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Polymorphic Expression of *UGT1A9* is Associated with Variable Acetaminophen Glucuronidation in Neonates: A Population Pharmacokinetic and Pharmacogenetic Study

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Abstract

Introduction—Acetaminophen (paracetamol, APAP) is widely used as an analgesic and antipyretic drug in children and neonates. A number of enzymes contribute to the metabolism of acetaminophen, and genetic factors might be important to explain variability in acetaminophen metabolism among individuals.

Methods—The current investigation utilized a previously published parent–metabolite population pharmacokinetic model describing acetaminophen glucuronidation, sulfation, and oxidation to examine the potential role of genetic variability on the relevant metabolic pathways. Neonates were administered 30-min intravenous infusions of acetaminophen 15 mg/kg every 12 h (<28 weeks' gestational age [GA]) or every 8 h (≥28 weeks GA) for 48 h. A total of 18 sequence variations (SVs) in UDP-glucuronosyl-transferase (*UGT*), sulfotransferase (*SULT*), and cytochrome P450 (*CYP*) genes from 33 neonates (aged 1–26 days) were examined in a stepwise manner for an effect on the metabolic formation clearance of acetaminophen by glucuronidation (*UGT*), sulfation (*SULT*), and oxidation (*CYP*). The stepwise covariate modeling procedure was performed using NONMEM[®] version 7.3.

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Compliance with Ethical Standards

Conflict of interest Matthew W. Linakis, Sarah F. Cook, Shaun S. Kumar, Xiaoxi Liu, Diana G. Wilkins, Roger Gaedigk, Andrea Gaedigk, Catherine M.T. Sherwin, and John N. van den Anker all declare that they have no conflicts of interest that might be relevant to the contents of this article.

Results—Incorporation of genotype as a covariate for one SV located in the *UGT1A9* gene promoter region (rs3832043, –118>insT, T₉>T₁₀) significantly improved model fit (likelihood ratio test, $p < 0.001$) and reduced between-subject variability in glucuronide formation clearance. Individuals with the *UGT1A9*T₁₀ polymorphism, indicating insertion of an additional thymidine nucleotide, had a 42% reduction in clearance to APAP-glucuronide as compared to their wild-type counterparts.

Conclusion—This study shows a pharmacogenetic effect of an SV in the *UGT1A9* promoter region on the metabolism of acetaminophen in neonates.

1 Introduction

Acetaminophen (paracetamol, APAP) is used as an analgesic and antipyretic drug in children and neonates. In recent years, its use as an analgesic in the neonatal intensive care unit (NICU) has increased significantly after an intravenous (IV) formulation of acetaminophen became available in the USA. Moreover, the opioid-sparing effect of acetaminophen after major surgery in newborn infants has further boosted its use in the NICU. This increasing use necessitates more research into the potential adverse effects of acetaminophen in this fragile patient population. The most concerning adverse effects of acetaminophen overdose in neonates include hepatotoxicity and hemodynamic complications [1]. Acetaminophen is metabolized via three main pathways to (1) a glucuronide metabolite (APAP-Gluc); (2) a sulfate metabolite (APAP-Sulf); and (3) oxidative pathway metabolites (Ox APAP) (Fig. 1). The reactive intermediate metabolite N-acetyl-p-benzoquinone-imine (NAPQI), which belongs to the oxidative pathway, is responsible for the hepatotoxic effects of acetaminophen overdose [2].

A complicating factor related to metabolism in neonates is the ontogeny of drug-metabolizing enzymes, i.e., changes of enzyme expression during human development. As shown in Fig. 1, the major enzymes responsible for metabolizing acetaminophen to APAP-Gluc are UDP-glucuronosyltransferases (UGTs) 1A1, 1A6, and 1A9. Sulfotransferases (SULTs) convert acetaminophen to APAP-Sulf, and SULTs 1A1, 1A3/4, 1E1, and 2A1 are all involved in neonatal acetaminophen metabolism [3, 4]. Finally, cytochrome P450 (CYP) 2E1 is the major enzyme that converts acetaminophen to NAPQI, which is subsequently converted to downstream Ox APAP (APAP-N-acetylcysteine and APAP-cysteine). It has been documented that the major metabolic pathway for acetaminophen in neonates is sulfation [1]. However, there is a shift in the major route of metabolism towards glucuronidation as the glucuronidation pathway matures [5–9]. Indeed, fetal *UGT1A9* messenger RNA (mRNA) levels are negligible even up to 40 weeks gestation, indicating that glucuronidation may be even less important (and therefore sulfation may be more important) in extremely preterm neonates [6, 10].

Another potentially important consideration related to acetaminophen metabolism in neonates is the fact that, in adults, it is known that the sulfation pathway is saturable [4]. Concentrations of acetaminophen in excess of the saturation point of the SULT-mediated pathway will place an extra metabolic burden on the UGT- and CYP-mediated pathways. In turn, additional reactive NAPQI metabolite may be generated, increasing the risk for

hepatotoxicity [4]. However, the extent and consequences of such saturation in neonates is currently unknown.

Despite studies in adults demonstrating pharmacogenetic variability in acetaminophen metabolism related to UGTs [11, 12], it has historically been thought that, in neonates, variability in SULTs would play a more significant role on acetaminophen metabolism and clearance as a result of non-existing or underdeveloped UGTs. However, the effects of sequence variation (SV) on neonatal UGT, SULT, and CYP activity have been largely unexplored. Pharmacogenetic factors leading to a reduction in UGT activity could present an additional concern in cases of SULT pathway saturation, as a compromised UGT pathway may lead to an even greater amount of acetaminophen metabolism being shunted towards the toxic oxidative metabolism pathway.

To address this knowledge gap, this investigation examined the role of genetic variability on the relevant metabolic pathways to determine which variants contribute to the variability observed in the pharmacokinetic profile of acetaminophen metabolites in neonates. The polymorphisms investigated in this study were chosen due to either previous literature demonstrating importance of the polymorphism in acetaminophen metabolism in adults [4, 7, 11], or due to the importance of the enzyme itself in neonatal acetaminophen metabolism combined with known (common) polymorphisms in that enzyme.

2 Methods

2.1 Study Design

This was a continuation of a prospective, single-center, open-label pharmacokinetic and pharmacogenetic study ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01328808) identifier [NCT01328808](https://clinicaltrials.gov/ct2/show/study/NCT01328808)) approved by the Institutional Review Board at the Children's National Health System (Washington, DC, USA). The study was conducted in accordance with good clinical practice. Written informed consent was obtained from a parent or legal guardian prior to study inclusion.

The original study, described by Cook et al. [13] was a prospective, single-center, open-label study. Neonates were recruited from intensive care units at the Children's National Health System. Inclusion criteria included patient postnatal age (PNA) <28 days, presence of an indwelling arterial line, and clinical indication for IV analgesia (surgical or non-surgical). Patients were excluded if there was evidence of hepatic or renal failure, severe asphyxia, grade III/IV intracranial hemorrhage, major congenital malformations, neurological disorders, or use of neuromuscular blockers. Patients were stratified by extreme preterm birth (<28 weeks' gestational age [GA]), preterm birth (28 to <37 weeks' GA), and term birth (≥37 weeks' GA). This a priori stratification was made on the basis of safety in order to prevent overdosing in younger (and lighter) neonates. In particular, the categories were based on existing pharmacokinetic data in neonates [14–17].

Extreme preterm patients received 30-min IV infusions of acetaminophen 15 mg/kg (Ofirmev® 10 mg/mL; Mallinckrodt Pharmaceuticals, Dublin, Ireland) every 12 h over a 48-h period (five doses) while preterm and term patients received the same treatment every 8 h (seven doses). Within each group, patients were further randomized to one of two plasma-

sampling schemes, each consisting of nine or ten sampling times. Urine samples were additionally collected via indwelling catheter or from gelfree study diapers (Cuddle Buns Preemie diapers, Small Beginnings Inc., Hesperia, CA, USA). A detailed scheme describing dosing and sampling in each age group is provided in Fig. 2.

2.2 Pharmacogenetic Analyses

Genomic DNA (gDNA) was extracted from whole blood with the QIAamp[®] DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. DNA quality was assessed by agarose gel electrophoresis and quantity was determined by spectrophotometry using a NanoDrop[™] 1000 instrument (ThermoFisher, Waltham, MA, USA) and diluted to 10 ng/μL for subsequent analyses.

Seven SVs were genotyped using commercially available TaqMan[™] genotyping assays. TaqMan[™] assays were performed on a QuantStudio[™] 12 K Flex instrument following manufacturer's instructions and results analyzed with the ABI QuantStudio[™] 12 K Flex software version 1.2.2 (ThermoFisher). The identity of each SV along with the frequency of variant genotypes is shown in Table 1. The Hardy–Weinberg equilibrium for each variant was tested using a Chi square (χ^2) test. The sampling distribution of the test statistic under the null hypothesis is approximately a χ^2 distribution with 1 degree of freedom (*df*). A *p* value <0.05 indicated that the genotype distribution is not of consistent with Hardy–Weinberg equilibrium.

*SULT1A1**2(rs9282861) was genotyped using a restriction fragment length polymorphism (RFLP) assay. Briefly, gDNA was amplified with primers shown in Table 2 and Red Jumpstart[™] REDTaq[®] polymerase (Sigma-Aldrich, Saint Louis, MO, USA). Polymerase chain reaction (PCR) conditions were as following: initial denaturation at 94 °C for 2 min, 42 cycles of denaturation at 94 °C for 10 s, annealing at 68 °C for 10 s, and extension at 72 °C for 20 s. The resulting 332 bp PCR product was subjected to the *Hae*II restriction enzyme (New England Biolabs, Ipswich, MA, USA) and products separated on a 4% agarose gel. PCR fragments carrying the *SULT1A1**2 SV remained uncut (332 bp), while fragments with the reference nucleotide were cut once, giving rise to 168 and 164 bp digestion products.

Ten SVs were identified by Sanger sequencing. Briefly, sequencing templates for the *UGT1A9* and *SULT1A1* upstream gene regions were generated using the primers specified in Table 2 with KAPA2G Fast HotStart Taq polymerase (KAPA Biosystems, Wilmington, MA, USA). PCR conditions were as follows: initial denaturation at 95 °C for 3 min, 42 cycles of denaturation at 95 °C for 15 s, annealing at 66 °C for 10 s, extension at 72 °C for 30 s, and final extension at 72 °C for 5 min. Amplified PCR products were analyzed by agarose gel and treated with Exo-SAP-IT[™] (ThermoFisher). Sequencing was carried out with BigDye Terminator version 3.1 chemistry (ThermoFisher). Primers used for sequencing are listed in Table 2. Sequence traces were aligned and analyzed using Sequencher software (Gene Codes Corp, Ann Arbor, MI, USA). ENSG00000242366 (*UGT1A*) and ENSG00000196502 (*SULT1A1*) served as reference sequences. Wild-type and variant designations were confirmed by Single Nucleotide Polymorphism Database (dbSNP) entries.

The number of (TA) repeats in the *UGT1A1* promoter region were determined characterizing the fragment lengths of PCR amplicons. gDNA was amplified under the following conditions: initial denaturation at 95 °C for 2 min, followed by ten cycles at 94 °C for 15 s, 55 °C for 15 s, 72 °C for 30 s; 20 cycles at 89 °C for 15 s, 55 °C for 15 s, 72 °C for 30 s; and final extension at 72 °C for 10 min. PCR product was diluted 20-fold and 1 µL was mixed with Hi-Di™ Formamide and the GeneScan™ 600 LIZ™ Size Standard (ThermoFisher). Subsequently, samples were incubated at 95 °C for 5 min, placed on ice, and analyzed on an ABI 3730x/DNA Analyzer (ThermoFisher). Fragments lengths were determined with the GeneMapper™ software version 4.0 (ThermoFisher). Fragment lengths were as following: (TA)6 (wild-type, 131 bp), (TA)5 (*36, 129 bp), (TA)7 (*28, 133 bp), and (TA)8 (*37, 135 bp). Primer sequences are provided in Table 2.

SULT1A1 copy number variation (CNV) was determined as previously described in detail by Gaedigk et al. [18] Essentially, *SULT1A1* and *SULT1A2* were amplified with a single primer pair (Table 2) and amplicons analyzed on an ABI 3730x/DNA analyzer and GeneMapper™ software. The two genes give rise to fragments of 215 and 211 bp, respectively. The *SULT1A1* peak height was normalized to that of *SULT1A2*, which is not subjected to CNV. Equal peak heights indicate two copies.

To determine the number of repeat elements in the *CYP2E1* upstream region [19], gDNA was amplified as follows: initial denaturation at 94 °C for 2 min, 45 cycles of denaturation at 94 °C for 15 s, annealing at 59 °C for 30 s, and extension at 72 °C for 1 min 15 s. The resulting PCR products were separated on a 2% agarose gel. The *CYP2E1**1C allele is most common and harbors six repeats while *CYP2E1**1A and *CYP2E1**1D feature five and eight repeats, respectively. Corresponding PCR products are 1120 bp (*1A), 1168 bp (*1C), and 1264 bp (*1D) in length.

2.3 Initial Pharmacokinetic Model

The present investigation built upon a population pharmacokinetic model developed by Cook et al. [13]. The original population pharmacokinetic model was developed based on data obtained from 35 neonates. The structure of the model (Fig. 1) involves single compartments for acetaminophen and its metabolites in plasma and urine, for a total of eight compartments. Estimated pharmacokinetic parameters and covariates included in the model are available in Table 4. Briefly, formation clearances for each metabolite as well as volumes of distribution and renal clearances for parent drug and metabolites were estimated. Weight was included as a covariate for each of these parameters, while PNA was included on APAP-Gluc formation clearance (CL_{Gluc}) and Ox APAP formation clearance (CL_{Ox}), and drug indication (surgical or non-surgical) was included on acetaminophen renal clearance. As an example of how these covariates are represented in the model, for individuals receiving acetaminophen for post-surgical pain, the acetaminophen renal clearance was approximated 57% lower than for those receiving acetaminophen for non-surgical sources of pain. The benefit of each of these covariates in the model is that they each help explain some of the variability associated with acetaminophen disposition in this population. All concentrations were expressed in acetaminophen equivalents (mg/L) via conversion based on molecular weights. The purpose of presenting the concentrations this way was to provide a mass-

balance between the original mass of drug administered (the dose) and the metabolite masses. Since it is difficult to quantitate the concentrations of the toxic oxidative precursor molecule, NAPQI, the concentration of Ox APAP (APAP-cysteine and APAP-*N*-acetylcysteine) could be summed to approximate the total concentration of metabolites derived from CYP-mediated oxidation.

2.4 Pharmacogenetic Marker Evaluation

Each SV was tested for statistical significance in the pharmacokinetic model with a stepwise covariate approach using NONMEM® (version 7.3; Icon Development Solutions, Ellicott City, MD, USA) interfaced with Perl-speaks- NONMEM (PsN) and Pirana® (version 2.9.2). In particular, the *UGT* SVs were checked for their effect on CL_{Gluc} , the *SULT* SVs were checked for their effect on CL_{Sulf} , and the *CYP2E1* SV was examined for its effect on CL_{Ox} . SVs were treated as categorical covariates, and separate clearance values were estimated for homozygous wild-type (Wt/ Wt), heterozygous (Wt/Var), and homozygous variant (Var/Var) genotypes. A proportional shift model (Eq. 1) was used to include SVs on their respective clearance estimates:

$$CL = TVCL \times (1 + \theta_{SV}) \quad (1)$$

where CL is the final metabolic clearance estimate, TVCL is the typical value for the clearance estimate that includes other covariates, including weight and PNA, and θ_{SV} is the estimate of the effect of a given SV. The θ_{SV} value was set to 0 for Wt/Wt while the value was estimated for Wt/Var and Var/Var individuals.

Differences in objective function value (OFV), a model fit parameter, were used to determine statistical significance of SV covariate parameters in nested models. Specifically, upon forward addition of SV parameters, a *p* value <0.05 (corresponding OFV>3.84 for *df* = 1, or OFV>5.99 for *df* = 2, likelihood ratio test) was considered significant for improvement in model fit. Once all significant SV parameters were included via forward addition, they were tested for elimination in the backwards elimination step with *p* value <0.01 (OFV>6.63 for *df*= 1, or OFV>9.21 for *df*= 2) required to retain parameters in the model. Parameters were also removed if the directionality of clearance values was not biologically plausible (e.g., clearance was highest in heterozygous individuals while being lower in both homozygous wildtype and homozygous variant individuals). Additionally included in the backwards elimination step were the PNA and treatment indication (SURG, binary covariate indicating surgical or non-surgical intervention), in order to determine if those covariates were still significant in the presence of any included SV covariates.

2.5 Model Evaluation

The updated model was evaluated through visual examination of goodness-of-fit plots, visual (VPC; *n* = 1000 simulations) and numerical predictive check (NPC; *n* = 1000 simulations), bootstrap (*n* = 500 simulations), and leave-one-out cross validation (LOOCV), examining both median absolute prediction error and non-parametric distribution error [20].

3 Results

3.1 Demographics

SV data were available for 33 neonates. Demographic information is summarized in Table 3. A total of 20 SVs were determined in the 33 neonates. Eighteen of these SVs had at least one individual with a variant allele. Of note, nearly half (49%) of the neonates in this cohort were born preterm, and ten of the 33 neonates (29%) were born extremely preterm (< 28 weeks GA). Acetaminophen was administered for surgical reasons about half the time (17/33).

3.2 Pharmacogenetic Evaluation

One SV (rs8175347) was removed from the analysis because the directionality of the effect on CL_{Gluc} was biologically implausible (e.g., effect was greatest for heterozygous individuals). Forward addition of the remaining 17 SVs determined that *UGT1A9* rs3832043, a length polymorphism of nine versus ten thymidine nucleotides ($T_9 > T_{10}$), was the most informative SV to the model fit. None of the other SVs were significant in the forward addition step after inclusion of rs3832043. In the backwards elimination step, rs3832043 was still significant at the $p < 0.01$ level. Additionally, PNA and SURG covariates were also significant during backwards elimination and therefore were left in the final model. In addition to improving model fit, inclusion of rs3832043 reduced the between-subject variability in CL_{Gluc} by 7% coefficient of variation.

Inclusion of the $T_9 > T_{10}$ genotype on CL_{Gluc} did not change estimates for other parameters (all other estimates changed <1% from base model parameter estimates). Finally, individuals heterozygous for the $T_9 > T_{10}$ variant ($n = 19$) had a 42% reduction in CL_{Gluc} compared to the wild-type, and individuals homozygous for the variant ($n = 5$) also had a 42% reduction in CL_{Gluc} compared to the wild-type (< 1% reduction in CL_{Gluc} relative to heterozygotes) (Table 4). These findings indicate that the T_{10} genotype is associated with decreased acetaminophen glucuronidation.

Diagnostic plots demonstrated good model fit with no evident bias, as did a VPC (Fig. 3). An NPC indicated that 7.24% of plasma observations were outside the model 90% prediction interval, consistent with the expected 10%. Bootstrap results (relevant parameters in Table 4) suggest that the model is stable and consistent. Bootstrap simulations were limited to 500 due to computational requirements and limitations. Median absolute prediction error for the LOOCV cohort model compared to the original cohort model was 25.2%. Additionally, the median normalized prediction distribution error (NPDE) for the LOOCV cohort model was -0.424 compared to -0.828 for the original model cohort. Similarity of these results support the bootstrap results that the model is stable.

4 Discussion

The model described herein demonstrates the influence of the $T_9 > T_{10}$ *UGT1A9* promoter SV (rs3832043) on the metabolic formation clearance of acetaminophen to APAP-Gluc. Specifically, our results suggest that individuals with the T_{10} allele (T_9/T_{10} or T_{10}/T_{10}) have a 42% reduced metabolic clearance to APAP-Gluc compared to those without the SV, i.e.,

T₉/T₉ genotypes. Previous literature has described inconsistent results with regards to the influence of the T-insertion (T₁₀) allele. One in vitro study using DNA extracted from peripheral lymphocytes of 87 Japanese, 50 Caucasian, and 50 African American individuals suggested that the T-insertion increased luciferase activity, indicating higher levels of gene expression [21]. However, another study examining DNA samples and liver microsomal fractions from 42 Caucasian, four African American, and two Hispanic individuals did not find an association between hepatic UGT1A9 protein content and the T₉>T₁₀ polymorphism, but rather attributed differences in expression levels and mycophenolic acid glucuronidation to variants at – 275 and – 2152 [22]. Court et al. [23] also used liver microsomes from 48 donors (non-Hispanic white, 37 male and 11 female) to demonstrate that three SVs in *UGT1A1* (rs8330, rs1042640, rs10929303, all in linkage disequilibrium) were statistically associated with acetaminophen glucuronidation at concentrations of 0.1–40 mM, while rs45625337 (another designation for rs3832043) was not, though there was a statistical trend for the effect of rs45625337 on glucuronidation ($p = 0.056$) at acetaminophen concentrations of 2.0–40 mM, suggesting potential activity. Of note, the three *UGT1A1* SVs from Court et al.'s study (rs8330, rs1042640, rs10929303) did demonstrate a non-significant effect in our model, and additional patients, particularly those homozygous for the SVs, may improve the ability of the model to resolve significant differences in acetaminophen metabolism stemming from those variations.

Biologically, it is not fully understood which transcription factors can impact UGT1A (and specifically *UGT1A9*) regulation, and to what extent. In this particular case, the TA insertion occurs within the TATA box, which is a crucial core promoter for about 25% of human genes [24]. Much of the available literature specifically examines promoter regulation with regards to UGT1A1 where, for example, a common TA insertion promoter polymorphism (*UGT1A1* *28) significantly decreases *UGT1A1* gene transcription [25]. In particular, it is believed that numerous transcription factors contribute to a complex mechanism of regulation that have not been fully elucidated, though DNA methylation may play a role in UGT1A promoter regulation [26, 27]. One study did suggest that hepatocyte nuclear factors 1 and 4 alpha were involved in *UGT1A9* mRNA expression [28], but specific factors have not yet been identified with regards to TATA box regulation [27].

Interestingly, a pair of studies performed by Miyagi et al. [29, 30] in human liver microsomes showed minimal expression and activity of UGT1A1 and UGT1A9 at birth, with rapid increase after birth until reaching near adult levels at 3.8 months of age, suggesting there may be some interplay between development and the functional consequences of the SVs investigated. An additional report that examined DNA, RNA, and liver microsomes from Caucasian individuals also suggested that the T₁₀ allele does not affect gene expression or activity, although this study did not specifically consider acetaminophen metabolism [31]. In contrast, several other studies have demonstrated an effect of the T-insertion on the metabolism of irinotecan and mycophenolate [32–34]. Two of the studies performed in adults with non-small cell lung cancer demonstrated a decrease in irinotecan exposure related to the T₁₀ allele, which translates to an apparent increase in metabolic formation related to that allele compared to the T₉ allele [32, 34]. Conversely, Zhang et al. [33] suggested that the presence of at least one T₁₀ allele increased the dose-normalized partial area under the concentration–time curve from 6 to 12 h (AUC_{6–12}) of

mycophenolic acid in 98 Chinese renal transplant recipients, though it did not affect the apparent clearance of the same drug. Lastly, one study described that adult subjects with metastatic colorectal adenocarcinoma and at least one T_{10} allele had higher toxicity and a decreased response when treated with capecitabine plus irinotecan, which would be consistent with higher UGT activity than in individuals with the T_9/T_9 genotype [35]. Nonetheless, it remains unclear whether $T_9 > T_{10}$ is the causal variation, or if these differences in drug exposures and responses are due to other SVs in *UGT1A9* and/or other *UGT* gene(s) that are in linkage disequilibrium with *UGT* $T_9 > T_{10}$ haplotypes. Of note, the T_{10} allele was originally termed *UGT1A9**22 [21] but is currently listed as part of the numerous *UGT1A9**1 subvariants by the *UGT* Alleles Nomenclature webpage [36]. Aside from the impact on CL_{Gluc} , there were no other apparent differences in acetaminophen or acetaminophen metabolite pharmacokinetics related to rs3832043. Because there was virtually no difference in CL_{Gluc} among individuals with T_9/T_{10} and T_{10}/T_{10} genotypes, future functional models in this population could simply stratify individuals into two groups, i.e., those with one or two T_{10} alleles and those who do not carry the variant allele.

The allele frequency of the T_{10} allele is relatively high at around 40% in most ethnic groups, but is notably higher (approximately 60%) in individuals of Japanese descent [37]. These frequencies are in line with the allele frequency of 44% seen in the present study. Interestingly, a recent physiologically based pharmacokinetic (PBPK) model built on data compiled from 24 studies ($n = 350$ adult subjects) compared acetaminophen disposition in Western Europeans ($n = 271$) and East Asians ($n = 79$) and demonstrated that differences in glucuronidation capacity may be a driving factor contributing to variation in acetaminophen pharmacokinetics between the two groups [38]. Nonetheless, given the ontogeny of UGTs and the relative importance of the sulfation pathway in neonates mentioned previously, it is difficult to know if this trend holds true for newborns.

A major limitation of this study is that, while a difference was found in the formation clearance of acetaminophen to APAP-Gluc, it is not clear what mechanisms, if any, compensate for the reduced metabolic clearance of the individuals with T_{10} alleles. It is possible that no compensatory changes were noted in relation to the SV of interest because glucuronidation is a minor metabolism pathway compared to sulfation in neonates, and therefore changes in glucuronidation don't significantly perturb the overall system. Another limitation to this study was a somewhat small sample size of only 33 neonatal patients.

Further elucidation of the metabolic profile of acetaminophen in neonates may require additional patients. Additionally, a validation cohort would help support these findings.

5 Conclusions

Overall, incorporation of the *UGT1A9* $T_9 > T_{10}$ SV significantly improved the model fit and reduced the between subject variability in the model. However, because a relatively small amount of acetaminophen clearance occurs via the glucuronidation pathway, it is unlikely to be of clinical significance as it accounts for such a small fraction of overall parent drug clearance. Nonetheless, the inclusion of this SV in the model provides a better description

for the observed data, and highlights the potential importance of further pharmacogenetic and pharmacogenomic studies in the neonatal population.

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Key Points

Pharmacogenetic variability in enzymes that metabolize acetaminophen are known to affect acetaminophen disposition in adults. However, ontogeny is also a factor in acetaminophen metabolizing enzymes, and the interplay between these two factors has not been well-elucidated.

Pharmacogenetic variability in UDP-glucuronosyltransferase 1A9 (*UGT1A9*; specifically rs3832043), which was previously thought to be of little consequence in neonates, may play a role in differential acetaminophen metabolism.

Study results suggest additional attention should be paid to pharmacogenetic variability in acetaminophen metabolism in neonates moving forward.

Fig. 1.

Model structure denoting compartments included in the model and pathways where studied metabolizing enzymes are relevant. *APAP* acetaminophen, *APAP-Gluc* acetaminophen glucuronide metabolite, *APAP-Sulf* acetaminophen sulfate metabolite, *CYP* cytochrome P450, *Ox APAP* acetaminophen oxidative pathway metabolite, *SULT* sulfotransferase, *UGT* UDP-glucuronosyltransferase

Fig. 2.

Dosing and sampling scheme for patients born preterm or term (<28 weeks' gestation; top) and those born extremely preterm (≥ 28 weeks gestation; bottom). Timelines are represented in hours. Within each group, study patients were randomized to one of two sampling schedules as denoted by the top and bottom set of dots for each group. Gray boxes below the lines represent urine sampling collection times and black arrows show dosing events (15 mg/kg, 30-min infusion)

Fig. 3.

Visual predictive check for plasma **a** acetaminophen, **b** acetaminophen-glucuronide, **c** acetaminophen-sulfate, and **d** oxidative pathway metabolite concentrations over time. Bold black lines represent the median observed concentrations, and the 5th and 95th percentiles of the observed concentrations are denoted by the lower and upper thin black lines, respectively. Shaded regions represent the 95% confidence intervals of the 5th, median, and 95th prediction percentiles. *APAP* acetaminophen

Table 1

Summary of sequence variations interrogated

Gene	Variant	rs number	Assay type	TaqMan™ assay ID	Number of individuals with the given genotype		
					Wt/Wt	Wt/Var	Var/Var
<i>UGT1A9</i>	*1	rs17868320	TaqMan™	C_34418857_10	32	1	0
<i>UGT1A9</i>	*1c	rs2741045	Sequencing		18	13	2
<i>UGT1A9</i>	*1c	rs2741046	Sequencing		18	13	2
<i>UGT1A9</i>	*1	rs6714486	TaqMan™	C_27843087_10	28	5	0
<i>UGT1A9</i>		rs3832043	Sequencing		9	19	5
<i>UGT1A9</i>	*1e	rs17868322	Sequencing		32	1	0
<i>UGT1A9</i>		rs377206386	Sequencing		32	1	0
<i>UGT1A9</i>	*3a	rs72551330	Sequencing		33	0	0
<i>UGT1A9</i>		N/A (novel SNP)	Sequencing		32	1	0
<i>UGT1A6</i>	*5	rs2070959	TaqMan™	C_15868110_30	15	15	3
<i>UGT1A1</i>	*28, *36	rs8175347	FLP		9	15	9
<i>UGT1A1</i>	*6	rs4148323	TaqMan™	C_559715_20	33	0	0
<i>UGT1A1</i>	*76	rs10929303	TaqMan™	C_25971205_10	19	13	1
<i>UGT1A1</i>	*78	rs1042640	TaqMan™	C_7607428_10	25	8	0
<i>UGT1A1</i>	*79	rs8330	TaqMan™	C_7607429_10	19	12	2
<i>SULT1A1</i>	*2	rs9282861	RFLP		18	11	4
<i>SULT1A1</i>		rs3760091	Sequencing		12	19	2
<i>SULT1A1</i>		rs750155	Sequencing		13	12	8
<i>SULT1A1</i>		rs35837227	Sequencing		31	2	0
<i>SULT1A1</i>		N/A	CNV		2:27		3:6
<i>CYP2E1</i>	*1C, D	N/A	FLP		30	3	0

The rs numbers are provided if available. Assay type indicates the method by which SVs were tested. Variant indicates in which allele a SV is found. *UGT* and *SULT* variants are according to the *UGT* Alleles Nomenclature webpage [36] and the *SULT* nomenclature proposed by Blanchard et al. [39]. All genotypes were in Hardy–Weinberg equilibrium

CNV copy number variation, *FLP* fragment length polymorphism, *N/A* not applicable, *RFLP* restriction fragment length polymorphism, *SULT* sulfotransferase, *SV* sequence variation, *UGT* UDP-glucuronosyltransferase, *Var* variant allele, *Wt* wild-type allele

Table 2
Summary of polymerase chain reaction primers used for amplification and sequencing

PCR primer	Sequence 5' to 3' ^d	Assay	PCR fragment length
UGT1A9-1 (F)	5'-GGGTTGTGTCAGTCTCATTTTCAGC	Sequencing template (variants see ESM Table S1)	
UGT1A9-2 (R)	5'-CACCGACCTCATGGTGAAC		
UGT1A9-3 (F)	5'-GGGTTGTGTCAGTCTCATTTTCAGC	Sequencing primers for <i>UGT1A9</i>	
UGT1A9-4 (R)	5'-TCCAGATCCTCCAGGGTATATG		
UGT1A1-5 (F)	5'-[6FAM]TCCCCTGCTACCTTTGTGGAC	<i>UGT1A1</i> (TA) repeat	(TA)6 = 131 bp (*1)
UGT1A1-6 (R)	5'-TGCTCTGCCAGAGGTTTC		(TA)5 = 129 bp (*36)
			(TA)7 = 133 bp (*28)
			(TA)8 = 135 bp (*37)
SULT1A1-1 (F)	5'-CCTCATCATCCTGCCTGGTTAATG	Sequencing template (variants see ESM Table S1)	322 bp
SULT1A1-2 (R)	5'-GAAAGCACCCCTCGTCGGGCAA		
SULT1A1-3 (F)	5'-CTTGGCTCTGCAGGTTTCTAGGA	<i>SULT*2</i> RFLP	
SULT1A1-4 (R)	5'-CCCAACCCCTGCTGGCCAGCACCC		
SULT1A1-5 (R)	5'-TCCAGGAGAGTCCAGCTGCA	Sequencing primer for <i>SULT1A1</i>	<i>SULT1A2</i> = 211 bp
SULT1A1-6 (F)	5'-[6FAM]TCACCGAGCTCCCATCTT		<i>SULT1A1</i> = 215 bp
SULT1A1-7 (R)	5'-GTTTGGGCAGGTTGTCTTCAG	Variable number of repeats	*1A = 1120 bp
CYP2E1-1 (F)	5'-CCCAGTCCACAGAGAAGACAGG		*1C = 1168 bp
CYP2E1-2 (R)	5'-GGGTGAGAACAGGAAGCATC		*1D = 1264 bp

CNV copy number variation, *ESM* Electronic Supplementary Material, *PCR* polymerase chain reaction, *RFLP* restriction fragment length polymorphism, *SULT* sulfotransferase, *UGT* UDP-glucuronosyltransferase

^d 6FAM denotes that the primer was labeled with fluorescent dye 6-carboxyfluorescein

Table 3

Demographic information of patients included in analysis

Characteristic	Value (<i>n</i> = 33)
Current body weight (kg) [median (range)]	2.70 (0.46–4.20)
Postnatal age (days) [median (range)]	6 (1–26)
Gestational status [<i>n</i> (%)]	
Extreme preterm (<28 weeks' GA)	10 (30)
Preterm (28 to < 37 weeks' GA)	6 (18)
Term (≥ 37 weeks' GA)	17 (52)
Primary indication for intravenous paracetamol [<i>n</i> (%)]	
Postoperative analgesia	17 (52)
Non-surgical (medical) conditions	16 (48)
Sex [<i>n</i> (%)]	
Male	19 (58)
Female	14 (42)
Race [<i>n</i> (%)]	
Caucasian	14 (42)
African American	14 (42)
American Indian/Alaska Native	1 (3)
Asian	1 (3)
Declined to respond	3 (9)
Ethnicity [<i>n</i> (%)]	
Non-Hispanic	22 (67)
Hispanic	8 (24)
Declined to respond	3 (9)

GA gestational age

Table 4

Model parameter values and bootstrap results

Parameter	Model fit		Bootstrap results ($n = 500$) ^b			
	Estimate ^a	BSV (%CV)	Median ^a	95% CI	BSV (%CV)	95% CI
CL _{GluC} (L/h)	0.0688	61.6	0.0684	0.0477–0.101	58.1	42.3–80.0
Effect of weight ^c	1.91		1.93	1.64–2.34		
Effect of PNA ^c	0.333		0.361	0.00935–0.743		
Effect of T ₉ T ₉ ^d	0		N/A	N/A		
Effect of T ₉ T ₁₀ ^d	– 0.417		– 0.421	– 0.617 to – 0.0751		
Effect of T ₁₀ T ₁₀ ^d	– 0.420		– 0.414	– 0.641 to 0.0828		
CL _{Sulf} (L/h)	0.207	34.4	0.204	0.179–0.234	33.6	26.2–42.2
Effect of weight ^c	0.896		0.899	0.701–1.07		
CL _{Ox} (L/h)	0.0579	82.6	0.0578	0.0414–0.0791	78.4	62.0–99.3
Effect of weight ^c	1.74		1.76	1.43–2.10		
Effect of PNA ^c	0.689		0.652	0.208–0.991		
V _{APAP} (L)	2.74	11.2	2.74	2.53–3.01	12.5	7.24–20.9
Effect of weight ^c	0.901		0.899	0.810–1.03		
V _{GluC} (L)	1.15	50.7	1.17	0.904–1.41	48.0	30.3–72.0
Effect of weight ^c	1.07		1.06	0.779–1.38		
V _{Sulf} (L)	0.986	36.3	0.977	0.787–1.24	37.7	23.6–51.6
Effect of weight ^c	1.18		1.16	0.928–1.45		
V _{Ox} (L)	2.98	75.9	3.01	2.14–4.39	73.5	32.5–127
Effect of weight ^c	1.54		1.53	1.28–1.87		
CL _{APAPrenal} (L/h)	0.0159	31.8	0.0160	0.0114–0.0215	30.2	16.8–45.5
Effect of weight ^c	0.633		0.638	0.370–0.991		

Parameter	Model fit		Bootstrap results ($n = 500$) ^b		
	Estimate ^a	BSV (%CV)	Median ^a	95% CI	
				BSV (%CV)	95% CI
Effect of urine flow rate ^e	0.0603		0.0604	0.0458–0.0767	
Effect of postoperative indication ^f	– 0.56		– 0.561	– 0.744 to – 0.306	
CL _{Gluc,renal} (L/h)	0.103	38.8	0.101	0.0842–0.123	38.1
Effect of weight ^c	1.06		1.05	0.847–1.27	24.3–50.3
Effect of urine flow rate ^e	0.0153		0.0152	0.00353–0.0307	
CL _{Sulf,renal} (L/h)	0.118	47.4	0.117	0.0908–0.149	46.6
Effect of weight ^c	1.38		1.38	1.09–1.70	29.5–62.2
Effect of urine flow rate ^e	0.015		0.0148	0.00379–0.0308	
CL _{Ox,renal} (L/h)	0.174	42.0	0.173	0.144–0.212	41.2
Effect of weight ^c	1.30		1.30	1.09–1.60	26.2–54.7
Effect of urine flow rate ^e	0.0323		0.0329	0.0172–0.0449	
<hr/>					
Model fit		Bootstrap median ^b			
RUV		RUV	95% CI		
<hr/>					
Proportional RUV (%CV)					
Plasma APAP	26.9	26.4	21.5–33.0		
Plasma APAP-Gluc	27.9	27.6	23.1–34.0		
Plasma APAP-Sulf	23.0	23.9	17.0–31.4		
Plasma Ox APAP	35.9	35.2	27.5–43.2		
Urinary APAP	52.1	51.9	44.7–59.7		
Urinary APAP-Gluc	77.0	77.6	66.7–94.1		
Urinary APAP-Sulf	79.3	79.0	67.8–94.0		
Urinary Ox APAP	67.3	67.3	57.9–81.7		
Additive RUV (SD, mg/L) ^g					
Plasma APAP-Sulf	1.1	1.0	0.066–2.1		
Urinary APAP	1.3	1.2	0.024–3.1		

Parameter	Model fit		Bootstrap results (<i>n</i> = 500) ^b		
	Estimate ^a	BSV (%CV)	Median ^a	95% CI	95% CI
Urinary APAP-Sulf	14		12	0.25–33	

APAP, acetaminophen, APAP-*Cluc* acetaminophen glucuronide metabolite, APAP-*Sulf* acetaminophen sulfate metabolite, *BSV* between-subject variability, *CI* confidence interval, *CLAPAP*, renal-*CL Gluc* renal *CL Sulf*; renal, and *CL OX*, renal represent renal clearances for unchanged paracetamol, paracetamol-glucuronide, paracetamol-sulfate, and the oxidative pathway metabolites, respectively, *CL Gluc*, *CL Sulf*, and *CL OX* represent hepatic formation clearances for paracetamol-glucuronide, paracetamol-sulfate, and the oxidative pathway metabolites, respectively, *CV* coefficient of variation, *OX* APAP-acetaminophen oxidative pathway metabolite, *PNA* postnatal age, *RUV* residual unexplained variability, *SD* standard deviation, *VAPAP*, *V Gluc*, *V Sulf*, and *V OX* represent volumes of distribution for paracetamol, paracetamol-glucuronide, paracetamol-sulfate, and the oxidative pathway metabolites, respectively

^a Pharmacokinetic parameter estimates are typical values for patients with a procedural indication at the mean current body weight (2.3 kg), mean PNA (7.5 days), and median urine flow rate (6.5 mL/h)

^b Bootstrap success rate was 73% (*n* = 367 of 500)

^c Exponent on mean-centered weight or mean-centered PNA (see Burchell [11] for additional details)

^d Proportional change in *CL Gluc* for patients with a T10 allele (see Eq. 1 for additional details). T9 and T10 are alleles for the wild-type and variant copies of *UGT1A9* rs3832043, respectively

^e Coefficient on median-centered urine flow rate in the exponential covariate function (see Burchell [11] for additional details)

^f Proportional change in *CLAPAP*, renal for patients with a postoperative indication (see Burchell [11] for additional details)

^g mg APAP equivalents/L