5.3), and 9.8 ng/mL (SD, 8.6). There was a significant difference in plasma rosuvastatin concentration between those taking 5 versus 10, 20, or 40 mg ($P<0.001$ for all) and for those taking 10 versus 20 or 40 mg ($P<0.01$ and $P<0.001$, respectively; Figure 1B).

To assess the association of clinical and pharmacogenetic variables with the rosuvastatin levels observed, we performed multiple linear regression analysis. Only those patients with blood drawn ≥ 4 hours after their last oral dose were included in this analysis ($n=130$). Multiple linear regression analysis indicated that plasma rosuvastatin concentration was higher in individuals with the reduced function hepatic uptake transporter allele *SLCO1B1* c.521C ($P<0.001$) and the reduced function efflux transporter polymorphism *ABCG2* c.421A ($P<0.05$). Age also contributed to

Table 1. Population Characteristics of Prospective Cohort of Atorvastatin- and Rosuvastatin-Treated Patients

	Atorvastatin	Rosuvastatin
No. of patients	134	165
Male	83 (61.9%)	115 (69.7%)
Age at enrolment, y	59.5 (24–86)	59 (18–80)
White	113 (83.7%)	143 (86.7%)
Body mass index, kg/m ²	29.0 (5.2)	30.1 (6.8)
Number of concomitant medications	4.9 (3.1)	4.7 (3.1)
Statin dose, mg/kg	0.45 (0.31)	0.22 (0.15)
5 mg	...	24 (14.5%)
10 mg	22 (16.4%)	52 (31.5%)
20 mg	30 (22.4%)	47 (28.4%)
40 mg	58 (43.2%)	38 (23.0%)
80 mg	23 (17.1%)	...
Other	1 (0.7%)	4 (2.4%)
Hours from last dose	12.9 (5.0)	11.5 (5.3)
4 β -Hydroxycholesterol, ng/mL	22.0 (14.1)	18.7 (11.9)
Lathosterol, μ g/mL	3.9 (2.1)	3.4 (2.2)
Minor allelic frequency		
<i>ABCG2</i> c.421A	25/268 (9.3%)	36/330 (10.9%)
<i>SLCO1B1</i> c.388G	119/268 (44.4%)	145/330 (43.9%)
<i>SLCO1B1</i> c.521C	30/268 (11.2%)	61/330 (18.5%)

Data are presented as number (%), mean (SD), or median (range).

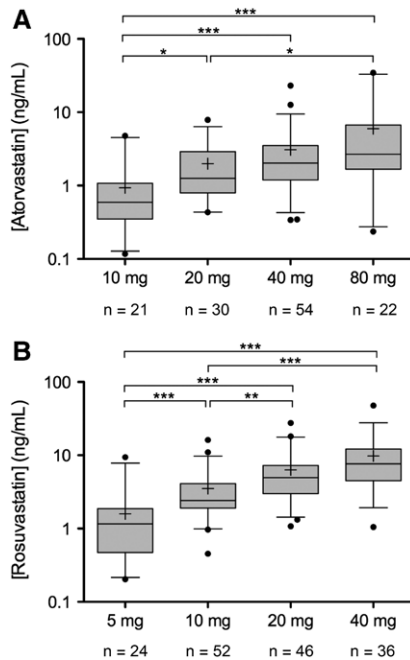


Figure 1. Prospective analysis of atorvastatin (A) plasma concentrations in patients taking 10, 20, 40, or 80 mg daily, and rosuvastatin (B) plasma concentrations in patients taking 5, 10, 20, or 40 mg daily. All concentrations were collected within 0 to 24 hours of the last oral dose. Levels are presented on a log-scale axis as box and whisker plots with the whiskers depicting 5th and 95th percentile; means are depicted by +. Significance of the mean difference between 2 groups is depicted by * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

plasma rosuvastatin level ($P<0.01$; Table 2). The adjusted R^2 value of the model was 0.56. Similar results were obtained using *SLCO1B1* haplotypes (Table V in the online-only Data Supplement). Polymorphisms in transporter genes *SLCO1B1* and *ABCG2* contributed to 88% of the explainable variability after adjusting for dose and time from last dose. The variables sex, ethnicity, body mass index, and *SLCO1B1* c.388, *SLCO1B3*, *SLCO2B1*, *ABCB1*, *ABCC2*, and *CYP2C9* genotype were not significantly associated with rosuvastatin concentration.

Table 2. Plasma Statin Concentration Linear Regression Model Coefficients From Prospective Cohort

Variable	Effect (B)	P Value
Atorvastatin-treated patients (n=128)		
Age, y	0.018	0.002
4 β -Hydroxycholesterol, ng/mL	-0.015	0.006
<i>SLCO1B1</i> c.521T>C	0.339	0.020
<i>SLCO1B1</i> c.388A>G	-0.278	0.009
Rosuvastatin-treated patients (n=130)		
Age, y	0.012	0.005
<i>SLCO1B1</i> c.521T>C	0.413	<0.001
<i>ABCG2</i> c.421C>A	0.310	0.020

Adjusted for sex, ethnicity, body mass index, dose, and time from last dose. Dose and time from last dose were significant in both models ($P<0.001$ for atorvastatin and rosuvastatin). Coefficients for all variables for atorvastatin and rosuvastatin are described in Tables III and IV in the online-only Data Supplement, respectively.

Atorvastatin Concentration

Similar to rosuvastatin, we observed 45-fold or higher variability between patients on the same daily atorvastatin dose (Figure 1A). In patients taking 10-, 20-, 40-, or 80-mg atorvastatin daily, mean plasma concentration of atorvastatin was 0.9 ng/mL (SD, 1.0), 2.0 ng/mL (SD, 1.7), 3.0 ng/mL (SD, 3.5), and 6.0 ng/mL (SD, 8.2). There was a significant difference in plasma atorvastatin concentration between those taking 20, 40, or 80 versus 10 mg ($P<0.05$, $P<0.001$, or $P<0.001$, respectively) and between those taking 20 versus 80 mg ($P<0.05$; Figure 1A).

Multiple linear regression analysis indicated that plasma atorvastatin concentration was higher in individuals with the *SLCO1B1* c.521C allele ($P<0.05$) but lower in those patients with the *SLCO1B1* c.388G allele ($P<0.01$). 4 β -Hydroxycholesterol also contributed significantly to the variability observed ($P<0.01$). In addition, age was a significant predictor of atorvastatin level ($P<0.01$; Table 2). The adjusted R^2 value of the model was 0.47. *SLCO1B1* haplotype-based analysis produced similar results (Table V in the online-only Data Supplement). In contrast to rosuvastatin, the genetic component of the model contributed only 38% of the explainable variability observed. Metabolism, as measured by 4 β -hydroxycholesterol concentration, accounted for an additional 30% of the explainable variability in atorvastatin concentration. A list of CYP3A inhibitors and inducers prescribed to patients taking atorvastatin is provided in Table VI in the online-only Data Supplement. Similar results were obtained when 4 β -hydroxycholesterol levels were normalized by total cholesterol. The following variables were not significantly associated with atorvastatin concentration: sex, ethnicity, body mass index, and *SLCO1B3*, *SLCO2B1*, *ABCB1*, *ABCC2*, *ABCG2*, *CYP3A4*, and *CYP3A5* genotype.

Lathosterol Concentration

The mean lathosterol concentration in patients taking atorvastatin was 3.9 μ g/mL (SD, 2.1) and rosuvastatin was 3.4 μ g/mL (SD, 2.2). In patients taking atorvastatin, lathosterol concentration was lower in patients taking a higher dose of atorvastatin ($P<0.01$); however, there was no significant association between rosuvastatin or atorvastatin concentrations and lathosterol concentration detected in this population. In both groups, lathosterol was associated with total cholesterol and was higher in patients taking ezetimibe (Table 3). The adjusted R^2 value of the model of lathosterol concentration in atorvastatin-treated patients was 0.08; for rosuvastatin-treated patients the adjusted R^2 value was 0.39.

LDL-C—Lowering Response to Rosuvastatin

Despite the lack of association between lathosterol level and statin level, some insight can be gained from this rare opportunity to examine lipid-lowering response in combination with plasma statin concentration. We examined rosuvastatin acid concentrations in patients taking 40-mg rosuvastatin daily, for whom no higher dose or more potent statin is available, and included only those with blood taken 9 to 24 hours after dose, to be within the linear range of statin elimination and minimize the variability associated with the peak statin absorption. For patients who were not at target,

Table 3. Lathosterol Plasma Concentration Linear Regression Model Coefficients From Prospective Cohort

Variable	Effect (B)	P Value
Atorvastatin-treated patients (n=128)		
Atorvastatin dose, mg	-0.02	0.009
Total cholesterol, mmol/L	0.23	0.032
Ezetimibe use	0.96	0.012
Rosuvastatin-treated patients (n=128)		
Total cholesterol, mmol/L	0.54	<0.001
Ezetimibe use	1.70	<0.001

Adjusted for sex, age, ethnicity, and body mass index.

LDL-C (n=12) had a mean plasma rosuvastatin concentration of 9.2 ng/mL (SD, 1.6; 13.6 hours after dose) compared with a mean plasma concentration of 7.5 ng/mL (SD, 1.8; 13.7 hours after dose) for those who were at target (n=13); the difference between the 2 groups was not significant (P=0.45). There was also a trend toward lower lathosterol level in those individuals at target compared with those not at target (3.4 µg/mL [SD, 0.49] versus 4.7 µg/mL [SD, 0.45], P=0.065). Notably, there is a higher proportion of *SLCO1B1* c.521T>C variants in the nonresponders (8 of 12 patients are *SLCO1B1* c.521CT heterozygotes) versus responders (3 of 13 heterozygotes; P=0.047, Fisher exact test).

Statin Dosing Algorithm

In Figure 2, we summarize recommendations for maximum atorvastatin and rosuvastatin doses, on the basis of a patient’s age and transporter genotype, and the linear regression analysis described above. These doses are predicted to result in plasma concentrations that remain lower than the 90th percentile, a value chosen to reflect the fact that 10% of individuals will experience statin-related muscle complaints.

Retrospective Analysis of Statin Dosing

We further examined the impact of genotype and clinical covariates on statin dose, retrospectively in 2 clinical populations (n=579). The first cohort contained 224 patients taking atorvastatin and 37 patients taking rosuvastatin in the context of routine clinical care at a large academic center in the United States; the second cohort contained 121 patients taking atorvastatin and 198 patients taking rosuvastatin treated in a lipid clinic at a large academic center in Canada. Thus, we were able to assess the potential clinical utility of our model in the context of both primary and specialty care. Population characteristics of each cohort are described in Table 4.

For these clinical practice-based cohorts, the relationship among genotype, age, and most recent statin dose has been summarized in Figure 3. We observed that patients taking 5-mg rosuvastatin were older than those taking 10 and 40 mg (P<0.05). The average ages are 64 years (SD, 13.7) for the group taking 5 mg daily versus 55 (SD, 13.3) and 54 years (SD, 14.4) for groups taking 10 and 40 mg, respectively. The transporter genotypes associated with statin concentration were not differently distributed among statin dose, implying that physicians may not yet have dosed each respective patient to his or her optimal serum statin level. Using the genotypes

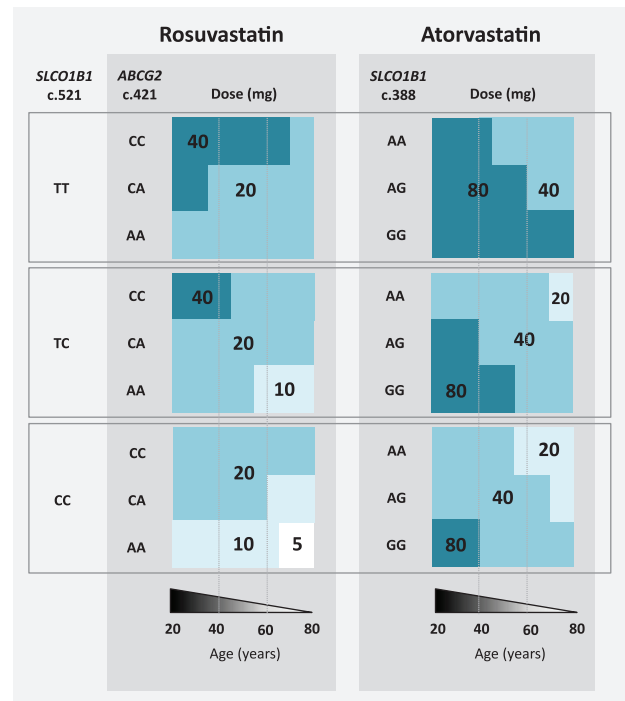


Figure 2. Rosuvastatin and atorvastatin dosing decision support algorithm. Doses are the maximum doses that result in a predicted rosuvastatin or atorvastatin concentration that is less than the 90th percentile. In patients taking atorvastatin, dose should be lowered if the patient is taking a CYP3A4 inhibitor, including an antifungal, macrolide antibiotic, or HIV protease inhibitor. The organic anion-transporting polypeptide inhibitors cyclosporine and gemfibrozil have also been associated with risk for statin-induced muscle toxicity; a dose reduction should be considered for both atorvastatin and rosuvastatin if cyclosporine or gemfibrozil are also prescribed. It should be noted that this algorithm is based on data collected from a predominantly white population and may not apply to other ethnicities, particularly Asians, who demonstrate increased sensitivity to statins.

and ages of these subjects to determine the dose recommended by our model indicates that only those patients at the highest doses exceeded the recommended dose (Table 5).

Table 4. Population Characteristics of Retrospective Atorvastatin and Rosuvastatin Dosing Cohort

	Atorvastatin	Rosuvastatin
No. of patients	345	234
Male	203 (58.8%)	141 (60.2%)
Age at enrolment, y	54 (14)	57 (13)
Statin dose, mg/kg		
5 mg	9 (2.6%)	25 (10.7%)
10 mg	131 (38.0%)	74 (31.6%)
20 mg	106 (30.7%)	98 (41.9%)
40 mg	69 (20.0%)	37 (15.8%)
80 mg	30 (8.7%)	0
Minor allelic frequency		
<i>SLCO1B1</i> c.388G	336/690 (48.7%)	Not determined
<i>SLCO1B1</i> c.521C	95/690 (13.8%)	86/468 (18.4%)
<i>ABCG2</i> c.421A	Not determined	50/468 (10.7%)

Data are presented as number (%) and mean (SD).

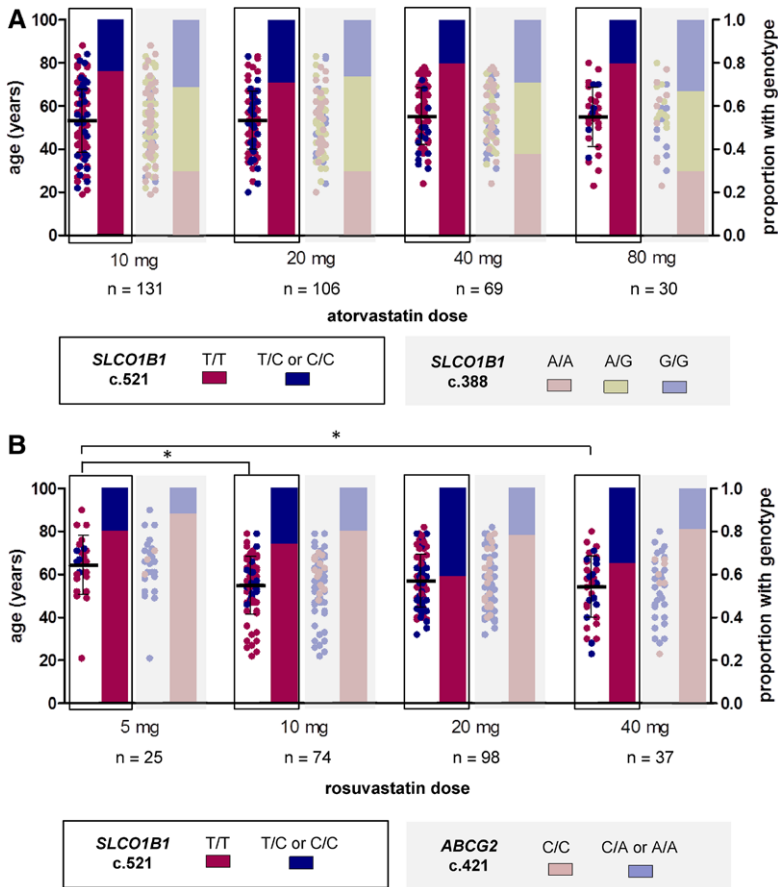


Figure 3. Distribution of transporter genotypes and age among atorvastatin (A) doses of 10, 20, 40, or 80 mg daily and rosuvastatin (B) doses of 5, 10, 20, or 40 mg daily in retrospective analysis of statin-treated patients. Age is depicted on the left y axis with points colored according to transporter genotype. The proportion of patients on any given dose with a particular genotype is depicted by the bars, colored according to transporter genotype and associated with the right y axis. *The age difference between 2 groups is significant (64 years [SD, 13.7] for the group taking 5 mg daily vs 55 [SD, 13.3] and 54 [SD, 14.4] for groups taking 10 and 40 mg, respectively; $P < 0.05$).

Among 67 patients on high-dose rosuvastatin (40 mg) or atorvastatin (80 mg), $\approx 50\%$ exceeded the maximum dose recommended by our model, suggesting that many were at risk of developing intolerance. Of the 16 patients taking high-dose atorvastatin (80 mg) within our cohort derived from an electronic medical records-linked biobank, 9 exceeded the maximum recommended dose, and only 7 of these patients were still on 80-mg atorvastatin 1 year later. Conversely, all (7 of 7) subjects predicted by our algorithm to tolerate 80 mg were

still on high-dose atorvastatin 1 year later; however, this result was not statistically significant ($P = 0.48$, Fisher exact test). Collectively, these observations suggest that clinicians may benefit from the use of this model, when weighing the risks and benefits of implementing a high dose prospectively.

Discussion

This study investigated the relationship between common drug transporter polymorphisms and plasma concentrations of atorvastatin and rosuvastatin in a real-world population. We found a marked, 45-fold interpatient variability in observed plasma level, especially at the higher doses. In our clinical situation, where statin dose has been titrated to effect, statin transporter polymorphisms are associated with a detectable change in statin level. Indeed, $\approx 90\%$ of the explainable variability in rosuvastatin concentration can be accounted for by 2 reduced function transporter polymorphisms: in the uptake transporter *SLCO1B1* and the efflux transporter *ABCG2*. In contrast, explainable variability in atorvastatin level is almost equally divided between 2 polymorphisms in *SLCO1B1* and the activity of *CYP3A* as measured by 4 β -hydroxycholesterol concentration. Taking our findings together, we propose a dosing algorithm for atorvastatin and rosuvastatin that, on the basis of our data on the association among transporter genotype, age, and statin concentration, would minimize risk for high plasma statin exposure.

Indeed, genetic polymorphisms in transport proteins contribute to interindividual variation in exposure to a number of

Table 5. Accuracy of Dose Prediction in Retrospective Analysis of Atorvastatin and Rosuvastatin Dosing

	At or Below Dose Recommended by Algorithm	Exceeds Dose Recommended by Algorithm
Atorvastatin dose, mg		
5	9 (100%)	0
10	131 (100%)	0
20	106 (100%)	0
40	69 (100%)	0
80	15 (50%)	15 (50%)
Rosuvastatin dose, mg		
5	25 (100%)	0
10	74 (100%)	0
20	98 (100%)	0
40	21 (56.8%)	16 (43.2%)

drugs, including the statins.^{15–17} The *SLCO1B1* gene encodes organic anion-transporting polypeptide 1B1 (OATP1B1; previously known as OATP-C or OATP2); in 2001, our group was the first to identify functionally relevant SNPs in this transporter.¹⁸ Healthy subjects harboring certain *SLCO1B1* SNPs had higher plasma concentrations of such statins as atorvastatin, rosuvastatin, simvastatin, pravastatin, and pitavastatin.^{19–22} Importantly, a genome-wide analysis revealed an association between susceptibility to biochemical myopathy on high-dose simvastatin and a common reduced function variant in *SLCO1B1*, namely, c.521T>C (rs4149056).⁴ *SLCO1B1* c.521T>C has also been associated with reduced LDL-C–lowering response to rosuvastatin therapy.²³ The other *SLCO1B1* polymorphism genotyped, c.388A>G (rs2306283), seems, *in vitro*, to have activity equivalent to the reference sequence¹⁸ and has been shown in some, but not all, healthy volunteer studies to be associated with a trend toward a lower plasma atorvastatin level.^{22,24} Interestingly, the Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine (SEARCH) showed a link between this SNP and reduced risk for simvastatin-associated myopathy.⁴ In our population, the *SLCO1B1* c.521T>C variant was not in Hardy–Weinberg equilibrium; it is possible that individuals homozygous for this variant are less likely to tolerate and remain on statin therapy. The *ABCB1* polymorphism c.3435 T>C was also not in equilibrium in our population; however, the reason for this result is unclear.

Polymorphisms in the ATP-binding cassette (ABC) efflux transporter *ABCG2* have been associated with higher rosuvastatin concentration in healthy volunteers²⁵ and recently with improved lipid-lowering response in Korean subjects²⁶ and whites.^{23,27} The effect of reduced activity *ABCG2* polymorphism on rosuvastatin concentration suggests that increased statin exposure is the mechanism resulting in the augmented lipid-lowering response observed by other studies.

It is important to note that this study was conducted in a predominantly white population, and that caution may be warranted in extrapolating these results to other ethnicities. In particular, ethnicity-dependent differences have been observed in studies comparing statin pharmacokinetics in healthy volunteers of Asian and white ethnicity.²⁴ Moreover, in Asian countries, such as Japan, the maximum approved dose of rosuvastatin is 20 mg/d compared with 40 mg/d in North American and European countries. Because the increase in rosuvastatin exposure is not strictly related to environment,²⁴ physicians in North America and Europe treating patients of Asian descent should be particularly aware that the maximum recommended dose of 40 mg/d may not be appropriate.

It has long been recognized that there is significant interindividual variation in CYP3A activity; however, the genetic basis for this variability has remained elusive. 4 β -Hydroxycholesterol is produced by CYP3A enzymes from cholesterol and has been proposed to be a marker of CYP3A activity *in vivo*.^{28,29} In our population, low 4 β -hydroxycholesterol level was associated with higher atorvastatin but not rosuvastatin concentration. Rosuvastatin concentration was also not associated with *CYP2C9* *2 and *CYP2C9* *3 genotypes, consistent with previous studies, which have indicated that rosuvastatin is predominantly

eliminated unchanged.³⁰ Previous reports have associated reduced CYP3A function with higher creatine kinase levels in patients taking atorvastatin, suggesting that these individuals are prone to more severe myopathy.^{31,32} Numerous drug interaction studies have described increased risk of adverse events resulting from the concomitant use of CYP3A inhibitors and statins metabolized by CYP3A, particularly atorvastatin and simvastatin. The US Food and Drug Administration recommendations advocate for a reduced dose of these statins if moderate CYP3A inhibitors are prescribed and for some potent CYP3A inhibitors contraindicate their use entirely.³³

Finally, our study identified age as a significant factor in predicting the concentrations of atorvastatin and rosuvastatin in patients. Age has been recognized as a clinical risk factor for statin-induced muscle toxicity.^{5,34} In early pharmacokinetic studies, age was associated with increased exposure to atorvastatin³⁵ but not rosuvastatin.³⁶ Rosuvastatin clearance, however, is partially mediated by tubular secretion in the kidney, thus the reduced renal function associated with advanced age may account for this effect.³⁰ Older patients are also more likely to take more medications, although the number of comedications was not a significant predictor of atorvastatin or rosuvastatin concentrations in our population.

Lathosterol is a late intermediate in cholesterol synthesis that can be used to measure the efficacy of statin-mediated HMG-CoA reductase inhibition.^{37,38} In our population, plasma concentrations of atorvastatin and rosuvastatin did not correlate with lathosterol levels. This suggests that statin concentration in the liver, not the plasma, is the most important factor in determining the inhibition of HMG-CoA reductase. Ezetimibe is a cholesterol absorption inhibitor that has been previously associated with lathosterol level³⁹; here, we observed that lathosterol level is increased in patients taking ezetimibe even when they are concurrently taking statins that limit lathosterol synthesis by inhibiting HMG-CoA reductase.

Our analysis of statin dosing patterns indicates that *SLCO1B1* and *ABCG2* variant carriers were distributed throughout the dosing groups (Figure 3), indicating that physicians did not adjust dose on the basis of the altered pharmacokinetics profile caused by these variants. This suggests that, based on clinical presentation alone, it is not possible for clinicians to detect those that are driven by pharmacokinetics-related mechanisms linked to polymorphisms in *SLCO1B1* and *ABCG2* and thus preventable by lowering the dose.

At the highest available dose, $\approx 50\%$ of patients taking atorvastatin or rosuvastatin exceeded the maximum genotype-based dose recommended by our algorithm. At lower doses, no patient in our cohort exceeded the maximum dose recommended by our algorithm, although it is important to note that rare individuals, not represented in our cohort, may in fact exceed their recommended dose even at lower doses. Taken together, genetic testing may be most useful when a patient is starting the highest dose of atorvastatin or rosuvastatin, bearing in mind that *SLCO1B1* c.521 T>C variant carriers in particular are more likely to require higher doses as a result of reduced hepatic uptake.

Here, we present the range of atorvastatin and rosuvastatin concentrations in a patient population providing a framework by which to assess normal variability in statin concentration,

and to identify the relationship between statin exposure and common statin transporter polymorphisms. In the clinical review of rosuvastatin originally submitted to the US Food and Drug Administration, all patients with serious adverse events for whom drug levels were available ($n=6$) had high rosuvastatin concentrations (>50 ng/mL; http://www.accessdata.fda.gov/drugsatfda_docs/nda/2003/21-366_Crestor_Medr_P4.pdf). The US Food and Drug Administration recently updated advice on statin risk to include memory loss and diabetes mellitus (<http://www.fda.gov/ForConsumers/ConsumerUpdates/ucm293330.htm>), indicating that we do not yet fully understand the consequences of statin exposure systemically. Although several groups have called for transporter genetic-guided statin dosing,^{4,8,26,27,40} to our knowledge, this study is the first to propose guidelines on the basis of interindividual differences in statin concentration. These guidelines provide a maximum starting dose to reduce the risk for high plasma statin concentration. Controlled, randomized trials are required to determine whether statin myopathy is reduced if statins are prescribed using this approach. In summary, this initial report of prospectively assessed plasma statin level and transporter genotypes in a patient care setting creates a framework for individualized statin selection and dosing.

Acknowledgments

We thank Brooke Kennedy and Julie Lorenzin for help with blood sampling, and Cameron Ross, Adam McIntyre, and Matthew Ban for technical assistance.

Sources of Funding

Dr Iwuchukwu is supported by NIH training grant T32 GM007569/GM/NIGMS NIH HHS/United States.

Disclosures

This work was supported by grants from the Canadian Institutes of Health Research (MOP-89753), the Drug Safety and Effectiveness Network (DSEN-PREVENT), and Wolfe Medical Research Chair in Pharmacogenomics to Dr Kim and Academic Medical Organization of Southwestern Ontario Alternate Funding Plan Innovation Fund to Drs Kim, Dresser, and Myers. BioVU is supported by the National Institutes of Health (UL1RR024975). Dr DeGorter is the recipient of a Vanier Canada Graduate Scholarship from the Canadian Institutes of Health Research. Dr Hegele serves on the advisory board and speaker's bureau of Abbott, Aegerion, Amgen, AstraZeneca, Merck, Pfizer, Roche, Sunovion, and Valeant. The other authors report no conflicts.

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CLINICAL PERSPECTIVE

Muscle pain and weakness are common side effects of statin use, and in some cases, statins can cause significant muscle injury, including a rare but life-threatening form of muscle damage known as rhabdomyolysis. Although exposure to high doses of statins has been linked to a greater likelihood for statin-associated muscle injury, not all patients prescribed high-dose statins experience muscle side effects, whereas some patients prescribed low doses of statins experience muscle damage. In this study, we measured atorvastatin and rosuvastatin concentration in 299 patients in a clinical setting and observed that statin concentrations vary by \approx 45-fold among patients taking the same dose. We sought to better understand the clinical and pharmacogenetics determinants that underlie such a wide interpatient variability in statin concentrations as a way to better identify patients at risk for statin myopathy. We report that common loss-of-function polymorphisms in the drug transporter genes, *SLCO1B1* and *ABCG2*, and patient age are highly associated with statin exposure. In a second group of 579 patients, we observed that \approx 50% of patients taking the highest statin doses are predicted to have statin concentrations higher than the 90th percentile of those observed in our prospective group. This suggests that current prescribing practices do not adequately identify patients at risk for high statin exposure. Taking these findings together, we propose a clinically feasible, relevant, and individualized statin dosing algorithm for reducing the risk of statin myopathy.

Clinical and Pharmacogenetic Predictors of Circulating Atorvastatin and Rosuvastatin Concentrations in Routine Clinical Care

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Circ Cardiovasc Genet. 2013;6:400-408; originally published online July 22, 2013;
doi: 10.1161/CIRCGENETICS.113.000099

Circulation: Cardiovascular Genetics is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

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Print ISSN: 1942-325X. Online ISSN: 1942-3268

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SUPPLEMENTAL MATERIAL

Supplemental Table 1. Genetic polymorphisms included in linear regression analysis of atorvastatin and rosuvastatin in prospective dosing cohort.

Gene	rs number	Location	Amino Acid	Consequence	Reference(s)
SLCO1B1 (c.388A>G)	rs2306283	12:21329737	Asn130Asp	Substrate-specific differences in transport activity	1
SLCO1B1 (c.521T>C)	rs4149056	12:21331548	Val174Ala	Reduced cell surface trafficking and changes in PK for some substrates, including statins	1-2
SLCO1B3 (c.699G>A)	rs7311358	12:21015759	Met233Ile	Reduced transport activity <i>in vitro</i>	3-4
SLCO2B1 (c.935G>A)	rs12422149	11:74883576	Arg312Gln	Reduced uptake of montelukast	5
ABCB1 (c.3435C>T)	rs1045642	7:87138644	Ile1145Ile	Reduced P-glycoprotein expression and changes in PK for some substrates, including statins	6-7
ABCC2 (c.1249G>A)	rs2273697	10:101563814	Val417Ile	Reduced MRP2 expression in some tissues	8
ABCG2 (c.421C>A)	rs2231142	4:89052322	Gln141Lys	Reduced BCRP expression in some tissues and changes in PK for some substrates, including statins	9-10
CYP3A4	rs35599367	7:99366315	Intronic	Reduced CYP3A4 mRNA expression in liver and lower atorvastatin and simvastatin doses required for lipid control	11
CYP3A5	rs776746	7:99270538	Intronic	Splicing variant resulting in nonfunctional allele	12
CYP2C9 (*2)	rs1799853	10:96702046	Arg144Cys	Substrate-specific reduction in activity	13
CYP2C9 (*3)	rs1057910	10:96741052	Ile359Leu	Substrate-specific reduction in activity	13

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Supplemental Table 2. Concomitant medications in prospective atorvastatin and rosuvastatin dosing cohorts.

acarbose (Prandase)
acebutolol
acetaminophen (Tylenol)
acetaminophen-oxycodone 5 mg (Percocet or Oxycocet)
acetylsalicylic acid (Aspirin)
aldactazide
alendronate (Fosamax)
alfuzosin ER (Xatral)
aliskiren (Rasilez)
allopurinol
alprazolam (Xanax)
amiloride (Novamilor)
amiodarone (Cordarone)
amitriptyline
amlodipine - atorvastatin (Caduet)
amlodipine (Norvasc)
amoxicillin
atenolol
atorvastatin (Lipitor)
azathioprine
baclofen
betahistine (Serc)
betaxolol (Betoptic S)
bethanechol (Duvoid)
bezafibrate SR (Bezalip)
bimatoprost 0.03% (Lumigan)
bisoprolol (Monacor)
brimonidine 0.2%-timolol 0.5% (Combigan)
budesonide (Pulmicort)
budesonide-formoterol (200-6 mcg/puff) (Symbicort)
buPROPion SR (Wellbutrin)
candesartan (Atacand)
carvedilol (Coreg)
celecoxib (Celebrex)
cephalexin (Keflex)
cetirizine (Reactine)
chlorthalidone
cholestyramine resin (Questran)
chondritin
cilazapril (Inhibace)
citalopram (Celexa)
clonazepam
clonidine
clopidogrel (Plavix)
clorazepam
colchicine
conjugated estrogens (Premarin)
Cosopt (dorzolamide 2%-timolol 0.5%)
cyclobenzaprine
cyclosporine
desloratadine (Aerius)
dexamethasone
dextroamphetamine (Dexedrine)
diclofenac - misoprostol (Arthrotec)
digoxin
diltiazem ER (Tiazac XC)
dimenhydrinate (Gravol)
diphenhydrAMINE (Benadryl)
domperidone
donepezil (Aricept)
doxazosin
duloxetine (Cymbalta)
dutasteride (Avodart)
enalapril (Vasotec)
eplerenone
escitalopram (Ciprallex)
esomeprazole EC (Nexium)
etidronate - calcium carbonate (Didrocal)
ezetimibe (Ezetrol)
famciclovir (Famvir)
famotidine (Pepcid)
felodipine (Plendil)
fenofibrate (Lipidil)
fentanyl
fluoxetine (Prozac)
fluticasone (Avamyl)
fosinopril
furosemide (Lasix)
gabapentin
gemfibrozil (Lopid)
gliclazide (Diamicon)
glucosamine
glyBURIDE
glyceryl trinitrate (Nitrolingual pump)
goserelin (Zoladex)
hydrochlorothiazide
hydromorphone
hydroxychloroquine (Plaquenil)
ibuprofen (Advil, Motrin)
indapamide
indomethacin (Indocid)
inositol hexaniacinate
insulin aspart (NovoRapid)
insulin detemir (Levemir)
insulin glargine (Lantus)
insulin lispro (Humalog)

Supplemental Table 2, continued

insulin NPH (Humulin N, Novolin ge NPH)
ipratropium (Atrovent)
irbesartan (Avapro)
isosorbide dinitrate
ketorolac (Toradol)
lansoprazole (Prevacid)
latanoprost 0.005% (Xalatan)
levothyroxine (Synthroid, Eltroxin)
lisinopril (Zestril)
lorazepam (Ativan)
losartan (Cozaar)
meloxicam (Mobicox)
metformin
methocarbamol (Robaxacet)
methotrexate
metoprolol tartrate (Lopressor)
mirtazapine (Remeron)
mometasone (50 mcg/spray) (Nasonex)
montelukast (Singulair)
morphine sulfate (Kadian)
mycophenolate mofetil (CellCept)
nabilone (Cesamet)
nadolol
naproxen
niacin (Niaspan)
nifedipine (Adalat)
nitroglycerin (0.4 mg/spray)
nitroglycerin patch (Nitroderm)
norgestimate-ethinyl estradiol (Tri-Cyclen)
nortriptyline
olmesartan (Olmotec)
omeprazole EC (Losec)
oxazepam
oxybutynin (Ditropan)
oxycodone CR (Oxycontin)
pantoprazole (Pantoloc, Tecta)
paracetamol (Tramacet)
paroxetine (Paxil)
peridone
perindopril (Coversyl)
phenobarbital
phenytoin (Dilantin)
pioglitazone (Actos)
prazosin
prednisone
progesterone cream
propafenone
quetiapine (Seroquel)
quinapril (Accupril)
quinine
rabeprazole EC (Pariet)
raloxifene (Evista)
ramipril (Altace)
ranitidine (Zantac)
repaglinide (Gluconorm)
risedronate (Actonel)
risperidone (Risperdal)
rizatriptan (Maxalt)
rosiglitazone - metformin (Avandamet)
rosiglitazone (Avandia)
rosuvastatin (Crestor)
salbutamol (100 mcg/puff) (Ventolin)
salmeterol - fluticasone (Advair)
senna (Senocot)
sertraline (Zoloft)
sildenafil (Viagra)
sirolimus (Rapamune)
sitagliptin (Januvia)
sodium citrate (Microlax)
sodium polystyrene sulfonate (Kayexalate)
sotalol
spironolactone (Aldactone)
sulfacetamide (Sulfex)
sulfasalazine
sumatriptan (Imitrex DF)
tadalafil (Cialis)
tamoxifen
tamsulosin (Flomax CR)
telmisartan (Micardis)
temazepam
terazosin
testosterone undecanoate (Andriol)
tetracycline
tiotropium (Spiriva)
tolterodine (Detrol)
topiramate
trandolapril (Mavik)
trazodone
ubiquinone (Co-Enzyme Q10)
valacyclovir (Valtrex)
valproic acid
valsartan (Diovan)
venlafaxine ER (Effexor)
verapamil
warfarin (Coumadin)
zoledronic (Aclasta)
zopiclone

Supplemental Table 3. Plasma atorvastatin concentration linear regression model from atorvastatin-treated patients of prospective cohort (n = 128)

	Variable	Effect (B)	p-value
1	Age (yr)	0.018	0.002
2	<i>SLCO1B1</i> c.521T>C	0.339	0.020
3	<i>SLCO1B1</i> c.388A>G	-0.278	0.009
4	4 β -hydroxycholesterol (ng/mL)	-0.015	0.006
5	Dose (mg)	0.020	<0.001
6	Time from last dose (hr)	-0.089	<0.001
7	Gender (male = 1)	0.132	>0.05
8	Ethnicity (non-Caucasian = 1)	0.295	>0.05
9	Body Mass Index (kg/m ²)	0.025	>0.05

Supplemental Table 4. Plasma rosuvastatin concentration linear regression model from rosuvastatin-treated patients of prospective cohort (n = 130)

	Variable	Effect (B)	p-value
1	Age (yr)	0.012	0.005
2	<i>SLCO1B1</i> c.521T>C	0.413	<0.001
3	<i>ABCG2</i> c.421C>A	0.310	0.020
4	Dose (mg)	0.048	<0.001
5	Time from last dose (hr)	-0.060	<0.001
6	Gender (male = 1)	-0.166	>0.05
7	Ethnicity (non-Caucasian = 1)	0.060	>0.05
8	Body Mass Index (kg/m ²)	-0.010	>0.05

Supplemental Table 5: Plasma statin concentration linear regression model coefficients from prospective cohort using *SLCO1B1* haplotypes

	Variable	Effect (B)	p-value
<i>Atorvastatin-treated patients (n = 128)</i>			
1	Age (yr)	0.018	0.001
2	4 β -hydroxycholesterol (ng/mL)	-0.014	0.012
3	<i>SLCO1B1</i> 388G-521T vs. 388A-521T	-0.246	0.025
4	<i>SLCO1B1</i> 388A-521C vs 388A-521T	0.783	0.060
<i>Rosuvastatin-treated patients (n = 130)</i>			
1	Age (yr)	0.012	0.004
2	<i>SLCO1B1</i> 388G-521C vs 388A-521T	0.413	<0.001
3	<i>ABCG2</i> c.421C>A	0.286	0.030

Adjusted for gender, ethnicity, BMI, dose and time from last dose. Dose and time from last dose were significant in both models ($p < 0.001$ for atorvastatin and rosuvastatin); BMI was significant in the atorvastatin model ($p < 0.05$).

The adjusted R^2 of the atorvastatin regression is 0.47; for rosuvastatin the adjusted R^2 is 0.57.

Supplemental Table 6. Concomitant CYP3A4 inhibitors and inducers in prospective atorvastatin dosing cohort. Inhibitors and inducers were from: Flockhart DA. Drug Interactions: Cytochrome P450 Drug Interaction Table. Indiana University School of Medicine (2007). <http://medicine.iupui.edu/clinpharm/ddis/table.aspx>. Accessed 27 April 2012.

Inhibitor (number of patients)	Inducer (number of patients)
Diltiazem (6)	Phenobarbital (1)
	Phenytoin (1)
	Pioglitazone (1)
	Prednisone (2)