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ARTICLE

Impact of *SLCO1B1* Genetic Variation on Rosuvastatin Systemic Exposure in Pediatric Hypercholesterolemia

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This study investigated the impact of *SLCO1B1* genotype on rosuvastatin systemic exposure in hypercholesterolemic children and adolescents. Participants (8–21 years) with at least one allelic variant of *SLCO1B1* c.521T>C (521TC, *n* = 13; 521CC, *n* = 2) and wild type controls (521TT, *n* = 13) completed a single oral dose pharmacokinetic study. The variability contributed by *SLCO1B1* c.521 sequence variation to rosuvastatin (RVA) systemic exposure among our pediatric cohort was comparable to previous studies in adults. RVA concentration-time curve from 0–24 hours (AUC_{0-24}) was 1.4-fold and 2.2-fold higher in participants with c.521TC and c.521CC genotype compared 521TT participants, respectively. Interindividual variability of RVA exposure within *SLCO1B1* genotype groups exceeded the ~ 1.5-fold to 2-fold difference in mean RVA exposure observed among *SLCO1B1* genotype groups, suggesting that other factors also contribute to interindividual variability in the rosuvastatin dose-exposure relationship. A multivariate model performed confirmed *SLCO1B1* c.521T>C genotype as the primary factor contributing to RVA systemic exposure in this pediatric cohort, accounting for ~ 30% of the variability RVA AUC_{0-24}. However, of the statins investigated to date in the pediatric population, RVA has the lowest magnitude of variability in systemic exposure.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ In children with hypercholesterolemia, response to 3-hydroxy-3-methyl-glutaryl Coenzyme A reductase inhibitors (statins) is variable. In adults, genetic variation of *SLCO1B1* influences the pharmacokinetics of statins. To date, the mechanisms that influence statin disposition in a developing child is unknown.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ In the present study, we investigated the impact of *SLCO1B1* c.521C>T genotype on rosuvastatin (RVA) systemic exposure in children and adolescents.

WHAT DOES THIS STUDY ADDS TO OUR KNOWLEDGE?

☑ Similar to adults, *SLCO1B1* c.521 allelic variation impacts the pharmacokinetics of RVA in children, however, other unknown factors contribute to interindividual variability in the dose-exposure relationship. There is less interindividual variability in RVA compared with pravastatin and simvastatin in the pediatric population.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ This study highlights that less variability in systemic exposure was observed with children and adolescents dosed RVA compared with pravastatin and simvastatin.

Rosuvastatin (RVA) is a hydrophilic, synthetic inhibitor of 3-hydroxy-3-methyl-glutaryl Coenzyme A reductase, labeled to treat children 8 years and older with heterozygous familial hypercholesterolemia.[1–3] RVA is administered in active acid form and undergoes hepatocellular uptake via the drug transporters, OATP1B1, OATP1B3, OATP2B1, and NTCP.[1,4] Minor cytochrome P450 (CYP)-mediated metabolism via CYP2C9 leads to the formation of a minimally active metabolite, N-desmethyl rosuvastatin (NDMRV; **Figure 1**).[1,2] RVA undergoes phase II metabolism via UGT1A3 leading to the formation of an inactive metabolite, rosuvastatin lactone (RVL).[5] RVA is also a substrate for BCRP, responsible for hepatic clearance into the bile.[6,7] Due to its hydrophilic nature, RVA, similar to pravastatin, may experience less passive diffusion across the blood brain barrier[8,9] and skeletal muscle,[1,10–15] making it a potentially safer statin alternative for maturing brains and myocytes in children compared with highly lipophilic statin agents (e.g., simvastatin).

Two double-blind, randomized, placebo-controlled trials of rosuvastatin in children (ages 6–17 years) have demonstrated

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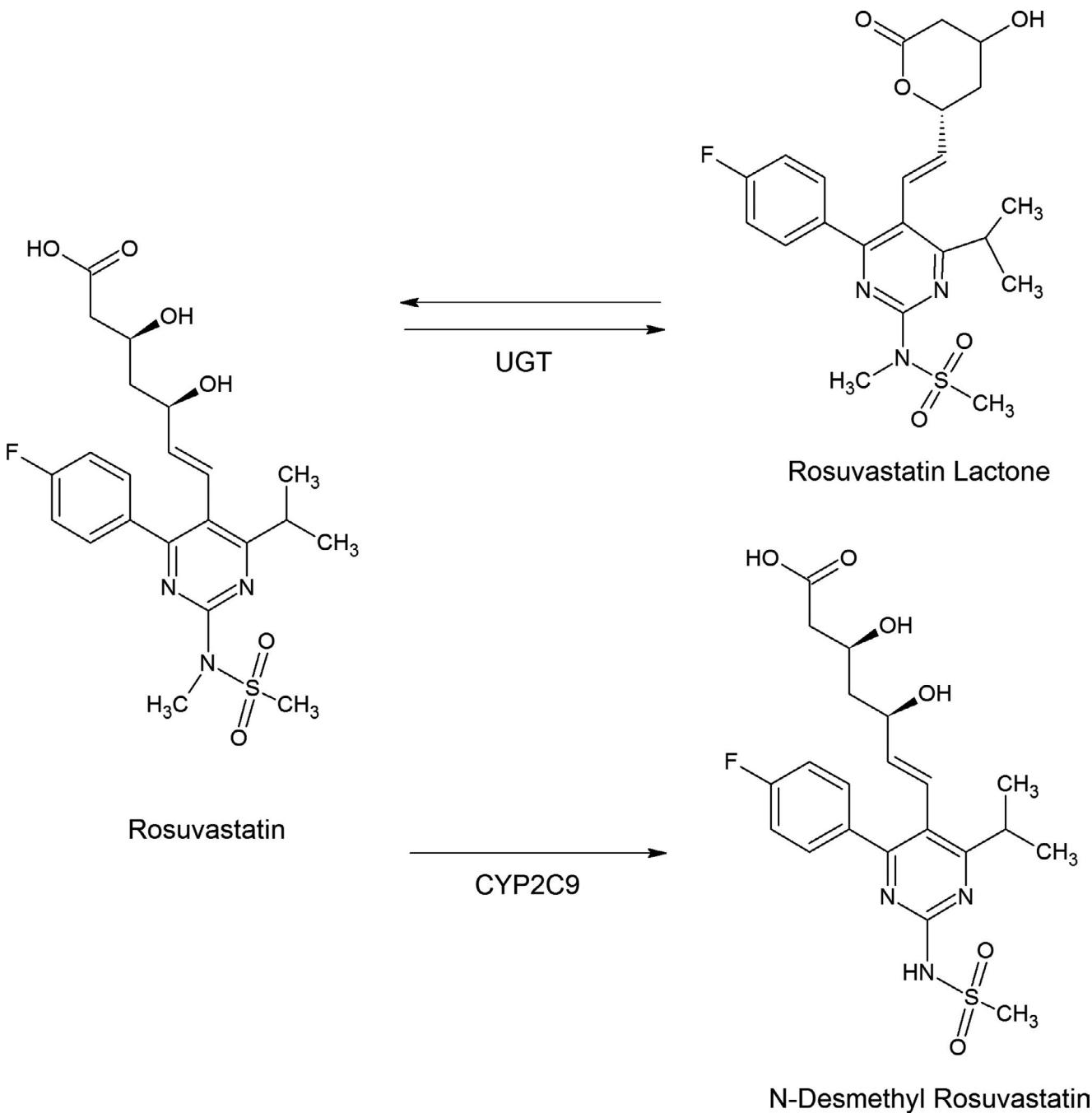

Figure 1 Rosuvastatin (RVA) pathway. RVA undergoes CYP2C9-mediated biotransformation to N-desmethyl rosuvastatin and UGT-mediated lactonization to form RVA lactone.

an ~ 35–50% reduction in low density lipoprotein cholesterol (LDL-C), validating its efficacy in this age group.[16,17] However, considerable (approximately three- to fivefold) interindividual variability in drug response, as indicated by reductions in LDL-C, has been observed.[18] Additionally, ~ 35–60% of participants failed to achieve the target LDL-C goal (< 130 mg/dL) despite documented adherence to the drug.[16,17] With this large degree of variability in response and unknown long-term effects of chronic statin exposure, providing dose-optimization (e.g., the lowest dose that achieves maximal response with minimal risk of toxicity) is essential to improving hypercholesterolemia treatment in the developing child.

In order to investigate the etiology of interindividual variability in statin response, however, we must determine if poor or no response is a function of inadequate drug exposure or altered drug target engagement due to diminished expression and/or function of the drug target proteins. One of the largest sources of variation in the dose-exposure relationship is hepatic uptake of statins to the site of action within the liver. The *SLCO1B1* gene encoding the

hepatic drug transporter OATP1B1 has been shown to be a major contributor to RVA transport.[4] Furthermore, the c.521T>C single nucleotide polymorphism (rs4149056) located in exon 5 of the *SLCO1B1* gene affects the localization of the transporter on the basolateral membrane of the human hepatocyte.[19] Functionally, this alteration leads to a nonsynonymous amino acid change (Val174Ala) that contributes to decreased human hepatocyte uptake[4] and increased RVA systemic exposure.[20,21] In adults, Pasanen *et al*.[20] demonstrated that a *SLCO1B1* single nucleotide polymorphism (SNP; c.521T>C in exon 5) was associated with an ~ 1.5-fold increase in RVA exposure compared with participants with the reference genotype (c.521TT). The association between *SLCO1B1* c.521T>C gene variation and clinical response to statins (e.g., LDL-C reduction, myopathy) has been demonstrated in several studies,[22–25] but in large part, the association regarding its impact on LDL-C reduction remains ambiguous.[26–28] The largest association of *SLCO1B1* gene variation and myopathy has been observed with simvastatin acid and current Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines are available for simvastatin dosing according to *SLCO1B1* genotype.[29]

Growth and development may modulate the magnitude of the genotype-phenotype relationship in children, limiting the extrapolation of adult data to hypercholesterolemic children. In our recent pediatric genotype-stratified, single-dose, pharmacokinetic study of simvastatin in children > 8 years, the genotype-phenotype relationship observed in adults was confirmed with each copy of the variant c.521C allele contributing to a 2.5-fold increase in simvastatin acid systemic exposure.[30] The magnitude of the genotype effect was twofold greater in children compared with adults, suggesting *SLCO1B1* c.521T>C is more influential in children. In contrast, the magnitude of genotype effect in the same cohort of children receiving pravastatin was comparable to adults.[31,32] Of concern though, was the extent of interindividual variability in simvastatin and pravastatin systemic exposure that was observed within genotype groups (~ 8-fold to 17-fold range).[31,33] Currently, the influence of ontogeny on the RVA dose-exposure relationship is not well-established.

Understanding the impact of ontogeny on the genotype-phenotype relationship for key drug transporters influencing statin disposition is of critical importance in optimal dose selection for pediatric patients. Thus, the primary goal of this investigation was to establish the role of genetic variation in *SLCO1B1* on the rosuvastatin dose-exposure relationship in hypercholesterolemic children and adolescents.

METHODS

Subjects

Subjects meeting inclusion and exclusion criteria for this study (**Supplemental Materials S1**) were recruited from the Children's Mercy Hospital Cardiology Pharmacogenomics Repository, a living biorepository and patient registry designed to facilitate genotype-guided clinical trial participant selection. Cardiology Pharmacogenomics Repository enrollees were invited to participate based on their *SLCO1B1* c.521T>C genotype status. Reference genotype (c.521TT) and heterozygous variant populations (c.521TC and c.521CC) were age-matched, ethnicity-matched, and gender-matched. The study protocol was reviewed and approved by the Children's Mercy Hospital Institutional Review Board and the study conducted in accordance with US and international standards of Good Clinical Practice (US Food and Drug Administration (FDA) regulations 21 Code of Federal Regulations (CFR) 312 for Investigational New Drug studies and FDA guidance E6).

Genetic analysis

Genomic DNA was isolated from biospecimens using Sigma GeneElute Mammalian Genomic DNA Miniprep Kit (St. Louis, MO) or QIAamp DNA Blood Mini Kit (Valencia, CA) according to manufacturer protocols. All DNA samples were genotyped for the common *SLCO1B1* SNPs –11187G>A (rs4149015), c.388A>G (rs2306283), and c.521T>C (rs4149056) using TaqMan SNP genotyping assays (LifeTechnologies, Carlsbad, CA) with KAPA Probe Fast qPCR Master Mix (2X) ABI Prism (KAPA Biosystems, Boston, MA) on a QuantStudio 12 k Flex Real-Time PCR System (Applied Biosystems, Foster City, CA). Five to 20 ng of DNA was used per reaction. The cycling conditions for all 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 reanalyzed samples were concordant with the original results.

To identify sequence variations in *SLCO1B1*, *SLCO1B3*, *SLCO2B1*, *SLC10A1*, *ABCG2*, *CYP2C9*, and *UGT1A3* next-generation sequencing (NGS) was performed. Briefly, a TruSeq Library was constructed according to manufacturer's protocol (Illumina, San Diego, CA). A custom targeted capture sequencing panel (Integrated DNA Technologies, Coralville, IA) was used for enrichment. Samples were sequenced on an MiSeq instrument (Illumina) with paired end 200 base pair reads. Read coverage was ~ 500×. *SLCO1B1* genotypes obtained by Taqman genotyping were 100% consistent with the NGS data.

CYP2C9 and *UGT1A3* genotype calls were made as following: *CYP2C9* genotype was called using Astrolabe, a bioinformatic tool that allows star allele calling from NGS data.[33,34] Astrolabe has been expanded to CYP2C9 and 2C19 (manuscript in preparation). For UGT1A3, an SNP report was generated and star alleles manually called using definitions per the UGT Nomenclature site at https://www.pharmacogenomics.pha.ulaval.ca/ugt-alleles-nomenclature/.

Study design

This was a single-center, open-label, genotype-stratified, single oral dose pharmacokinetic study, comparing the disposition of rosuvastatin among hypercholesterolemic children and adolescents with one or more *SLCO1B1* c.521C variant alleles to patients homozygous for the reference c.521TT genotype. Participants on current statin therapy withheld statin administration for a washout period of 7 days prior to the drug study visit. All participants had a screening physical examination (including Tanner

Staging) performed by a licensed physician prior to drug administration.

Subjects ingested a single oral dose of rosuvastatin (ages 8–21 years: 10 mg tablet, Glenmark Pharmaceuticals, Lot Number FC0259) with 150 mL of water after an overnight fast and resumed meals no earlier than 3 hours after administration of the study dose. Serial venous blood samples (1.5 mL each) were drawn from an indwelling venous cannula before pravastatin administration (time 0), and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 7, 9, 18, and 24 hours post-ingestion to measure plasma drug and metabolite concentrations. Samples were collected in a syringe, transferred to a tube containing potassium EDTA, gently mixed by inversion, and immediately centrifuged at 4°C for 10 minutes at 600 g. Plasma was removed and stored at –80°C until analysis. Predose urine and pooled postdose urine were collected through the duration of the study (24 hours).

Analytical methods

Plasma and urine concentrations of RVA, RVL, and NDMRV were measured on a Waters TQ-S triple quadrupole tandem mass spectrometer with a novel ultra-high pressure liquid chromatography-tandem mass spectrometric method previously developed and validated in our laboratory.[35] In brief, the dynamic range of the assay was from 0.5–100 nM for all analytes. The method was linear for all analytes in the concentration range 0.5–100 nM with intraday and interday precisions (as relative SD) of ≤ 10.3% and accuracy (as relative error) ranging from 97–111% at all quality control levels (1, 10, and 75 nM). Plasma samples were analyzed in duplicate. For instances where the coefficient of variation in the duplicates exceeded 20%, the samples were rerun (< 5% of samples).

Pharmacokinetic parameters

Power calculations to determine sample size for our study are detailed in **Supplemental Materials S1**. Pharmacokinetic analyses were conducted using Kinetica version 5.0 (ThermoFisher Scientific, Philadelphia, PA). Plasma concentration vs. time data for RVA, RVL, and NDMRV were curve fit using a peeling algorithm to generate initial monoexponential 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 determined as follows. Individual peak plasma concentration (C_{max}) and time to maximal concentration (T_{max}) were obtained by direct examination of the plasma concentration vs. time profile. The area under the plasma concentration vs. 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 not performed; secondary peaking occurred in a significant number of subjects within the cohort and, therefore, insufficient data points to accurately capture the terminal elimination phase for RVA were available.

Statistical analysis

Pharmacokinetic data for the study cohort were examined using standard descriptive statistics in JMP version 14 (SAS, Marlow, UK). Pharmacokinetic parameters reflective of systemic exposure (C_{max} and AUC_{0-n}) were log-transformed using the natural logarithm. The significance limit accepted for all statistical analyses was $\alpha = 0.05$. *SLCO1B1* genotype (c.521TT vs. c.521TC/CC) was treated as an independent variable. Pharmacokinetic parameters were compared between demographic and genotype groups, using Welch's *t*-test and Kruskal–Wallis test given the non-normality of the dependent variables. For the pharmacokinetic parameters related to *SLCO1B1* genotype groups (**Table 2**), Kruskal–Wallis test was utilized. For the pharmacokinetic parameters related to demographics (**Table S3**), both Welch's *t*-test and Kruskal–Wallis test were utilized.

We used linear regression to examine associations between RVA AUC_n and individual SNPs. For each SNP, we fit one model where the SNP was the only predictor of RVA AUC_n, and a second model where sex, body mass index (BMI) percentile, and Tanner stage were added as covariates. For comparison, we also fit a base model with only sex, BMI percentile, and Tanner stage as predictors, allowing us to compute how much additional variability in RVA AUC_n could be accounted for by adding each SNP variable to the base model. Tanner stage was computed as the average of the Pubic Hair Scale score and Breast Development/External Genitalia Scale score. CYP2C9 genotype was used to create two variables for use in modeling: number of *1 alleles (0, 1, or 2) and number of *2 alleles (0, 1, or 2). For all other SNPs, the count of variant alleles (0, 1, or 2) was computed for use in modeling.

Because administration of a fixed dose of RVA resulted in an almost fourfold range of weight-based doses (0.07–0.28 mg/kg), pharmacokinetic exposure parameters (e.g., C_{max} and AUC) were normalized to dose for each individual participant by dividing the exposure parameter value by actual mg/kg dose received then multiplied by the mean mg/kg dose for the entire cohort (e.g., participant AUC (ng*hour/mL)/participant dose (mg/kg) * cohort mean dose (mg/kg)).

RESULTS

Participant characteristics and adverse events

A total of 28 children and adolescents (15 male and 13 female participants) were enrolled in this investigation. The demographic and genetic constitution of the participant population is detailed in **Table 1**. Demographic parameters were similar between *SLCO1B1* c.521T>C variant-containing genotypes and c.521TT controls. Of note, the weight-based dose received was similar between the genotype groups (**Table 1**). No adverse events were reported during the study.

Drug disposition profiles

The drug disposition profiles of RVA, RVL, and NDMRV (**Figure 2a–c**) were consistent with first-order absorption and elimination. RVA, RVL, and NDMRV were detected in all participants. Secondary peaking, suggestive of enterohepatic recirculation, was noted on the RVA profiles of 11 participants (39%) precluding accurate assessment

Table 1 Characteristics of participants stratified by *SLCO1B1* genotypes

	SLCO1B1 521 TT (*n* = 13)	*SLCO1B1* 521 TC (*n* = 13)	*SLCO1B1* 521 CC (*n* = 2)	*P* value
Age, years[a]	15.2 (± 3.0)	15.0 (± 2.8)	14.4 (± 4.3)	0.965
Weight, kg[a]	86.0 (± 34.2)	87.0 (± 25.8)	61.8 (± 1.6)	0.314
Height, cm[a]	164.2 (± 8.6)	165.6 (± 11.9)	157.5 (± 20.0)	0.659
BMI, kg/m^2[a]	31.6 (± 11.8)	31.1 (± 6.7)	25.5 (± 5.8)	0.695
Sex[b]				
Female	6	6	1	0.994
Male	7	7	1	
Ethnicity[b]				
White, non-Hispanic	7	7	1	0.951
White, Hispanic	5	5	1	
African American	1	1	0	
Tanner breast/testicular[b]				
Stage 1	0	0	0	0.165
Stage 2	2	2	1	
Stage 3	0	1	0	
Stage 4	4	0	0	
Stage 5	7	10	1	
Pubic[b]				
Stage 1	1	0	0	0.235
Stage 2	0	3	1	
Stage 3	1	0	0	
Stage 4	3	0	0	
Stage 5	8	10	1	
Dose, mg/kg[a]	0.14 (± 0.06)	0.13 (± 0.04)	0.16 (± 0.00)	0.402

BMI, body mass index.
All data expressed as mean (± SD).
[a]Designates Kruskal–Wallis test. [b]Designates Chi-square test performed.

of the mean terminal elimination rate constant across the population.

Overall, a relatively large degree of variability (approximately eightfold) was observed across the entire population with respect to measures of systemic exposure for RVA (median interquartile range (IQR), C_{max}: 5.4 ng/mL (IQR 3.4–7.3 ng/mL); and AUC_{0-24}: 42.7 ng*hour/mL (IQR 31.1–60.3; **Table S1**). Significant variability was also observed for RVL (median IQR, C_{max}: 1.1 ng/mL (IQR 0.4–1.5); AUC_{0-24}: 12.9 ng*hour/mL (IQR 7.3–21.5; **Table S1**) and NDMRV (median IQR C_{max}: 0.8 ng/mL (IQR 0.5–1.1 ng/mL); AUC_{0-24}: 4.7 ng*hour/mL (IQR 3.0–6.3); **Table S1**).

Effect of *SLCO1B1* genotype on systemic exposure

Maximum mean RVA concentrations (C_{max}) were 2.3-fold higher in the c.521CC (*n* = 2) group and 1.4-fold higher in the c.521TC (*n* = 13) group relative to the c.521TT (*n* = 13) group (**Figure 3**a, **Table 2**). Mean RVA AUC_{0-24} was 2.2-fold higher in the c.521CC group and 1.4-fold higher in the c.521TC group relative to subjects with reference genotype (**Figure 3**b, **Table 2**).

RVL exposure was similar among the *SLCO1B1* genotype groups (*P* = 0.17; **Table 2**). Similarly, NDMRV exposure was similar among c.521TC and c.521CC groups compared with c.521TT (reference) participants (*P* = 0.44; **Table 2**).

There was no relationship between RVA or related isomers and the presence of *SLCO1B1* –11187G>A (rs4149015) or c.388A>G (rs2306283).

In addition to differences in RVA exposure among *SLCO1B1* c.521 genotype groups, variability within genotype groups was observed. The largest range of variability occurred in the c.521TT group (5.5-fold) compared with the c.521TC group (2.2-fold). Overall, these data indicate that additional patient-specific variables, in conjunction with *SLCO1B1* genotype, influence RVA systemic exposure. RVA exposure was not normally distributed within the entire cohort or even within the c.521TT and TC genotype groups. In fact, there were four participants within each group that deviated from normality in the normal quantile plot (**Figure S1**) and, subsequently, these were identified as a potential subgroup classified as "high outliers."

Secondary *post hoc* analyses were conducted to seek insight into additional factors contributing to high systemic RVA exposure within *SLCO1B1* genotype groups.

Effect of demographic and developmental factors on systemic exposure

Simple linear regression analyses of RVA, RVL, NDMRV AUC_{0-24} on independent variables (age, height, lean body weight, and BMI) revealed only a few weak, positive correlations (**Table S2**). RVA and RVL AUC_{0-24} were similar among sex and ethnicities (**Table S3**).

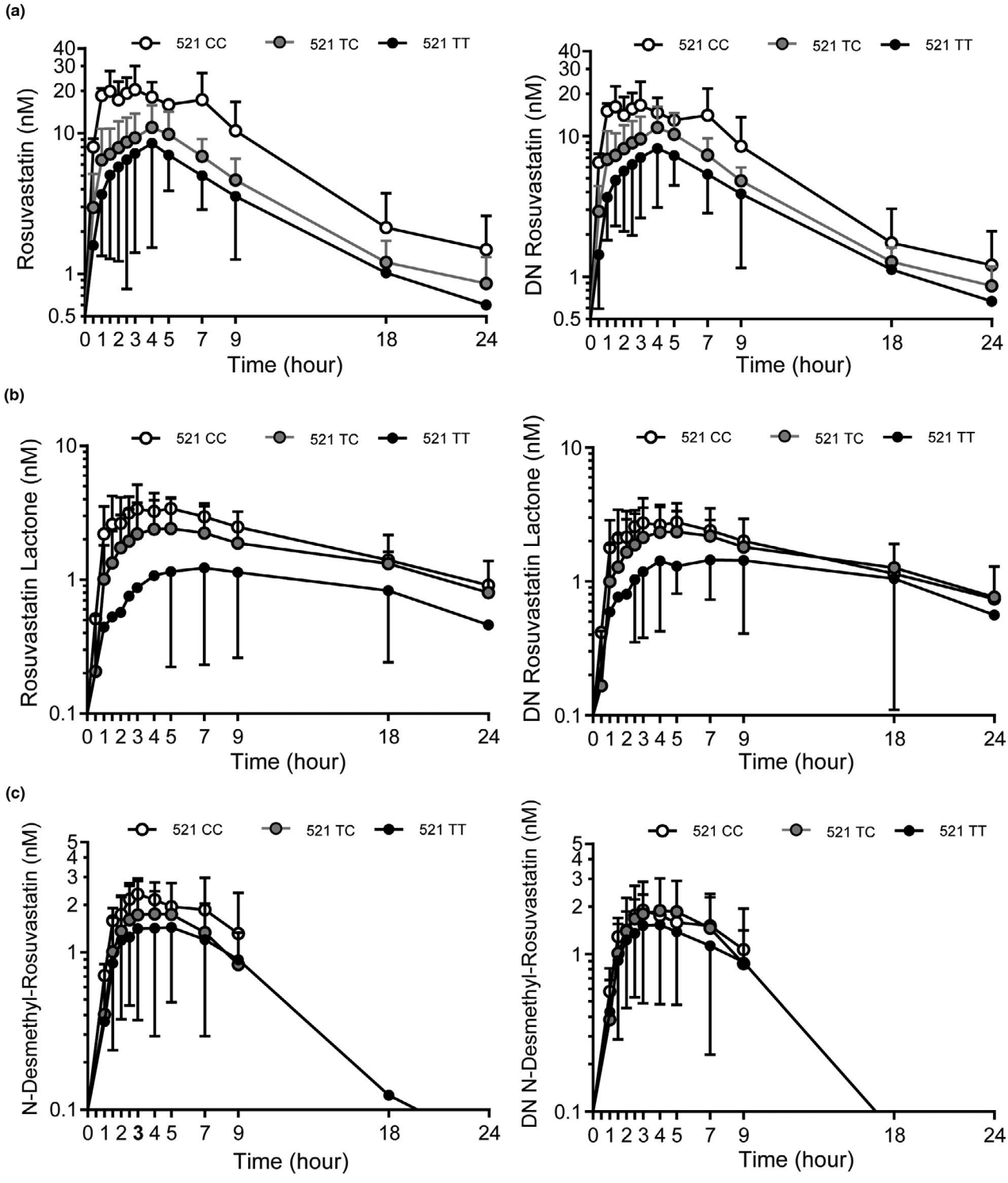

Figure 2 Rosuvastatin (RVA) and related metabolite pharmacokinetic profiles (**a**) mean ± SD plasma concentrations (nM) of RVA and dose-normalized RVA and (**b**) RVA lactone and dose-normalized rosuvastatin lactone (**c**) N-Desmethyl rosuvastatin and dose-normalized N-Desmethyl RVA after a single dose of RVA in 28 healthy pediatric participants. Black, gray, and open white circles represent participants with the c.521TT (n = 13), c.521TC (n = 13), and c.521CC (n = 2) genotypes, respectively.

Effect of non-*SLCO1B1* sources of genetic variation on systemic exposure

Additional gene sequencing of hepatic transporters associated with RVA uptake (*SLCO1B1*, *SLCO1B3*, *SLCO2B1*, and *SLC10A1)* and efflux (*ABCG2*) was performed on all participants (**Table S4**). Gene sequencing of *SLCO1B1 and SLCO2B1* revealed no additional sequence variations implicating altered OATP1B1 or OATP2B1 expression

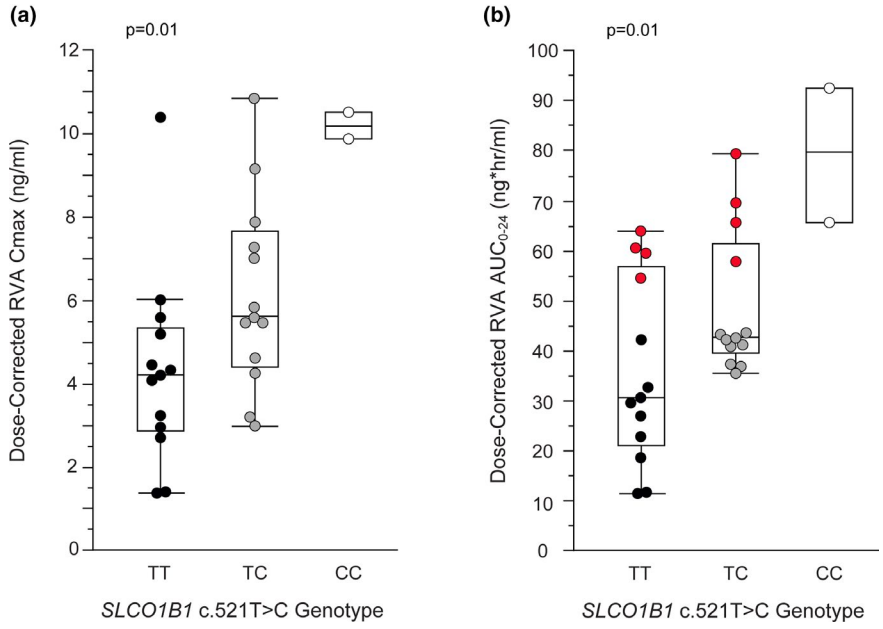

Figure 3 Rosuvastatin (RVA) exposure (**a**) comparison of RVA peak plasma concentration (C_{max}; ng/mL; $P = 0.01$) and (**b**) area under the curve from 0 to 24 hours ($AUC_{0\rightarrow24}$; ng*hr/mL) normalized for dose among *SLCO1B1* c.521 genotypes ($P = 0.01$). Black, gray, and open white circles represent participants with the c.521TT ($n = 13$), c.521TC ($n = 13$), and c.521CC ($n = 2$) genotypes, respectively. Red circles represent potential "high outlier" participants (c.521TT, $n = 4$; c.521TC, $n = 4$).

Table 2 Dose-normalized pharmacokinetic variables of RVA and related analytes after a single dose of RVA in relationship to *SLCO1B1* genotype

	***SLCO1B1* c.521TT (*n* = 13)**	***SLCO1B1* c.521TC (*n* = 13)**	***SLCO1B1* c.521CC (*n* = 2)**	***P* value**
RVA				
C_{max}, ng/mL	4.3 (± 2.3)	6.2 (± 2.2)	10.1 (± 0.6)	0.01
T_{max}, hour	5.0 (4.0–5.0)	4.0 (1.0–5.0)	4.0 (3.0–7.0)	N/A
AUC_{0-24}, ng/mL*hour	36.0 (± 18.5)	49.2 (± 14.0)	79.7 (± 18.2)	0.01
RVA lactone				
C_{max}, ng/mL	0.8 (± 0.5)	1.2 (± 0.7)	1.4 (± 0.4)	0.09
T_{max}, hour	5.0 (1.0–9.0)	4.5 (2.5–7.0)	5.0 (1.0–9.0)	N/A
AUC_{0-24}, ng/mL*hour	11.8 (± 8.6)	17.2 (± 10.2)	18.5 (± 2.0)	0.17
N-Desmethyl RVA				
C_{max}, ng/mL	0.8 (± 0.5)	1.0 (± 0.5)	1.0 (± 0.0)	0.50
T_{max}, hour	4.0 (2.5–7.0)	4.0 (2.0–7.0)	5.0 (3.0–7.0)	N/A
AUC_{0-24}, ng/mL*hour	5.4 (± 5.5)	5.8 (± 3.4)	5.7 (± 0.9)	0.44

AUC_{0-24}, area under the curve from 0 to 24 hours; C_{max}, peak plasma concentration; N/A, not applicable; RVA, rosuvastan; T_{max}, time to maximal concentration.
Data expressed as mean (± SD), T_{max} expressed as median (range). Kruskal–Wallis test utilized for all statistical analysis.

and/or function. There were seven participants with the *SLCO1B1* c.463C>A (rs11045819) known to result in increased OATP1B1 expression and function. However, two "high outliers" had the rs11045819 variant (one c.521TT and one c.521TC) and the other five participants (three c.521TT and two c.521TC) were in the lower half of their respective genotype. Thus, rs11045819 variation alone does not explain the dichotomy of the cohort within *SLCO1B1* c.521T>C genotype groups. Eight participants had a nonsynonymous sequence variation in *SLCO1B3* (c.767G>C; rs60140950) that alters transporter expression. The c.767G>C was detected in two "high outliers" in the c.521TC group and one subject in the c.521TT group. There was one additional "high outlier" in the c.521TC group with a nonsynonymous sequence variation in *SLC10A1* (c.957G>A; rs149272163). In the entire cohort, three c.521TT participants had a sequence variation in *ABCG2* (c.421C>A) with two of these participants being "high outliers." Six participants had a sequence variation in *ABCG2* (c.34G>A) including one c.521TC and one c.521TT "high outlier." There were no participants having both SNPs, *ABCG2* c.34G>A and c.421C>A. Comparison of each of these variants to wild type participants among the entire cohort and within *SLCO1B1* c.521T>C subgroups did not achieve significance. Although these aforementioned sequence variations may potentially explain higher RVA exposure for six of the eight outliers, none were solely unique to the "high outlier" group.

Effect of *CYP2C9* and UGT on systemic exposure

To determine if altered CYP-mediated metabolism contributed to the extreme phenotype results within genotype groups, differences in NDMRV metabolite formation was assessed using postdose, pooled urine samples. However, the recovery of NDMRV as a percentage of the total analyte (RVA + RVL + NDMRV) in the aforementioned "high outliers" of both genotype groups was not as a whole lower compared with others. Of note, three "high outliers," one c.521TC and two c.521TT participants,

were within the normal quantile distribution plot (**Figure S2**) with only one "high outlier" c.521TT participant in the lower group having SNPs associated with one nonfunctional *CYP2C9* allele (**1/*3*). Collectively, *CYP2C9* genotype may have contributed to higher RVA systemic exposure for only one "high outlier," but does not independently contribute to the variability within *SLCO1B1* c.521T>C genotype groups.

Similarly, to determine if diminished lactonization in the "high outliers" led to increased RVA systemic exposure, plasma RVL as a percentage of the total analyte (RVA + RVL + NDMRV) was quantitated. However, the percentage RVL/total in the aforementioned "high outliers" of both genotype groups was not as a whole lower compared with others (**Figure S3**). Of note, two "high outliers," one c.521TC and one c.521TT participant within the lower end of the normal quantile distribution plot, were present and none of them had a sequence variation suggestive of diminished UGT1A3 function. Collectively, *UGT1A3* genotype does not seem to contribute to the variability within *SLCO1B1* c.521T>C genotype groups.

RVA multivariate model

We explored a number of multiple regression models (e.g., models for *SLCO1B1* genotypes and demographic parameters), including multivariate models to quantify associations between the aforementioned SNPs and RVA AUC_{0-24}, controlling for BMI percentile, sex, and Tanner Staging. The most significant SNP associated with RVA AUC_{0-24} outcome was *SLCO1B1* c.521T>C genotype, yielding an *R*-squared value of 0.39 in the multivariate model, as compared with an R-squared of 0.10 when only the 3 covariates were included (**Table S5**). Collectively, *SLCO1B1* genotype seems to be the primary factor contributing to RVA systemic exposure among those evaluated in our analysis.

DISCUSSION

The present study investigated the impact of *SLCO1B1* genetic variation and developmental factors on RVA pharmacokinetics in children and adolescents. The magnitude of effect for the c.521TC group compared with the reference genotype in children was similar to that reported in adults (i.e., 1.4-fold in the current study vs. 1.6-fold as observed by Pasanen *et al.*[20]. In contrast to the adult study where only a difference was observed between reference genotype and c.521CC, we observed a difference among each genotype group. We do recognize that our conclusion with regard to c.521CC genotype is limited given the small sample size ($n = 2$), this observation is, however, concordant with our previous simvastatin acid and pravastatin acid analyses.[31,33] In these studies, systemic exposure increased with each variant "C" allele that was present.[31,33] The magnitude of the *SLCO1B1* genotype effect for RVA is less compared with that found for SVA but similar to PVA in this pediatric cohort.[31,33] Collectively, *SLCO1B1* c.521T>C explained nearly three times more variability in RVA exposure compared with BMI percentile, sex, and Tanner Staging in our multivariate model.

Particularly striking was the observation of an ~ 5.5-fold and 2.2-fold range of RVA systemic exposures within the c.521TT and TC genotype groups, respectively (TT: 11.7–64.2 ng*hour/mL; TC: 35.3–78.9 11.7–64.2 ng*hour/mL; **Figure 3b**). A similar observation has been described in adults with coefficients of variation for RVA AUC ranging from 36–51% within *SLCO1B1* genotype groups.[20,21] An unexpected observation was the putative existence of two separate groups within each *SLCO1B1* c.521T>C genotype group, as shown in **Figure 3b** and **Figure S1**. Eight of 28 participants (~ 30%) were deemed as "high outliers." Collectively, these data indicate that additional patient-specific variables, in conjunction with *SLCO1B1* genotype, influence RVA exposure and these need to be identified in order to effectively tailor RVA treatment to the individual patient.

Our previous investigation involving pravastatin observed that "high outliers" in the c.521TT group had the highest BMI values (*Z*-score > +2.5) in the entire cohort, suggesting that liver adiposity may compromise statin transport.[31] Previous investigations demonstrate a link between nonalcoholic fatty liver disease and diminished hepatic uptake transporter expression,[36–38] subsequently altering simvastatin and pravastatin transport.[38,39] This association of obesity and diminished RVA transport (e.g., larger systemic exposure) was not observed in this similar pediatric cohort. However, we acknowledge that our studies were not specifically powered to answer this particular *post hoc* analysis. Collectively, the role of liver adiposity on pediatric statin disposition requires further elucidation.

RVA is a known substrate of several other transporters, including OATP1B1, OATP1B3, OATP2B1, NTCP, and ABCG2[1,4] and, thus, additional gene sequencing was performed to ascertain if other sequence variants existed in the "high outliers" to explain the discordant results in our study population (**Table S4**). There were seven participants that harbored the *SLCO1B1* c.463C>A (rs11045819) SNP associated with enhanced expression and function. As shown in **Table S4**, two of our "high outliers" had rs11045819 and five in the "lower" group had the SNP. Thus, rs11045819 did not explain the dichotomy within c.521T>C groups.

Of the eight outliers, three were found to have nonsynonymous sequence variants in *SLCO1B3* (c.767G>C; rs60140950) with one having homozygosity of the variant allele. This SNP was previously investigated by Schwarz *et al.*[40] and was found to cause diminished total protein and cell surface expression of OATP1B3, but was not implicated to result in diminished uptake of cholecystokinin-8, a specific OATP1B3 substrate. Of note, they observed other sequence variants that were associated with diminished cholecystokinin-8 and RVA uptake, but these were not found in our "high outliers." The role of *SLCO1B3* c.767G>C on RVA uptake was not investigated as a part of the aforementioned study but should be investigated in future analyses.

One additional participant in the "high outlier" group had a nonsynonymous *SLC10A1* c.957G>A SNP. Previously, Ho *et al.* reported discordant results with the *NTCP*2* allele (*SLC10A1* c.800C>T), despite known to result in complete loss of function, having enhanced RVA uptake

and *NTCP*3* (*SLC10A1* c.668T>C), known to alter cell surface trafficking, having diminished RVA uptake. The *SLC10A1* c.957G>A SNP was not investigated as a part of this study, but its effect on RVA should be validated in future investigations.

ABCG2 (BCRP) is expressed on the apical membranes of a variety of tissues, including the liver and small intestine,[41] and decreased activity of ABCG2 secondary to the c.34G>A and c.421C>A SNPs at the small intestine leads to enhanced absorption. This resultant increase in bioavailability leads to increased RVA systemic exposure, which has been confirmed in adult cohorts.[42–44] Two of the six participants with the *ABCG2* c.34G>A SNP and two of three with the *ABCG2* c.421C>A SNP were "high outliers." Although this could lead to the higher exposure within each genotype group for some participants, these SNPs were not unique to the "high outliers" (i.e., were also found in 5 other participants) and, therefore, cannot be definitively concluded as the etiology leading to RVA exposure above the 99% for the cohort.

The role of *CYP2C9* genotype on RVA systemic exposure was evaluated in our study cohort, implying that if RVA metabolism was impaired, it could result in higher RVA systemic exposure. There was one "high outlier" with an intermediate metabolizer *CYP2C9* genotype. However, this finding did not explain the clear separation of the two groups within each *SLCO1B1* genotype. Collectively, *CYP2C9* and *UGT1A3* genotypes do not seem to have an impact on the interindividual variability observed in the rosuvastatin dose-exposure relationship in the pediatric population.

LIMITATIONS OF STUDY

The primary goal of this investigation was to establish the role of genetic variation in *SLCO1B1* on the RVA dose-exposure relationship in hypercholesterolemic children and adolescents. Secondary *post hoc* analyses were conducted to ascertain if additional SNPs contributed to high systemic RVA exposure within *SLCO1B1* genotype groups. We acknowledge that several of these tested SNPs had low variant allele frequency and may not have been observed in our cohort. In summary, we did observe some SNPs associated with altered RVA disposition, but no single sequence variant consistently accounted for the presence of unexpectantly high RVA AUC.

CONCLUSION

Collectively, our study demonstrates that *SLCO1B1* genotype contributed to the variability in the dose-exposure relationship observed in the pediatric cohort, yet, within each genotype group there exists variability that cannot be explained by *SLCO1B1* genotype alone. Alternative proteins involved in RVA disposition may contribute to the separation observed within the genotype groups, however, no single SNP tested consistently accounted for the presence of expectantly high RVA AUC. Perhaps the most insight comes from the finding that of the statins investigated in the pediatric population, RVA had the lowest magnitude of variability of systemic exposure of individuals within genotype groups compared with pravastatin and simvastatin.[31,33] Moving forward, RVA may be the more ideal agent for future investigations that develop clinical decision support tools to optimize outcomes for children prescribed a statin for the treatment of hypercholesterolemia. Future analyses determining the impact of less variable systemic exposure, as seen with RVA, on the markers of short-term and long-term response, needs investigation prior to making it a preferred agent in pediatric populations.

Supporting Information. Supplementary information accompanies this paper on the *Clinical and Translational Science* website (www.cts-journal.com).

Supplemental Figure 1.
Supplemental Figure 2.
Supplemental Figure 3.
Supplemental Tables 1-5.
Supplemental Materials 1.
Supplemental Figure Legends.

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