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Impact of *SLCO1B1* Genotype on Pediatric Simvastatin Acid Pharmacokinetics

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Abstract

This study investigated the impact of allelic variation in *SLCO1B1*, a gene encoding for the liver-specific solute carrier organic anion transporter family member 1B1 protein (SLCO1B1), on simvastatin and simvastatin acid (SVA) systemic exposure in children and adolescents. Participants (8–20 years old) with at least 1 variant *SLCO1B1* c.521T>C allele (521TC, n = 15; 521CC, n = 2) and 2 wild-type alleles (521TT, n = 15) completed a single oral dose pharmacokinetic study. At equivalent doses, SVA exposure was 6.3- and 2.5-fold greater in 521CC and TC genotypes relative to 521TT (C_{max}, 2.1 ± 0.2 vs 1.0 ± 0.5 vs 0.4 ± 0.3 ng/mL; $P < .0001$; and AUC, 12.1 ± 0.3 vs 4.5 ± 2.5 vs 1.9 ± 1.8 ng·h/mL; $P < .0001$). The impact of the *SLCO1B1* c.521 genotype was more pronounced in children, although considerable interindividual variability in SVA exposure was observed within genotype groups. In addition, SVA systemic exposure was negligible in 25% of pediatric participants. Further investigation of the ontogeny and genetic variation of SVA formation and SLCO1B1-mediated hepatic uptake is necessary to better understand the variability in SVA exposure in children and its clinical consequences.

Keywords

cholesterol, lipids, pediatrics, pharmacogenomics, pharmacokinetics, statin

Although symptoms typically appear in later life, atherosclerotic coronary artery disease (CAD) has its origins in childhood.[1–3] The Pathobiological Determinants of Atherosclerosis in Youth study and the Bogalusa Heart Study noted varying stages of atherosclerosis in children and adolescents, with elevated low-density lipoprotein cholesterol (LDL-C) and risk factors such as obesity, hypertension, tobacco smoke exposure, and diabetes.[4,5] These impactful studies highlighted the need for preventive cardiovascular surveillance in childhood.[6–8] With the increasing prevalence of overweight and obese children in the United States,[9] clinically diagnosed CAD in young to middle-aged adults is expected to increase 5%–16% by 2035, contributing more than 100 000 excess cases of early coronary heart disease.[9,10] Strategies to reverse obesity or familial-related increases in LDL-C could potentially blunt the anticipated increase in CAD and thereby substantially reduce morbidity and mortality in younger adults.

Increasingly, children and adolescents qualify for pharmacologic intervention for dyslipidemia.[11,12] Similar to in adults, 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) are the mainstay of pediatric dyslipidemia treatment when lifestyle modifications have failed.[8] Simvastatin, introduced in the early 1990s, is a lipophilic semisynthetic statin.[13–15] It is formulated as a lactone prodrug (SVL) that requires hydrolysis to its active hydroxyl acid (simvastatin acid [SVA]) for HMG-CoA reductase inhibition[13] (Figure 1). Following SVL hydrolysis in plasma, hepatocellular uptake of SVA is thought to occur via the hepatic drug transporter SLCO1B1, encoded by the *SLCO1B1* gene.[16] Termination of biological activity occurs by biotransformation via CYP3A[17] and hepatocellular elimination primarily via p-glycoprotein (MDR1).[16,18]

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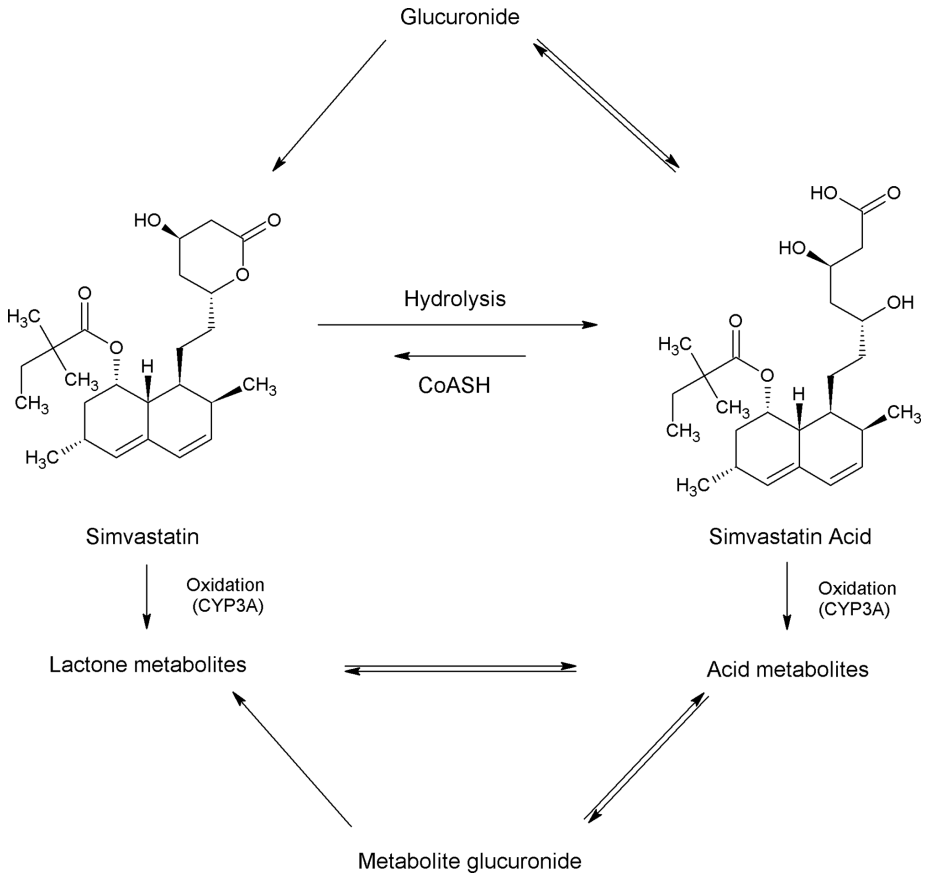

Figure 1. Simvastatin pathway. Simvastatin undergoes hydrolysis to form simvastatin acid and can be alternatively relactonized by the CoASH-dependent pathway. Simvastatin disposition is complex, with several biotransformation pathways involved.

The largest study, a double-blind, randomized, placebo-controlled multicenter trial of simvastatin in children demonstrated a 41% reduction in LDL-C.[19] However, daily administration of relatively high doses (40 mg) for 48 weeks was accompanied by considerable (~4-fold) interindividual variability in LDL-C reduction.

One potential source of interindividual variability in SVA response is genetic variation in the drug transporters that alter circulating plasma statin concentrations, referred to as "systemic exposure." The c.521T>C single-nucleotide variant (SNV; rs4149056) in *SLCO1B1,* represented at allele frequencies of 8%–20%, 8%–16%, and 1%–8% of the white, Asian, and African populations, respectively,[20] leads to a nonsynonymous amino acid change (Val174Ala), resulting in decreased hepatocyte uptake[21] and increased systemic statin exposure.[22] Pasanen et al demonstrated that homozygous variants (c.521CC genotype) experience significantly increased SVA plasma concentrations compared with subjects homozygous for the fully functional, reference allele (c.521TT genotype).[22] Consequently, clinically significant adverse events (ie, myalgias) are reported to be greatest in the presence of c.521C alleles.[23,24] Selected studies have also demonstrated an association between lipid-lowering attenuation and this SNV.[25,26] However, it should be appreciated that there are an equal number of publications that contradict the role of *SLCO1B1* c.521T>C on myopathy[27–29] and the LDL-C-lowering effect in adults.[30,31]

The role of the aforementioned SNV on pediatric hepatic uptake of statins is not well established but will depend on genotype and the ontogenic expression of SLCO1B1.[32,33] Increasingly, pharmacokinetic studies in children involving medications dependent on genetically polymorphic pathways for their elimination (eg, atomoxetine) are beginning to reveal the consequences of genetic variation in systemic drug exposure, namely, area under the curve (AUC) values ranging over 50-fold between children with the highest and the lowest exposures.[34] As simvastatin disposition is also dependent on a genetically polymorphic pathway, interindividual variability in SVA exposure may contribute to the observed interindividual variability in LDL-C reduction in pediatric patients.[19] At present, there are no data describing the magnitude of effect of *SLCO1B1* genetic variation on the dose–exposure relationship of simvastatin in children or the extent of interindividual

variability in simvastatin exposure in a patient population characterized by dramatic changes during growth and development. The primary goal of this investigation was to establish the role of genetic variation in *SLCO1B1* on the dose–exposure relationship for a commonly used statin in children and adolescents, with secondary analyses investigating CYP3A5 genotype as a potential source of interindividual variability in SVA exposure within *SLCO1B1* genotype groups.

Methods

Subjects

The study protocol and informed consent were reviewed and approved by the Children's Mercy Hospital Institutional Review Board, and the study was conducted in accordance with US and international standards of Good Clinical Practice (FDA regulations 21 CFR 312 for IND studies and FDA guidance E6). Subjects meeting inclusion and exclusion criteria for this study (Supplement 1) were recruited from the Children's Mercy Hospital Cardiology Pharmacogenomics Repository (CPR), a living biorepository and patient registry designed to facilitate genotype-guided clinical trial participant selection. CPR enrollees were invited to participate based on their *SLCO1B1* c.521T>C genotype, ensuring that the wild-type and heterozygous variant populations were age-, ethnicity-, and sex-matched. Written, informed consent was obtained from all participants.

Genetic Analysis

Deoxyribonucleic acid (DNA) was isolated from biospecimens using a Sigma GeneElute Mammalian Genomic DNA Minprep Kit (St. Louis, Missouri) or a QIAamp DNA Blood Mini Kit (250; Valencia, California) according to the manufacturers' protocols. All DNA samples were genotyped for the common *SLCO1B1* SNVs -11187 G>A (rs4149015), c.388 A>G (rs2306283), and c.521 T>C (rs4149056) and SNVs defining the *CYP3A5*1D* (rs15524), *CYP3A5*3* (rs776746), and *CYP3A5*6* (rs10264272) alleles using TaqMan single-nucleotide polymorphism genotyping assays (Life Technologies, Carlsbad, California) with KAPA Probe Fast qPCR Master Mix (2X) ABI Prism (KAPA Biosystems, Boston, Massachusetts) on an AB 7900HT Fast Real-Time PCR System (Applied Biosystems). For each reaction, 5 to 20 ng of DNA was used, in a total of 8 μL. The cycling conditions for all the assays were as recommended by the manufacturer. DNA samples from the Coriell Institute for Medical Research were used as controls. Twenty percent of samples were randomly selected and repeated for quality control. All the reanalyzed samples were concordant with the original results.

Study Design

This was a single-center, open-label, genotype-stratified single oral dose pharmacokinetic study comparing the disposition of simvastatin among hyperlipidemic children and adolescents with 1 or more *SLCO1B1* c.521C variant alleles with patients homozygous for the reference c.521TT genotype. All participants had a laboratory evaluation, physical examination, and Tanner staging for development performed at a screening visit prior to the pharmacokinetic study visit. Participants on statin therapy held their statin agent for a washout period of 7 days prior to the drug study visit.

Subjects ingested a single oral dose of simvastatin (aged 8–17 years: 10-mg tablet; Accord Health, lot number R09859; aged ≥ 18 years: 20-mg tablet; Accord Health, lot number PR02304) with 150 mL of water after an overnight fast and did not eat earlier than 2 hours after administration of the dose. Serial venous blood samples (1.5 mL each) were drawn from an indwelling venous cannula before administration (time 0), and 0.5, 1, 1.5, 2, 3, 4, 6, and 8 hours postingestion to measure plasma drug and metabolite concentrations. Samples were collected in a syringe, transferred to a tube containing potassium ethylenediaminetetraacetic acid and gently mixed by inversion. They were immediately centrifuged at 4°C for 10 minutes at 600 *g* (~2000 rpm). The plasma component of each blood sample was removed by manual aspiration, transferred to a labeled polypropylene cryovial, and stored at −80°C until analysis.

Analytical Methods

Plasma concentrations of SVL, SVA, 6-hydroxymethyl SVL, 6-hydroxymethyl SVA, and 3,5-dihydrodiol SVL were measured on a Waters TQ-S triple quadrupole tandem mass spectrometer with a novel ultra-high-pressure liquid chromatography–tandem mass spectrometric method developed in our laboratory.[35] In brief, analytes were extracted from 100 μL of plasma using hydrophilic lipophilic balance solid-phase extraction on 96-well elution plates. Extracted samples were evaporated to dryness under nitrogen at 37°C (Glas-Col, Terre Haute, Indiana). pH-dependent analyte interconversion, including lactonization or lactone ring hydrolysis, were minimized by adjusting the pH of the plasma sample to 4.5 prior to extraction and maintaining this pH throughout the extraction procedure. Samples were separated on a Waters Cortecs C18 column (1.8 μM, d_p 2.1 × 100 mm). Mass spectrometric detection was performed using multiple reaction monitoring switching between positive/negative electrospray ionization (ESI). SVL, 6-hydroxymethyl SVL, and 3,5-dihydrodiol SVL were ionized in positive ESI mode (m/z 450.40→285.00, 466.40→197.00, and 484.40→319.00, respectively).

SVA and 6-hydroxymethyl SVA were ionized in negative ESI mode (m/z 435.30→319.00 and 451.20→335.00, respectively). Deuterated versions of SVL and SVA were used as internal standards. The dynamic range of the assay was 0.5 to 100 nM for all analytes. The assay was validated according to the United States Food and Drug Administration "Guidance for Industry: Bioanalytical Method Validation" (http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm368107.pdf). The method was linear for all analytes in the concentration range 0.5–100 nM with intra- and interday precision (as relative standard deviation) of ≤10% and accuracy (as relative error) ranging from 90.1% to 112% at all quality control levels (0.5, 1, 10, and 75 nM). Plasma samples were analyzed in duplicate. In the rare instances in which the coefficient of variation in the duplicates exceeded 20%, the samples were rerun.

Pharmacokinetic Parameters

Pharmacokinetic analyses were conducted using Kinetica version 5.0 (Thermo Fisher Scientific, Philadelphia, Pennsylvania). Plasma concentration-versus-time data for SVL, SVA, and metabolites were curve-fitted using a peeling algorithm to generate initial mono-exponential parameter estimates. Final estimates of the terminal elimination rate constant (λ_z) were determined from an iterative linear least-squares regression algorithm. A model-independent approach was used, and parameters of interest were determined as follows. Individual peak plasma concentration (C_{max}) and T_{max} were obtained by direct examination of the plasma concentration-versus-time profile. The area under the plasma concentration-versus-time curve during the sampling period (AUC_{0-n}) was calculated using the mixed log-linear method, where n refers to the final sampling time with quantifiable drug or metabolite concentrations. Extrapolation of the AUC to infinity ($AUC_{0-\infty}$) was achieved by the summation of $AUC_{0-n} + Cp_n/\lambda_z$, where Cp_n is the last observable plasma concentration, calculated from the curve fit of the terminal slope of the plasma concentration-versus-time curve and λ_z is the apparent terminal elimination rate constant.

Statistical Analysis

Pharmacokinetic data for the study cohort were examined using standard descriptive statistics (ie, arithmetic mean, geometric mean, standard deviation) in JMP version 11 (SAS, Marlow, UK). Pharmacokinetic parameters reflective of systemic exposure (C_{max}, AUC_{0-n}, $AUC_{0-\infty}$) were log-transformed using the natural logarithm, the 90% confidence intervals for the difference in the means calculated, and the antilog of the confidence limits evaluated against the preestablished bioequivalence criteria as defined in our power calculations (Supplement 2). The significance limit accepted for all statistical analyses was $\alpha = 0.05$. The contribution of age, developmental stage, and allelic variation to the pharmacokinetic parameters was investigated with multiple regression analysis. Genotype class (noncarriers and carriers) was treated as an independent variable. Pharmacokinetic parameters were compared between genotype groups, using the independent *t* test and analysis of variance. All authors had full access to all the data generated from the study and take responsibility for its integrity and the data analysis.

Table 1. Characteristics of Participants Between *SLCO1B1* genotypes

	SLCO1B1 521 TT (n = 15)	SLCO1B1 521 TC (n = 15)	SLCO1B1 521 CC (n = 2)	*P*
Age (years)[a]	13.9 ± 3.3	14.2 ± 3.6	14.2 ± 4.2	.982
Weight (kg)[a]	82.8 ± 36.8	78.4 ± 29.4	61.1 ± 1.2	.672
Height (cm)[a]	160.3 ± 10.9	160.5 ± 14.9	157.3 ± 19.4	.949
BMI (kg/m^2)[a]	31.6 ± 12.4	29.4 ± 7.9	24.7 ± 5.7	.659
Sex[b]				
Female	8	8	1	.996
Male	7	7	1	
Ethnicity[b]				
White, non-Hispanic	9	9	1	.980
White, Hispanic	5	5	1	
African American	1	1	0	
Tanner				
Testicular/breast[b]				
Stage 1	1	4	0	.229
Stage 2	4	1	1	
Stage 3	1	0	0	
Stage 4	4	1	0	
Stage 5	5	9	1	
Pubic[b]				
Stage 1	1	4	0	.119
Stage 2	4	1	1	
Stage 3	0	0	0	
Stage 4	5	1	0	
Stage 5	5	9	1	
Dose (mg/kg)[a]	0.16 ± 0.06	0.17 ± 0.08	0.16 ± 0.003	.889

All data expressed as mean ± SD.
[a]Analysis of variance (ANOVA),
[b]Chi-square test performed.

Results

Participant Characteristics and Adverse Events

A total of 32 children and adolescents (15 males, 17 females) were enrolled in this investigation. The demographic and genetic constitution of the participant population is detailed in Table 1. No significant demographic differences existed between participants with *SLCO1B1* c.521T>C variant–containing genotypes and wild-type controls. Of note, there was no significant difference in the weight-based dose received

between the genotype groups (Table 1). There were no adverse events during the course of the trial.

Drug Disposition Profiles

A large degree of variability was observed for the mean systemic exposure of simvastatin (C_{max}, 3.5 ± 2.5 ng/mL; $AUC_{0–8}$, 10.7 ± 6.5 ng·h/mL) and SVA (C_{max}, 2.0 ± 1.4 ng/mL; $AUC_{0–8}$, 3.8 ± 3.3 ng·h/mL). Notably, the concentrations of SVA in our cohort were less than those observed in adults; however, this was largely attributed to the lower doses administered in this lower-weight population. When scaled to reflect adult doses, the dose–exposure profiles appear comparable in children and adults. When the large degree of variability in SVA concentrations was examined in greater detail, we observed that SVA was not detectable during the sampling period in 3 participants and only marginally higher than the lower limit of detection (0.5 nM) in an additional 5 participants. Cumulatively, this translated to approximately 25% of the patient cohort with negligible to minimal plasma SVA exposure over the 8-hour study period. There were insufficient data points to adequately capture the terminal elimination phase for SVA in approximately 63% of the participants (20 of 32), precluding accurate determination of $AUC_{0–\infty}$. Therefore, the $AUC_{0–8}$ was used for statistical analysis.

The 6-hydroxymethyl SVL was the most abundant metabolite recovered, with a mean systemic exposure of 18.7 ± 13.1 ng·h/mL. Mean 6-hydroxymethyl SVA and 3,5-dihydrodiol SVL systemic exposure (8.7 ± 5.8 and 9.3 ± 6.0 ng·h/mL) were comparable with SVL. However, we observed significant interindividual variability in our cohort for all drug analytes.

Effect of *SLCO1B1* Genotype on SVA Systemic Exposure

The effect of the *SLCO1B1* genotype on mean SVA plasma concentration profiles is presented in Figure 2. Peak (C_{max}) SVA concentrations were significantly higher in the c.521CC (n = 2) and c.521TC (n = 15) groups relative to the c.521TT (n = 15) group (2.1 ± 0.2 vs 1.0 ± 0.5 vs 0.4 ± 0.3 ng/mL, respectively; $P < .0001$; Figure 3A, Table 2), translating to a 5.2- and 2.3-fold higher mean C_{max}, respectively.

In participants with the *SLCO1B1* c.521TC genotype, the mean SVA $AUC_{0–8}$ was 2.4-fold ($P < .0001$) higher compared with subjects with the reference (4.5 ± 2.5 vs 1.9 ± 1.8 ng·h/mL); see Figure 4 and Table 2. c.521CC participants had a 6.3-fold higher mean $AUC_{0–8}$ compared with the c.521TT genotype (12.1 ± 0.3 vs 1.9 ± 1.8 ng·h/mL); see Figure 4A and Table 2. Correcting the simvastatin acid C_{max} and $AUC_{0–8}$ for the dose received in each individual participant does not change the relationship (Figures 3A and 4B). SVA plasma concentrations were not detected in 3 of 15 *SLCO1B1* c.521TT participants and was negligible in an additional 4 c.521TT and 1 c.521TC participants (Figure 4A,B).

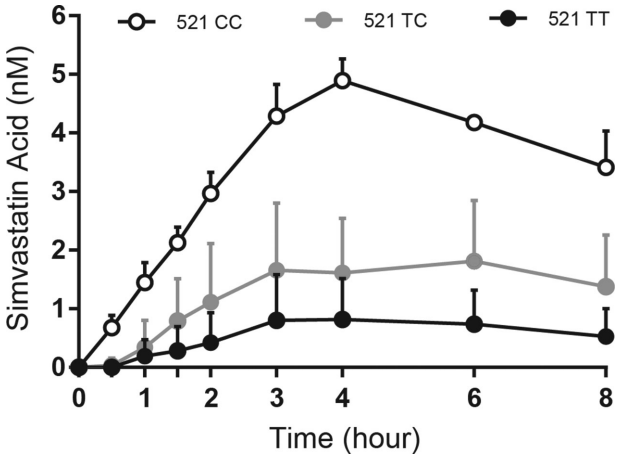

Figure 2. Mean ± SD plasma concentration (nM) of simvastatin acid after a single dose of simvastatin in 32 healthy pediatric participants. Black circles represent participants with the c.521TT genotype (n = 15). Gray *circles* represent participants with the c.521TC genotype (n = 15). Open white *circles* represent participants with the c.521CC genotype (n = 2).

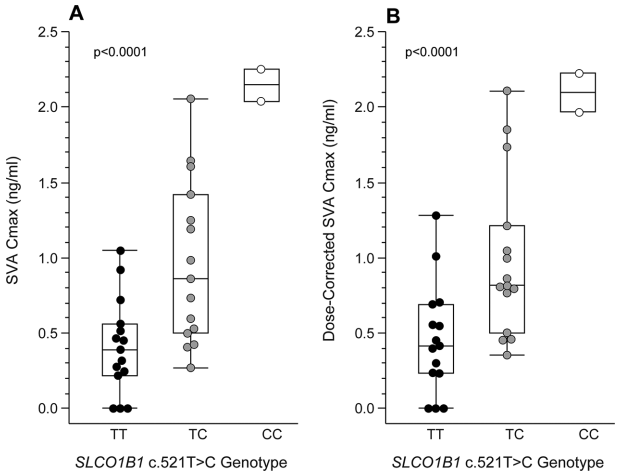

Figure 3. (A) Comparison of simvastatin acid C_{max} (ng/mL) and (B) C_{max} (ng/mL) corrected for dose among *SLCO1B1* c.521 genotypes ($P < .0001$). Black circles represent participants with the c.521TT genotype (n = 15). Gray circles represent participants with the c.521TC genotype (n = 15). Open white circles represent participants with the c.521CC genotype (n = 2).

Although SVA C_{max} and AUC tended to be ~64% lower in subjects with the *SLCO1B1* −11187G>A genotype (n = 2) compared with subjects with the -11187GG (reference) genotype (n = 30), these differences did not achieve statistical significance ($P = .29$ and $P = .25$, respectively).

SVL exposure was not significantly different among the *SLCO1B1* genotype groups (C_{max}: c.521CC, 2.7 ± 1.2 ng/mL: c.521TC, 3.6 ± 2.6 ng/mL; c.521TT, 3.6 ± 2.5 ng/mL; $P = 0.892$; $AUC_{0–8}$ [ng · h/mL]: c.521CC, 11.6 ± 7.6; c.521TC, 11.3 ± 7.6; c.521TT,

Table 2. Pharmacokinetic Variables of Simvastatin and Simvastatin Acid After a Single Dose of Simvastatin in Relation to *SLCO1B1* Genotype

	SLCO1B1 c.521TT (n = 15)	SLCO1B1 c.521TC (n = 15)	SLCO1B1 c.521CC (n = 2)	*P*
Simvastatin				
C_{max} (ng/mL)	3.6 ± 2.5	3.6 ± 2.6	2.7 ± 1.2	.892
t_{max} (h)	1.50 (0.5–4.0)	2.00 (0.5–3.0)	2.50 (2.0–3.0)	N/A
$t_{1/2}$ (h)	4.2 ± 2.9	2.9 ± 1.7	2.9 ± 0.7	.334
AUC_{0-8} (ng·h/mL)	10.0 ± 5.6	11.3 ± 7.6	11.6 ± 7.6	.857
Simvastatin acid				
C_{max} (ng/mL)	0.41 ± 0.32	0.96 ± 0.54	2.14 ± 0.15	< .0001
t_{max} (h)	N/A	N/A	N/A	N/A
$t_{1/2}$ (h)	N/A	N/A	N/A	N/A
AUC_{0-8} (ng·h/mL)	1.9 ± 1.8	4.5 ± 2.5	12.1 ± 0.3	< .0001

Data expressed as mean ± SD; t_{max} expressed as median (range). ANOVA was used for all statistical analyses. N/A, not applicable.

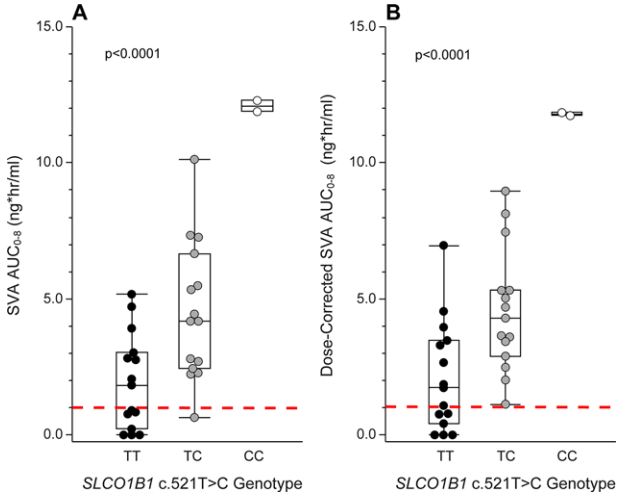

Figure 4. (A) Comparison of simvastatin acid AUC_{0-8} (ng·h/mL) and (B) AUC (ng·h/mL) corrected for dose among *SLCO1B1* c.521 genotypes ($P < .0001$). Black circles represent participants with the c.521TT genotype (n = 15). Gray circles represent participants with the c.521TC genotype (n = 15). Open white circles represent participants with the c.521CC genotype (n = 2). Red dashed line denotes negligible SVA exposure.

10.0 ± 5.6; $P = .857$); see Table 2 and Supplemental Figure 1. In addition, there was no significant difference in the 6-hydroxymethyl SVL, 6-hydroxymethyl SVA, and 3,5-dihydrodiol SVL exposure among c.521TC and c.521CC groups compared with reference participants (Supplemental Figures 2–4).

Effect of CYP3A5 Genotype on SVA Systemic Exposure

CYP3A genotype had no effect on participants with negligible or detectable SVA concentration (Supplemental Table 1).

Effect of Age and Nongenetic Factors

No correlation was evident between AUC_{0-8} for SVA or SVL and age, weight, sex, or ethnicity. A nonmonotonic relationship between SVL AUC_{0-8}, and Tanner stage for breast/testicular and pubic hair groups ($P = .001$ and $P = .005$, respectively) was observed, with significantly lower AUC_{0-8} values in stage 3 relative to stages 1 and 5. However, this observation did not persist when exposure was corrected for the weight-adjusted dose administered. There were no significant difference between unadjusted or dose-corrected SVA AUC and body mass index (BMI) percentile or *Z* score.

Discussion

The present study investigated the impact of *SLCO1B1* genetic variation on simvastatin pharmacokinetics in children and adolescents. We confirmed significant associations between *SLCO1B1* c.521 variants and the dose–exposure relationship of SVA in the pediatric cohort. The magnitude of the genotype effect in children was almost *2-fold greater compared with that observed in adults* (6.3-fold vs 3.2-fold as observed by Pasanen et al[22]). In our study, each copy of the variant *SLCO1B1* c.521C allele was associated with a 2.5-fold increase in SVA AUC. This is in direct contrast to the only previously reported investigation on the effect of *SLCO1B1* genotype on pravastatin disposition in children, where c.521 variants were associated with decreased systemic exposure.[36] However, the pediatric pravastatin study was complicated by the coadministration of inhibitory immunosuppressive agents, a small variant population (c.521 T>C: family hypercholesterolemia, n = 6; cardiac transplantation, n = 3) and failure to analyze the full spectrum of metabolites so as to more completely characterize the relative contribution of pathways other than *SLCO1B1*.

There are notable difference in our study compared with the largest existing adult study. First, this study was conducted in dyslipidemic (LDL >130 mg/dl) children and adolescents (n = 32), a pediatric patient population for whom statin treatment is prescribed or considered, and a population that is likely to increase in the future; the adult reference study conducted by Pasanen et al[22] was conducted in young healthy volunteers (n = 32), a population unlikely to be treated with statins. Thus, the sample size is the same as the adult study, and in fact, we would argue that the data from our pediatric study is likely to have a greater impact given that the data are derived from a patient population that might directly benefit from the drug. Second, the pharmacogenetic association — the magnitude of the effect of each copy of the *SLCO1B1 c.521C* allele — was actually *double* that observed in the Pasanen study. Whether the genotype-phenotype relationship observed in our study is "similar" to what is observed in adults or is indicative of a true developmental influence on the genotype-

phenotype relationship will require replication of our study in additional pediatric cohorts.

SLCO1B1 polymorphism did not have an effect on SVL, 6-hydroxymethyl SVA, or the other lactone metabolites. Because of their lipophilic nature, passive diffusion appears to be the mechanism by which the lactone substrates cross the lipid bilayer membrane into the hepatocyte, as opposed to transport mediated via SLCO1B1. Few studies, if any, have addressed cellular transport of SVA metabolites, such as 6-hydroxymethyl SVA. However, the lack of association between *SLCO1B1* genotype and plasma 6-hydroxymethyl SVA concentrations may be because of 6-hydroxymethyl SVA being formed intracellularly via CYP-mediated biotransformation of SVA, and thus cellular uptake by SLCO1B1 is less important than cellular efflux, as a determinant of plasma concentrations.

Further inspection of the interindividual variability within genotype groups, especially the c.521TC group, revealed that factors other than *SLCO1B1* genotype contribute to variability in the dose–exposure relationship for simvastatin. In this investigation, SVA concentration was negligible in 25% of the study cohort but would likely be larger in the general population, such as our biorepository of dyslipidemic children in whom the c.521TT genotype was present in 75% of participants, similar to the reported frequencies of approximately 64%–77% reported in Europeans.[20,37] It remains unknown if this observation represents developmental or genetic differences between children and adults with respect to SVL hydrolysis or more efficient uptake in the liver, resulting in lower circulating SVA concentrations. Published data from adults revealed a degree of interindividual variability in SVA exposure consistent with the variability observed in our study.[22] Assuming the adult data are normally distributed, the total body exposure of SVA would be negligible in a subset of this population as well. Even when scaling our pediatric data to approximate the dose used in the previous adult study (40 mg), concentrations in the "lower-exposure" participants remain negligible. However, scaling the AUC in c.521TT participants with "detectable" exposure results in AUC values within the range reported in adults, thereby eliminating the influence of dose administered as the reason behind low exposure. Overall, the observation of low simvastatin acid exposure in a subset of patients represents a new finding that may have important clinical consequences in treatment response for both pediatric and adult patients.[19,38,39]

Analysis of those 8 subjects with negligible SVA $AUC_{0–8}$ compared with those with detectable exposure revealed no significant associations with age, sex, ethnicity, development stage, weight, height, or BMI (Supplemental Table 1). However, we acknowledge that this study was not specifically powered to address this particular post hoc analysis.

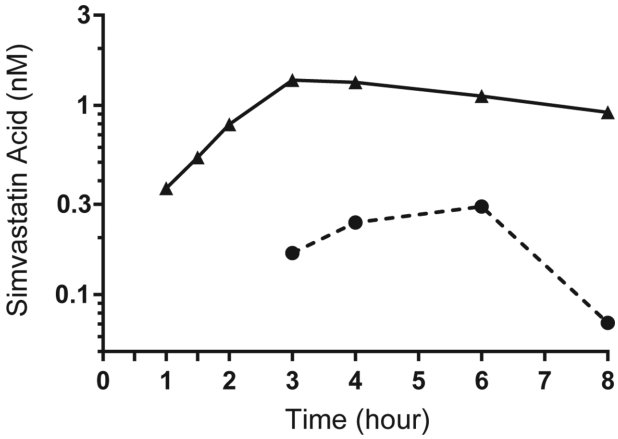

Figure 5. Comparison of c.521TT participants with negligible and detectable simvastatin acid $AUC_{0–8}$. Black triangle represents participant with c.521TT genotype with detectable simvastatin acid $AUC_{0–8}$ (n = 7). Black circle with dashed line represents participants with negligible simvastatin acid $AUC_{0–8}$ (n = 8).

In contrast to published adult data,[22] simvastatin acid formation was delayed and prolonged in a significant number of children and adolescents, as evidenced by a direct comparison of the pharmacokinetic profiles (Figure 5). It is generally assumed that hydrolysis of SVL to SVA is rapid, such that the initial increases in SVA concentration represent formation, and the slower decline in concentrations represents the rate-limiting elimination phase. However, in situations in which SVA formation is rate limiting, as may occur in the presence of genetic variation or developmental changes in expression of the genes coding for the hydrolysis pathways, the terminal rate process in the disposition profiles may reflect SVA formation. In adults, SVA formation is considered rapid, (ie, not rate limiting), whereas we observed the SVA disposition profile in our pediatric participants to be characterized by more gradual increases in concentration, consistent with slower formation rates.

Low systemic concentrations of SVA may be the consequence of changes in multiple pathways involved in SVA formation and disposition (Figure 1). Presystemic metabolism at the level of the enterocyte prior to plasma hydrolysis of simvastatin could contribute to variable SVA exposure. CYP3A4 and CYP3A5 have been identified as the primary CYP isoforms involved in the presystemic and hepatic metabolism of simvastatin and SVA.[17] Coadministration of a potent CYP3A inhibitor itraconazole increased SVL and SVA $AUC_{0–\infty}$ 5- and 20-fold, respectively,[40] and CYP3A5 nonexpressers (*CYP3A5*3/*3*) demonstrated increased simvastatin exposure[41] and enhanced lipid lowering, compared with subjects with *CYP3A5*1/*1 or *1/*3*

genotypes.[42] We genotyped for this variation in our population and noted that all the participants with negligible SVA possessed homozygous *CYP3A5*3/*3* genotypes, thus, eliminating the potential for increased CYP3A5 expression to account for low SVA systemic exposure; this does not preclude involvement of a non-CYP3A5 pathway.

Reduced conversion of the lactone prodrug to SVA is another potential explanation for the significant percentage of children with negligible systemic exposure. Although hydrolysis of SVL to SVA is considered to occur via a combination of enzymatic and nonenzymatic hydrolysis, there is a paucity of published data in this regard. In vitro data suggest that simvastatin conversion to SVA in human plasma occurs via carboxylesterases (CES) or paraoxonase (PON) reactions.[13,43] CES, comprising 2 subtypes, is a hydrolyzing enzyme responsible for phase 1 metabolism of many xenobiotics. It is minimally expressed in neonates, it increases during early childhood, and expression plateaus during middle childhood and adolescence to 75% of adult levels.[44] PON, comprising 3 subtypes, is an antioxidant with roles in detoxifying organophosphates and inhibiting oxidation of LDL-C,[45,46] and is reported to hydrolyze simvastatin in vitro. Additional data regarding the relative contributions of CES and PON subtypes toward SVA formation and the ontogeny of PON expression are essential to better understand the factors contributing to SVA disposition during growth and development.

Low plasma SVA concentrations could be because of accelerated uptake into the liver in a subset of the c.521TT study participants. Although we did not conduct a quantitative analysis of urinary metabolites in this study, the most abundant metabolite detected was 6-hydroxymethyl SVA, implying that SVA was taken up into the liver and extensively metabolized. Thus, a major unanswered question is whether the subgroup of participants with low plasma concentrations of SVA represents patients likely to respond better than anticipated because of enhanced hepatic uptake. Ultimately, though, therapeutic response is a function of SVA concentration within hepatocytes and is dependent on both uptake into the cells and clearance via biotransformation and cellular efflux; whichever of these components is rate limiting with respect to hepatocyte exposure will represent the primary determinant of pharmacodynamic response.[47]

Limitations of the Study

These findings illustrate the potential pitfalls that may occur when relying on adult experience to inform the design of pediatric studies. More specifically, the sampling strategy we used was based on adult data suggesting a mean terminal half-life ($t_{1/2}$) of ~3 hours[20] and an assumption that clearance may be equivalent to, or increased in children based on reported differences in the clearance of other CYP3A substrates.[48–50] The reduced and delayed SVA exposure that we observed in our study was unanticipated based on available adult data and precluded accurate estimation of the terminal elimination rate constant (λ_z) for SVA, $AUC_{0-\infty}$, and elimination $t_{1/2}$. It is unlikely that the results of our study could be predicted by applying some sort of scaling factor to existing adult data, confirming the necessity of pediatric pharmacokinetic studies to determine the range of drug exposure anticipated in children. This is especially important given recent findings regarding the ontogeny of hepatic *SLCO1B1* expression by Prasad et al[33] in which proteomic analysis revealed age-dependent changes in *SLCO1B1*, but only for the *SLCO1B1* c.521TT genotype group. The implication of these data is that the *SLCO1B1* genotype–statin exposure phenotype relationship may change during growth and development and, thus, that genotype-phenotype relationships may change with age, thereby limiting the value of adult experience to inform SVA disposition in children. In this context, it is tempting to speculate that genotype-dependent ontogeny effects may contribute to the 6.3-fold difference in SVA AUC between the c.521CC and c.521TT genotypes in our study — an effect that is approximately twice that observed by Pasanen and colleagues in adults.

Conclusion

The effect of allelic variation in *SLCO1B1* on the systemic exposure of SVA in hyperlipidemic children and adolescents is similar to that observed in adults, with exposure increasing significantly according to the number of variant c.521C alleles present. However, the magnitude of the effect of the *SLCO1B1* genotype on SVA systemic exposure was more pronounced in children compared with adults. Perhaps more important from a precision therapeutics perspective is the observation of considerable interindividual variability in SVA systemic exposure within *SLCO1B1* genotype groups, implying that factors in addition to the *SLCO1B1* genotype contribute to variability in simvastatin acid disposition. Further investigation of the ontogeny and genetic variation of SVA formation is necessary to better understand the variability in SVA exposure in children and its clinical consequences.

The significance of the results of our study can be further appreciated by considering the implications from the perspective of a prescriber initiating the labeled 10-mg dose to an individual child younger than 18 years of age. Without the knowledge of that patient's *SLCO1B1* genotype, systemic exposure of therapeutically active SVA can be expected to fall anywhere within a 60-fold range of systemic exposure, assuming that

the range of exposures in this study (0.2–12.3 ng·h/mL) reasonably reflect the range of exposures in the treated population. Even when correcting systemic exposure for weight-based dose, there remains an alarming 30-fold range of systemic exposure (0.4 to 11.8 ng·h/mL). Notably, within that 30-fold range exists a subset of patients (25%) who would have negligible exposure. In the context of clinical practice (or clinical trials), a significant proportion of pediatric participants may be classified as poor- or nonresponders to simvastatin because they are unable to form sufficient amounts of therapeutically active SVA or clear it more efficiently compared with the other study participants. Early identification of individuals unlikely to form sufficient simvastatin acid would allow selection of an alternative statin and thereby minimize the risk of delayed implementation of effect treatment. The apparent variability in SVA exposure within *SLCO1B1* genotype groups provides evidence of the need for additional investigation into sources of variability in the simvastatin dose–exposure relationship. Until this relationship is clearly established, it will not be possible to control the exposure in a manner that will improve the potential for clinical trials to provide actionable data to inform the use of the drug in pediatric patients and to implement effective precision therapeutics in this vulnerable population.

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Declaration of Conflicting Interests

The authors have no conflicts of interest relevant to the topic of this manuscript to disclose.

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Author Contributions

Wagner: primary investigator, designed research, wrote manuscript, analyzed data. Rahman: designed research, wrote manuscript, analyzed data. Van Haandel: contributed to new analytical method, wrote manuscript. Gaedigk A: designed research, performed research, edited manuscript. Gaedigk R: performed research, edited manuscript. Raghuveer: designed research, wrote manuscript. Kauffman: designed research. Leeder: primary mentor, designed research, wrote manuscript, analyzed data.

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