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Age- and genotype-dependent variability in the protein abundance and activity of six major uridine diphosphate-glucuronosyltransferases in human liver

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Abstract

The ontogeny of hepatic uridine diphosphate-glucuronosyltransferases (UGTs) was investigated by LC-MS/MS proteomics to determine UGT protein abundance in human liver microsomes isolated from 136 pediatric (0–18 years) and 35 adult (age 18–67 years) donors. Microsomal protein abundances of UGT1A1, UGT1A4, UGT1A6, UGT1A9, UGT2B7 and UGT2B15 increased by ~8, 55, 35, 33, 8 and 3-fold from neonates to adults, respectively. The estimated age at which 50% of the adult protein abundance is observed for UGT isoforms was between 2.6 to 10.3 years. Measured *in vitro* activity was generally consistent with the protein data. UGT1A1 protein abundance was associated with multiple genetic variants possessing noticeable ontogeny-genotype interplay. *UGT2B15* rs1902023 (*2) was associated with decreased protein activity without any change in protein abundance. Taken together, these data are invaluable to facilitate the prediction of drug disposition in children using physiologically based pharmacokinetic modeling as demonstrated for zidovudine and morphine.

Keywords

Glucuronidation; UGTs; ontogeny; LC-MS/MS proteomics; ADME; drug metabolism; pharmacogenomics

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CONFLICT OF INTEREST

The authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

D.K.B., A.M., A.G., R.C., A.B., H.Z., M.B., R.E.P., R.G., J.S.L., and B.P. wrote the manuscript; D.K.B., A.G., R.E.P., J.S.L., and B.P. designed the research; D.K.B., A.M., A.G., R.C., A.B., H.Z., P.C., M.B., R.G., and B.P. performed the research; D.K.B., A.M., A.G., R.C., A.B., P.C., U.B., J.S.L., and B.P. analyzed the data; A.G., R.E.P., R.G., and J.S.L. contributed new reagents/analytical tools.

INTRODUCTION

Uridine diphosphate-glucuronosyltransferases (UGTs) are involved in the clearance (CL) of 10% of the top 200 prescribed drugs and constitute an important detoxification mechanism for diverse, potentially toxic xenobiotics such as polycyclic aromatic hydrocarbons, steroid hormones, nitrosamines, heterocyclic amines, fungal toxins, and aromatic amines (1). Importantly, drugs administered to children such as acetaminophen (2), valproic acid (3), lamotrigine (4), zidovudine (5), morphine (6), oxazepam (7), lorazepam (7), and furosemide (8) are primarily metabolized by UGTs and have been shown to exhibit age-dependent hepatic CL and pharmacokinetics (PK). The plasma CL of morphine in neonates is significantly lower than in adults (4.7 ± 2.8 vs 25.7 ± 4.7 ml min⁻¹ kg⁻¹) with >5 fold lower metabolite (glucuronides)/parent ratios observed in neonates (6). Similarly, lorazepam CL in neonates and infants is markedly lower compared to older children and adults (9). Such immature glucuronidation activity can lead to toxicity of UGT substrate drugs in children if these drugs are prescribed at the body-weight or -surface area-normalized doses, as classically demonstrated in the case of chloramphenicol, which was associated with cardiovascular collapse, reported as Gray baby syndrome (10). Lower levels of UGT activities have also been associated with higher zidovudine plasma exposure in infants, which has been shown to contribute to increased hematologic toxicity (5). Taken together, when a drug candidate is a UGT substrate, first-in-children dosing should consider the ontogeny of individual UGT enzymes to optimize dosing regimens.

The currently available ontogeny data for UGT enzymes are derived from conventional mRNA quantification (11), protein expression by immunoquantification (12) and in vitro activity (13). These methodologies are often associated with limitations such as poor correlation between mRNA and protein, use of non-specific antibodies and non-selective probe substrates (14). Moreover, the potential confounding effect of genetic polymorphisms on interpretation of ontogeny data has not been well studied. Recently, UGT quantification in human liver microsomes was reported by multiple groups as summarized elsewhere (15). However, the majority of these data were focused on adults, and there is still a significant knowledge gap with respect to age-dependent protein abundance of UGTs in drug disposition.

To fill this knowledge gap, we quantified four members of the UGT1A family (1A1, 1A4, 1A6 and 1A9) and two members of the UGT2B family (2B7 and 2B15) using a robust and selective LC-MS/MS proteomics approach (15), in a panel of well characterized (Table S1) microsomal samples isolated from human liver samples representing age categories, neonatal (0 to 27 days), infancy (28 to 364 days), early childhood (1 to <6y), middle childhood (6 to <12y), adolescents (12 to <18y) and adulthood (>18y). Activity data for the six UGT isoforms were also generated in the present study using selective probe substrates (16) and compared with the protein abundance data. The effects of genotype and gender on protein abundance and activity was also investigated. These ontogeny data, along with our previously published data on transporters (17), were integrated into physiologically-based pharmacokinetic (PBPK) models to predict the pediatric disposition of zidovudine and morphine, cleared primarily by metabolism and metabolism/transport, respectively.

RESULTS

Ontogeny of UGT enzymes

The relative abundance of UGTs in adult liver was present in the following order: UGT1A4>UGT2B7>UGT2B15>UGT1A1>UGT1A9>UGT1A6 (Table S2, Figs. 1 and 2). Neonatal abundances of UGT1A1, UGT1A4, UGT1A6, UGT1A9, UGT2B7 and UGT2B15 were 12.2, 1.8, 2.9, 3.0, 13.0 and 38.6% of adult levels, respectively, whereas infant abundances (% of adult abundance) of these enzymes were 43, 16, 15, 24, 41 and 60, respectively (Table S2, Fig. 1). Using an allosteric ontogeny equation (18), the age at which 50% abundance (Age_{50}) is achieved for UGT1A1, UGT1A4, UGT1A6, UGT1A9, and UGT2B7 was calculated to be at 7.5, 3.6, 10.3, 8.2 and 2.8 years of age, respectively (Table 1 and Fig. S1). Due to the high variability in neonatal and infant data, ontogeny parameters (F_{birth} and Age_{50}) could not be accurately estimated in case of UGT2B15. On a relative scale, UGT1A1 is the most abundant of the UGT1A (~50% of 4 measured UGTAs) in neonates whereas UGT1A4 contributes ~50% of all the UGT1As in adults (Fig. 2). UGT1A4 protein abundance was most variable (~223-fold) (Table S2, Fig. S1). Reasonably good correlations were observed between protein abundances and activities for all the investigated UGTs (Fig. S2, Fig. 3). Consistent with the abundance data, the glucuronidation activity of UGTs in neonatal samples was 13% that found in adult samples (Table S2, Fig. 3). In addition, several significant protein-protein correlations ($p<0.0001$) were observed between the abundance levels of multiple UGT isoforms, as shown in Fig. S3. Likely, the UGT ontogeny data are not confounded by gender and ethnicity because these factors are not associated with changes in the UGT abundance (Fig. 1) and because most of the liver donors are Caucasians (Table S3).

Effect of genetic variation on protein abundance and activity

Significantly lower abundance of UGT1A1 was observed in human liver microsomes from individuals with variant alleles carrying rs10929302 (*93; c.3156G>A), rs4124874 (*60; c.3279T>G), rs111741722 (c.2950A>G), rs8175347 (*28; c.TATA-box) and rs887829 (*80; c.364C>T) compared to those homozygous for the *UGT1A1**1 reference allele (Fig. 4; Table S4). UGT1A1 abundance was significantly associated with age in samples carrying the reference allele *UGT1A1**1/*1. Interestingly, samples carrying both homozygous and heterozygous variants for the rs10929302, rs4124874, rs111741722, rs887829 or rs8175347 showed little association of UGT1A1 abundance with age (Fig. S4).

An allelic variant of UGT2B15, rs1902023 of UGT2B15 (D85Y; c.253G>T), was associated with lower rates of oxazepam glucuronidation, but it was not significantly associated with protein abundance. Neither protein abundances nor activities of UGT1A4, UGT1A6, UGT1A9 and UGT2B7 were significantly associated with the high frequency SNPs in the respective genes (Table S4).

Zidovudine and morphine pharmacokinetic prediction in neonates and children

Because majority of age-related changes in the UGT abundance were observed during first year of life (i.e., neonates and infants), our primary objective in this study was to demonstrate the utility of UGT proteomics data to predict PK in this special population

using zidovudine and morphine as model examples. The UGT2B7 ontogeny data were integrated into the existing pediatric module of Simcyp software to predict PK of zidovudine and morphine in neonates and children. First, the adult PBPK model was constructed and validated against the observed PK following single 200 mg oral dose of zidovudine and 3.55 mg intravenous (IV) dose of morphine free base (Figs. 5 and 6). Predicted mean plasma concentrations reasonably matched the observed mean values (19–21) (Figs. 5 and 6). Inclusion of the ontogeny data significantly improved the plasma concentration-time profile of zidovudine in neonates following a 2 mg/kg IV infusion with the predicted AUC which was within 2-fold of the observed data (22). Similarly, inclusion of UGT2B7 ontogeny data significantly improved the population PK prediction of zidovudine in children when compared to the observed data (23) (Fig. 5). Morphine PK prediction in newborns (age 0 day) was significantly improved by integrating UGT2B7 abundance at birth into the model where majority of the observed data points were between predicted 95th and 5th confidence intervals (Fig. 6) (24). Further, the mean and range (minimum and maximum) predicted CL for zidovudine and morphine in each category were reasonably predicted using the UGT2B7 abundance data (Fig S5). However, PBPK models developed with and without considering ontogeny equation did not show any significant difference in morphine PK prediction in children (age 1–18 years) (25) likely because minimal changes in the UGT2B7 abundance are observed during this age range, as shown in Figs 1 and S1.

DISCUSSION

Maturation of expression and activity of drug metabolizing enzyme and transporter expression occurs throughout prenatal, neonatal to childhood periods thereby affecting functional drug metabolism and transport capabilities. A thorough characterization of the ontogeny of drug metabolizing enzymes and transporters is essential for predicting optimum dosing regimens for effective and safe drug therapy, and for evaluating toxicity risk in children. While previous studies have reported comprehensive ontogeny data utilizing absolute protein quantitation of CYP450 enzymes (26), data are limited or non-existent for the ontogeny of non-CYP450 enzymes and transporters. To fill this knowledge gap, we have recently conducted a series of studies to quantify the absolute ontogeny of hepatic drug transporters (17), carboxylesterases (27), flavin monooxygenase 3 (28) and aldehyde/alcohol dehydrogenases (18) at the protein level using validated LC-MS/MS proteomics methods in our well characterized pediatric and adult liver banks. Because LC-MS/MS proteomics can distinguish highly homologous proteins from one another, we report here for the first time the selective and absolute ontogeny of six clinically important UGTs. Protein abundance data are supplemented by activity data. Importantly, comprehensive genotype analyses of these samples allowed us to characterize the individual contributions of age and genetic variation on protein expression levels and/or activities of the studied UGT enzymes.

Overall, low rates of hepatic glucuronidation were observed in children, particularly in neonates and infants (age, <1 year), which was consistent with the literature (29–31). Most notably, the neonatal levels of UGT1A4, 1A6, 1A9 and 2B7 were 10-fold lower than the adult levels. This implies that neonates and infants may have very low intrinsic CL of drugs that are primarily metabolized by UGT1A4, UGT1A6, UGT1A9, and UGT2B7 in isoform-specific patterns. Our data are also consistent with published mRNA and activity data (11).

Strassberg et al. reported no detectable expression of UGT transcripts before birth in fetal liver (20 weeks gestation) and protein was only detected after 6 months of age (29). In another study, Onishi et al. did not detect bilirubin glucuronidation activity in fetal and neonatal samples (32), which explains greater susceptibility of jaundice in neonates. Similarly, in vitro acetaminophen glucuronidation activity is negligible in fetal liver, which gradually increases in neonates and matches adult levels only at 10 years of age (33). UGT abundance and activity data in children (age 1–12 years) and adolescents were lower than in adults, which are corroborated by literature data on rate of glucuronidation of drugs such as chloramphenicol, morphine, zidovudine, and trifluoperazine (10, 22–25, 31).

Lower abundances of UGT1A1 were significantly related with the presence of genetic variation (i.e. rs10929302, rs4124874, rs8175347, rs887829 and rs111741722), which is consistent with the literature (34). UGT2B15 rs1902023 was associated with decreased oxazepam activity but not with decreased protein abundance (Fig 4), perhaps due to altered substrate affinity (Km). Regardless, our data explains the observed reduced oxazepam CL in the tissue samples carrying *UGT2B15* rs1902023 (35).

Although little is known about the mechanisms of age-dependent UGT regulation, various genetic, transcription factors and epigenetic mechanisms have been shown to alter UGT expression (36–38). Liu et al. demonstrated that pregnane X receptor (PXR) and estrogen receptor (ESR1) are important factors for the transcriptional regulation of UGTs (37). Since these transcription factors can be activated by steroids, it has been suggested that hormonal regulation may be a critical factor that mediates the developmental expression of UGTs (37). The constitutive androstane receptor (CAR) and peroxisome proliferator-activated receptor alpha (PPAR α) have been associated with nutritional status and shown to influence the expression of UGT1A4 and UGT1A6 during development (39). This is corroborated by a differential effect of breast and formula milk on the induction of intestinal UGT1A1 and a reduction in total serum bilirubin levels in neonates (40). The latter is regulated by the IkappaB kinase/NF-kappaB (IKK/NF-kappaB) signaling pathway (41), which can be influenced by the redox state in intestinal epithelial cells during development due to environmental factors (41). We have also shown age-dependent increase in PXR or CAR previously, which correlates well with CYP3A4 expression (42). Other studies have revealed that the hepatocyte nuclear factor 1 homeobox (HNF1 α and HNF4 α) can also influence UGT expression (43). All the above-mentioned transcription factors, as well as retinoid X receptor alpha (RXRA), glucocorticoid receptor (GR), CCAAT/enhancer binding protein alpha and beta (CEBPA and CEBPB), exhibit dynamic expression patterns during hepatic development (44). Moreover, regulatory circuits between UGT substrates (bilirubin, lithocholic acid, hyodeoxycholic acid, eicosanoids and dietary polyphenols) and transcription factors (aryl hydrocarbon receptor (AhR), PXR, CAR, and PPAR α), is another plausible mechanism of ontogeny (45). Better understanding of transcriptional regulation of UGTs would also explain inter-correlation of UGTs observed in this study.

Distinct absolute age-dependent UGT abundance data (opposed to non-selective activity data) are essential to predict drug glucuronidation in children. For example, if a drug is predicted to be a substrate of specific UGT enzymes using a recombinant *in vitro* system, absolute ontogeny data along with genotype correlation data can be used to estimate the

individual contribution of each isoform to total glucuronidation of the substrate. With this respect, it is important to point out that scaling from recombinant enzyme data is challenging because of variable proportion of non-functional enzyme in recombinant UGTs as well as lack of specific model substrates for relative activity factor (RAF) approach. Despite this limitation, the ontogeny data presented here are useful in predicting metabolism of UGT substrates in children if the adult metabolism data are available. For instance, we have shown that the incorporation of the mean UGT2B7 ontogeny data significantly improved the performance of zidovudine and morphine neonatal and newborn (age, 0 day) PBPK models, respectively. However, few observed data points were out of 95th confidence interval, likely because of the high variability in UGT2B7 abundance in neonates, which perhaps was not captured by only four samples used in this study. Nevertheless, as shown in Fig 5S, the predicted CL data of zidovudine and morphine in each age group were also consistent with the reported observed data (references shown in Fig. S5). The morphine PBPK model, however, did not show any significant difference in PK prediction (and weight normalized CL) in children (age, 1–18 years) with or without ontogeny data, which agrees with a minimal change in UGT2B7 in this age range (Fig 1).

Our study has several limitations. For example, we could not accurately quantify UGT1A3, UGT2B4, and UGT2B10 in these samples because of the poor sensitivity of the selected surrogate peptides (Table S5) or the low levels of these isoforms. Similarly, UGT2B17 abundance was below LLOQ in >50% of the samples and needed a larger sample size for reliable data interpretation. A targeted UGT2B17 analysis was separately conducted on 455 samples, which is published recently (46). Further, because the major changes in UGT abundance are observed during the neonatal age, the availability of only four samples from this age-group is a critical limitation of our study. Finally, while we report here the first comprehensive UGT proteomics data in pediatric liver samples, the UGT proteomics data in adult samples are published by multiple groups as summarized in Table S6. Interestingly, our absolute UGT quantification data are up to two-fold lower than the reported data. While interindividual variability is one of the reasons for such variability, these disagreements could be because of the methodological differences. Although targeted proteomics results are precise, different laboratories use different in-house optimized protocols, which can result in variability of the accuracy of protein abundance (Table S1). Nevertheless, because our pediatric and adult data were generated using a precise single method, which utilized the same quality controls, we do not anticipate any confounding effect of inter-laboratory methodological variability on the relative ontogeny data, i.e., relative age-dependent maturation of UGTs. Because microsomes used in this study were isolated in different sites, quality of microsomal isolation can also affect the abundance of UGTs. This limitation was addressed by conducting principal component analysis (PCA) of abundance of major UGTs plus a marker protein (CYP reductase or POR) in all samples. The PCA plot suggests no significant changes on the microsomal recovery based on sites of microsome isolation (Fig. S6). Similarly, no apparent association was detected between UGT protein abundance with gender or ethnicities.

In summary, individual contributions of specific UGT pathways in drug disposition across the entire pediatric age range can be predicted using absolute ontogeny and genotype correlation data as presented here. Such information is invaluable to develop full mechanistic

pediatric PBPK models that accurately predict disposition of a drug before designing first-in-children clinical studies.

MATERIAL AND METHODS

Materials

Synthetic light and heavy labeled peptides (Table S5) were obtained from New England Peptides (Boston, MA) and Thermo-Fisher Scientific (Rockford, IL), respectively. 17- β -estradiol, trifluoperazine, 4-hydroxyindole, propofol, naloxone, oxazepam, chloroform, Optima MS-grade acetonitrile/methanol and formic acid were purchased from Fischer Scientific (Fair Lawn, NJ). 17- β -Estradiol 3-glucuronide was purchased from Cayman chemicals (Ann Arbor, MI), whereas 17- β -Estradiol 17-glucuronide was purchased from Steraloid Inc. (Newport, RI). Propofol β -D-glucuronide, naloxone 3 β -D-glucuronide and oxazepam-glucuronide were purchased from Sigma Aldrich, Inc. (St. Louis, MO). Ammonium bicarbonate (98% purity) and sodium deoxycholate (98% purity) were obtained from Thermo Fisher Scientific (Rockford, IL) and MP Biomedicals (Santa Ana, CA), respectively.

Human liver samples

Liver tissue samples from thirty-five adult and seven pediatric donors were procured from the liver bank of the University of Washington School of Pharmacy and from 108 pediatric donors from the National Institute of Child Health and Human Development Brain and Tissue Bank for Developmental Disorders at the University of Maryland, the Liver Tissue Cell Distribution System at the University of Minnesota and the University of Pittsburgh and from Vitron Inc. (Tucson, AZ) In addition, DNA and microsomes isolated from 21 livers acquired by XenoTech LLC were donated for use in this study. The samples were stratified based on the following age categories: neonatal (0 to 27 days, n=4), infancy (28 days to 364 days; n=17), early childhood (1 year to <6 years; n=30), middle childhood (6 years to <12 years; n=37), adolescence (12 years to 18 years; n=48) and adulthood (>18 years; n=35). The use of these tissues has been classified as non-human subject research by the Institutional Review Boards of the University of Washington, Seattle, WA and Children's Mercy Kansas City, Kansas City, MO. Demographic information of the tissue donors is provided in Table S3.

Isolation of liver microsomes, protein denaturation, reduction, alkylation, enrichment and trypsin digestion

Microsomal fractions were prepared and trypsin-digested following established protocols (27, 47). Briefly, microsomal samples were mixed with dithiothreitol, ammonium bicarbonate buffer (pH 7.8), sodium deoxycholate and human serum albumin (27). Proteins were then denatured at 95° C for 10 mins. After cooling the samples to room temperature, iodoacetamide was added and mixtures incubated in the dark for 30 mins at room temperature. Sequentially, ice-cold methanol (500 μ l), chloroform (100 μ l) and deionized water (400 μ l) were added to each sample, vortex mixed and centrifuged at 16,000 $\times g$ (4°C) for 5 mins. The liquid layers were removed using vacuum suction and pellets were dried at the room temperature. The pellets were washed with ice-cold methanol (500 μ l) and

centrifuged at 8000 g (4°C) for 5 mins before the supernatant layers were removed using vacuum suction. Pellets were left to dry at room temperature for 30 mins followed by re-suspension in 60 µl ammonium bicarbonate buffer (50 mM, pH 7.8) and 20 µl trypsin (0.16 µg/µl). In the next step, the samples were incubated at 37 °C with gentle shaking at 300 rpm for 16 hours following which the digestion was quenched by placing samples in dry ice and adding heavy peptide internal standards (20 µl, prepared in acetonitrile: water, 80:20 (v/v) with 0.5% formic acid) and acetonitrile: water (10 µl, 80:20 (v/v) containing 0.5 % formic acid) added to each sample. After mixing and centrifugation at 4000 ×g (4°C) for 5 mins, the samples were transferred to LC-MS auto-sampler vials. Separately, a cocktail of light peptides was prepared and diluted in acetonitrile:water, 80:20 (v/v) containing 0.5% formic acid to create ten calibration standards resulting in final concentrations ranging from ~0.1 to 200.0 fmol (on-column) in the phosphate buffer.

LC-MS analysis of UGT and POR surrogate peptides

The LC-MS/MS system consisted of an Acquity UPLC (Waters Technologies, Milford, MA) coupled to a Sciex Triple Quadrupole 6500 system (Framingham, MA) was used. Peptide separation was achieved on an Acquity UPLC column (HSS T3 1.8 µm, 2.1×100 mm, Waters). Mobile phase consisted of water containing formic acid 0.1% (v/v) (A) and acetonitrile containing formic acid 0.1% (v/v), respectively (B). Peptides were eluted under gradient conditions at a flow rate of 0.3 ml/min (Table S5). Multiple reaction monitoring (MRM) parameters for the surrogate peptides for UGTs and POR are provided in Table S5. Peak integration and quantification were performed using Analyst software (v.1.6, Framingham, MA, USA). A previously described robust strategy (15) to ensure reproducible quantification of UGT proteins was employed.

DNA isolation, gene re-sequencing and genotype analysis

DNA isolation, gene-sequencing and genotype analysis were performed as described previously (28). Briefly, the University of Washington tissue samples were sequenced using a targeted next-generation sequencing approach (PGRN-Seq V1) while all samples from the Children's Mercy liver collection were genotyped using the PharmacoScan array platform (Affymetrix, Santa Clara, CA, USA).

Activity assays for UGT1A1, 1A4, 1A6, 1A9, 2B7 and 2B15

A UGT cocktail assay was performed using a previously validated protocol (16). Detailed activity assay method is discussed in supplementary information.

PBPK prediction of zidovudine and morphine PK in neonates and children

Morphine and zidovudine were selected as representative examples to demonstrate utility of UGT2B7 ontogeny data in PBPK modeling because good pediatric (especially neonatal and infant) PK and in vitro metabolism and transport data were available for these drugs. Because neonatal data were available from 14 days old neonate for zidovudine and from term-infant (newborn) in case of morphine, UGT2B7 abundance data used for neonatal modeling was different for the two drugs, i.e., average neonatal vs. estimated newborn abundance.

For zidovudine, first, a minimal PBPK model describing adult PK (200 mg, oral dose) was developed using the population-based Simcyp simulator (Version 15, Sheffield, United Kingdom) similar to as described elsewhere (48). The model was consisted of liver compartment and a single adjusting compartment connected to a systemic compartment. Physicochemical and blood binding parameters such as molecular weight, lipophilicity (log P), acid dissociation constant (pKa), blood to plasma ratio (B/P), and fraction unbound in plasma (fu) were used directly from the Simcyp library (Table S10). First-order absorption model was employed, and fraction of drug absorbed (fa), absorption rate constant (Ka) and steady-state volume of distribution (Vss) were obtained from literature (49) (Table S10). UGT2B7-mediated zidovudine CL was estimated by the Simcyp retrograde enzyme kinetics model considering fa, total oral CL (CL_{po}), renal CL (CL_r), and additional non-UGT2B7 CL data from adult subjects (18–65 years) (20, 21). The simulation trial size consisted of 100 virtual individuals with 10 trials of 10 subjects with equal number of males and females (age 18–65 years). The model was evaluated by visual predictive checks and by comparison of the observed and predicted PK parameters (20, 21). The model was considered accurate if the observed plasma concentrations were within the 90% prediction interval (5th–95th percentile range of the virtual population).

Once the adult PBPK model for zidovudine was validated, we predicted zidovudine PK in pediatric subjects by using the UGT2B7 ontogeny data from the present study in the Simcyp Pediatric simulator. Particularly, we estimated intrinsic CL, Vmax or relative expression factor (REF) in neonates or children based on the age-dependent ontogeny profile obtained in this study. Then the pediatric PK profiles were simulated using the Simcyp Pediatric simulator, in order to take into account age-dependent anatomical and physiological changes such as organ size, cardiac output, microsomal protein concentration per organ, plasma protein concentration, hematocrit level, and glomerular filtration rate. Zidovudine PK in neonates (age 14 days) was predicted using the validated adult PBPK model with adjusted UGT2B7-mediated zidovudine CL calculated as per equations 1 and 2, where Km is the substrate affinity to the protein, [S] is the substrate concentration, [E] is the protein abundance, and, k_{cat} is the turnover number. Protein abundance in neonates was the average UGT2B7 abundance derived from four neonatal liver specimens.

$$CL_{UGT2B7(neonates)} = \frac{CL_{UGT2B7(adults)}}{UGT2B7\ abundance_{(adults)}} \times UGT2B7\ abundance_{(neonates)} \quad \text{Equation 1}$$

$$CL = \frac{V_{max}}{Km + [S]} \times [S], \text{ and } V_{max} = [E] \times K_{cat} \quad \text{Equation 2}$$

While UGT2B7 is also expressed in the kidney and intestine, the age-dependent changes in non-hepatic UGT2B7 expression were assumed to be the same as in the liver on the basis that extrahepatic expression of UGT2B7 is considerably lower compared to the liver. We also predicted the population PK of zidovudine in the pediatric population (age 0.15–18 years) employing a refined ontogeny equation (Equation 3) based on the UGT2B7 protein

abundance data generated in this investigation where, Y is the fractional protein abundance (of adult) at age X, F_{birth} is the fractional protein abundance (of adult) at birth, Adult_max is the maximum fractional average relative protein abundance, (i.e., 1), X is age in years, h is Hill coefficient, and Age_{50} is age in years at which 50% abundance is reached.

$$Y = F_{\text{birth}} + (\text{Adult_max} - F_{\text{birth}}) * X^h / (\text{Age}_{50}^h + X^h) \quad \text{Equation 3}$$

Visual inspection and statistical analyses were conducted to assess accuracy of both the neonatal and pediatric population PBPK models. Similar to the adult model, the pediatric PBPK model predictions were determined to be successful if the predicted mean plasma concentration overlapped the observed *in vivo* values obtained from the literature (22, 23) and were between the predicted 5th to 95th percentiles of the plasma concentrations for zidovudine.

For morphine, an approach similar to zidovudine was used for PBPK modeling with few exceptions (48). A previously published adult PBPK model (50) was used with input parameters described in Table S11. Because OCT1 is involved in hepatic uptake of morphine (50), a full PBPK model was employed to describe the permeability-limited uptake. We verified this model by comparing PK prediction using free base dose (3.55 mg) with reported adult data (19). Then, the pediatric model was developed by integrating UGT2B7 and OCT1 ontogeny data from this study and data previously published (17). Free base doses of 0.133 and 0.222 mg/kg were used for PK prediction in newborns and children, respectively. Because majority of the age-dependent changes in UGT abundance are observed during the first few months of the life, unlike zidovudine, we used extrapolated newborn level of UGT2B7 for modeling of newborn morphine PK as discussed in Table S11. Visual inspection was conducted to assess accuracy of the model. The PBPK model predictions were determined to be successful if the predicted mean plasma concentration overlapped the observed *in vivo* values from the literature (24, 25) between the predicted 5th to 95th percentiles of the plasma concentrations for morphine.

Statistical analysis

Detailed statistical analysis is discussed in the Supplementary information.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

ADME	Absorption, distribution, metabolism and excretion
CL	clearance
IVIVE	in vitro to in vivo extrapolation
LC-MS/MS	liquid chromatography tandem mass spectrometry
PBPK	physiologically based pharmacokinetic

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STUDY HIGHLIGHT

- **What is the current knowledge on the topic?**
 - mRNA expression, immunoquantification and glucuronidation activity-based ontogeny data are available on some hepatic UGTs, however, the existing data lack selectivity and these data are generally available using limited number of human samples.
- **What question did this study address?**
 - We determined individual ontogeny patterns of six major hepatic UGTs (1A1, 1A4, 1A6, 1A9, 2B7 and 2B15) using LC-MS/MS proteomics in 171 pediatric and adult liver samples. The selective quantification also allowed us to investigate the effect of age-genotype interplay on UGT protein abundance and activity.
- **What does this study add to our knowledge?**
 - Individual effect of age and genotype on each of the six UGT isoforms is the major knowledge gap addressed by this study.
- **How might this change clinical pharmacology or translational science?**
 - Individual UGT ontogeny data can be used to develop physiologically-based pharmacokinetic (PBPK) models to predict hepatic metabolism of UGT substrates in children including neonates and infants as demonstrated for zidovudine and morphine in this study. This approach can predict first-in-children dosing of new drugs before pediatric clinical trials.

Fig. 1.

Protein contents (pmol/mg microsomal protein) of the major UGT1As and UGT2Bs in human liver in different age-categories. Age range for the corresponding categories are shown in the table. Abbreviations in the table indicate, male (M), female (F) unknown (U), Asian (A), African American (AA), Caucasian (C), Hispanic (His), Native American (NA), and Pacific Islander (PI). The number of samples used in the study are shown in parentheses in x-axis and in the table. Dot plots are displayed with mean protein abundance and standard deviation as the horizontal line and error bar, respectively. *, ** and *** indicate p values of <0.05, <0.01 and <0.0001, respectively based on the Kruskal-Wallis test followed by Dunn's multiple comparison test.

Fig. 2.

Pie charts showing fractional abundance of individual hepatic UGTs in different age categories. UGT2B15 is the major UGT isoform in neonates, whereas UGT2B7 and UGT1A4 are the predominant UGTs in adults. Age ranges in individual categories are: 1) neonatal (0 to 27 days), 2) infancy (28 to 364 days), 3) early childhood (1 to < 6 years), 4) middle childhood (6 to < 12 years), 5) adolescence (12 to 18 years) and 6) adulthood (>18 years).

Fig. 3.

Glucuronide formation of probe substrates for UGT1As and UGT2Bs in human liver microsomes in different age-categories. Age categories 1 to 6 (x-axis) represent, 1) neonatal (0 to 27 days), 2) infancy (28 to 364 days), 3) early childhood (1 to < 6 years), 4) middle childhood (6 to < 12 years), 5) adolescence (12 to 18 years) and 6) adulthood (>18 years), respectively. The number of samples used in the study are shown in parentheses in x-axis. The data are presented as absolute glucuronide formation (pmol/min/mg of microsomal protein) or relative glucuronide formation to pooled adult sample. Dot plots are displayed with mean and standard deviation as the horizontal line and error bar, respectively. *, ** and

*** indicate p values of <0.05 , <0.01 and <0.0001 , respectively based on the Kruskal-Wallis test followed by Dunn's multiple comparison test.

Fig. 4. Effect of genotype on protein abundance and activity of hepatic UGT1A1 and UGT2B15. UGT1A1 SNPs rs10929302, rs4124874, rs11741722, rs887829 and rs8175347 were significantly associated with the protein abundance change. UGT2B15, rs1902023, decreases rates of oxazepam-glucuronidation, but not protein expression. *, ** and *** indicate p values of <0.05, <0.01 and <0.0001, respectively based on the Kruskal-Wallis test followed by Dunn's multiple comparison test.

Fig 5.

Observed (data points) and predicted (lines) zidovudine PK profiles after an oral dose of 200 mg in adults (A), dose of 2.0 mg/kg in neonates (age 14 days; B and C), and 300 mg in infants plus children (age 0.15–18 yrs; D and E). Because pediatric population PK data were available from different doses, the observed PK data were normalized to 300 mg dose. B or D and C or E are predictions without and with consideration of DME ontogeny. Solid lines represent mean predictions and the dotted lines are predicted 95th and 5th confidence intervals. Once the adult model was validated by comparison with the observed data (20, 21), ontogeny data of UGT2B7 improved the performance of the neonatal and pediatric models to predict the observed data (22, 23) within 95th and 5th confidence intervals (B vs. C and D vs. E).

Fig. 6.

Observed (data points) and predicted (lines) morphine PK profiles after an intravenous dose (free base) of 3.55 mg in adults (A), dose of 0.133 mg/kg in newborns (age 0 day) (B and C), and 0.222 mg/kg in children (age 1–18 yrs; D and E). B or D and C or E are predictions without and with consideration of DME ontogeny. Solid lines represent mean predictions and the dotted lines are predicted 95th and 5th confidence intervals. Once the adult model was validated by comparison with the observed data (19), ontogeny data of UGT2B7 improved the performance of the newborn (age, 0 day) model to predict the observed data (24) within 95th and 5th confidence interval (B vs. C). However, PBPK models developed with and without considering ontogeny equation did not show any significant difference in morphine PK prediction in children (age 1–18 years) (25) as there was minimal change in the UGT2B7 abundance during this age range, as shown in Figs 1 and S1.

Key parameters describing the abundance-based developmental trajectories of the major hepatic UGTs in liver microsomes. Interindividual variability is expressed as fold difference between the maximum and minimum values across the population.

Table 1

Key parameters (mean ± standard error (SE)) describing protein abundance-based developmental trajectories of UGT enzymes		UGT1A1	UGT1A4	UGT1A6	UGT1A9	UGT2B7	UGT2B15
F _{birth}	Mean±SE	0.27 ± 0.10	0.01 ± 0.01	0.03 ± 0.01	0.00	0.02 ± 0.08	ND
	95% CI	0.08 – 0.46	0.0 – 0.02	0.003 – 0.05	ND	0.0 – 0.18	ND
Age ₅₀ (yr)	Mean±SE	7.5 ± 6.4	3.6 ± 1.7	10.3 ± 2.0	8.2 ± 2.1	2.6 ± 1.4	ND
	95% CI	<0 – 20.0	0.2 – 7.0	6.4 – 14.2	4.1 – 12.4	<0 – 5.4	ND
h	Mean±SE	0.5 ± 0.2	0.9 ± 0.2	0.6 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	ND
	95% CI	0.02 – 0.9	0.6 – 1.2	0.5 – 0.8	0.3 – 0.7	0.3 – 0.6	ND

ND: estimated values were either negative or unreliable due to high variability in neonatal and infant data.