Incorporating ontogeny in physiologically-based pharmacokinetic modeling to improve pediatric drug development: what we know about developmental changes in membrane transporters

Kit Wun Kathy Cheung, Bianca D van Groen, Gilbert J Burckart, Lei Zhang, Saskia N de Wildt, Shiew-Mei Huang

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ABSTRACT

Developmental changes in the biological processes involved in the disposition of drugs, such as membrane transporter expression and activity, may alter the drug exposure and clearance in pediatric patients. Physiologically-based pharmacokinetic (PBPK) models take these age-dependent changes into account and may be used to predict drug exposure in children. As such, this mechanistic-based tool has increasingly been applied to improve pediatric drug development. Under the Prescription Drug User Fee Act VI, the U.S. Food and Drug Administration has committed to facilitate the advancement of PBPK modeling in the drug application review process. Yet, significant knowledge gaps on developmental biology still exist, which must be addressed to increase the confidence of prediction. Recently, more data on ontogeny of transporters have emerged and supplied a missing piece of the puzzle. This review highlights the recent findings on the ontogeny of transporters specifically in the intestine, liver and kidney. It also provides a case study, which illustrates the utility of incorporating this information in predicting drug exposure in children using a PBPK approach. Collaborative work has greatly improved the understanding of the interplay between developmental physiology and drug disposition. Such efforts will continually be needed to address the remaining knowledge gaps to enhance the application of PBPK modeling in drug development for children.

INTRODUCTION

The off-label use of drugs in doses that are insufficiently studied is extensive in pediatric medicine.[1] This is mainly because drug development for treatment in pediatric patients is challenged by ethical concerns and logistical issues.[2] As children widely differ from adults due to developmental changes in the biological processes involved in the disposition of drugs, this leaves them at risk for subtherapeutic or toxic exposures. [3] The establishment of the Best Pharmaceuticals for Children Act (BPCA) in 2002 and the Pediatric Research Equity Act (PREA) in 2003, which were made permanent under the Food and Drug Administration Safety and Innovation Act (FDASIA) in 2012, and the European 'Paediatric Regulation' (regulation no 1901-2/2006) in 2006 have highlighted the commitment of the U.S. Food and Drug Administration (FDA) and the European parliament and council to conduct studies in pediatric patients, and thereby fill the pediatric gaps in drug development to increase the safety and efficacy of pediatric drug therapy.[4-6]

With the advancement of *in silico* technologies, novel methodologies such as model-informed drug development (MIDD) can leverage our existing understanding of pediatric physiology, disease states and pharmacology. This provides quantitative information to streamline decision-making in drug development, such as clinical trial design and dose optimization, which can increase the success of pediatric clinical trials.[7] To support this, FDA has committed to advance MIDD under the Prescription Drug User Fee Act (PDUFA) VI, with approaches that include convening a series of workshops to identify best practices for MIDD, conducting a pilot meeting program for MIDD approaches, publishing or revising an existing draft guidance on MIDD and engaging in regulatory science research to develop expertise and capacity in MIDD approaches.[7,8]

Physiologically-based pharmacokinetic (PBPK) modeling is one of the mechanistic-based MIDD tools that has been increasingly incorporated into drug development programs to support submissions to the FDA and European Medicines Agency (EMA).[9,10] Of all the PBPK analyses that were included in the New Drug Application (NDA) submissions to the FDA between 2008 and 2017, 60% were utilized to assess enzyme-mediated drug-drug interactions (DDIs). This was followed by 15% of the submissions that supported the evaluation of pediatric-related issues such as initial dose recommendation for clinical trials, and 7% that analyzed transporter-mediated DDIs.[9] During the FDA Advisory Committee for Pharmaceutical Science and Clinical Pharmacology Meeting in March 2012, some experts expressed concerns regarding the routine use of PBPK modeling in pediatric drug development as pediatric PBPK models still had significant knowledge

gaps in areas such as the ontogeny of membrane transporters, and thereby may not predict drug exposure well.[11]

Given that new data on the ontogeny of membrane transporters has emerged since 2012, the objective of this article is to review findings from recent studies that have evaluated pediatric developmental changes in the membrane transporters.

ONTOGENY OF MEMBRANE TRANSPORTERS

Membrane transporters facilitate the active movement of drug molecules and endogenous compounds into and out of cells of various organs, affecting drug absorption, distribution and excretion.[12] Hence, they have a critical role in impacting pharmacokinetics (PK) and pharmacodynamics (PD) of drugs, and should be considered and assessed carefully during drug development. In the 2017 FDA's draft *in vitro* DDI guidance, FDA recommended the evaluation of DDI potential by studying whether a

Table 1. The full name, protein names and gene names of the membrane transporters that are discussed in this review

Full name	Protein name	Gene name
P-glycoprotein	P-gp	*ABCB1*
Breast Cancer Resistance Protein	BCRP	*ABCG2*
Multidrug and Toxin Extrusion 1	MATE1	*SLC47A1*
Multidrug and Toxin Extrusion 2-K	MATE2-K	*SLC47A2*
Organic Anion Transporting Polypeptide 1B1	OATP1B1	*SLCO1B1*
Organic Anion Transporting Polypeptide 1B3	OATP1B3	*SLCO1B3*
Organic Anion Transporter 1	OAT1	*SLC22A6*
Organic Anion Transporter 3	OAT3	*SLC22A8*
Organic Cation Transporter 2	OCT2	*SLC22A2*
Multidrug Resistance-Associated Protein 2	MRP2	*ABCC2*
Multidrug Resistance-Associated Protein 4	MRP4	*ABCC4*
Peptide Transporter 1	PEPT1	*SLC15A1*
Sodium/taurocholate Cotransporting Polypeptide	NTCP	*SLC10A1*
Bile salt export pump	BSEP	*ABCB11*
Glucose transporter 1	GLUT1	*SLC2A1*
Glucose transporter 2	GLUT2	*SLC2A2*
Monocarboxylate transporter 1	MCT1	*SLC16A1*
Uric acid transporter 1	URAT1	*SLC22A12*

new drug is a potential substrate or inhibitor of the following nine transporters (see Table 1 for full, protein and gene names): P-gp, BCRP, MATE1, MATE2-K, OATP1B1, OATP1B3, OAT1, OAT3, OCT2.[13]

There is a wealth of information on how alterations in the transporter activity, mainly due to genetic polymorphisms and DDIs, can lead to variability in drug safety and efficacy in adults. However, less is known about age-related changes in transporter expression levels and activities, and how that relates to the safety and efficacy of pediatric drug use. In 2015, the Pediatric Transporter Working Group performed a comprehensive review on the data available for the ontogeny of clinically relevant membrane transporters.[14] Further, the working group also provided recommendations to address and overcome some of the challenges in filling the pediatric knowledge.[14] These include building multidisciplinary and international collaborative networks to facilitate data sharing, increasing awareness of clinicians about the importance of transporters in pediatric drug disposition and identifying biomarkers for transporter activity in children. In the following discussion and in Table 2, human data presented in that review are highlighted, and updated information from recent literature is provided. Figure 1 also depicts the human membrane transporters in the intestine, liver and kidneys that are mentioned in this article.

Ontogeny of intestinal transporters

Most drugs prescribed to children are administered orally.[37] The intestine is a major absorption site of drugs that are administered via oral route. Transporters that are present in the enterocytes on the gut wall mucosa govern the initial access into the systemic circulation of molecules such as sugars, amino acids, vitamins, but also of drug substrates.[38,39] P-gp, multidrug resistance-associated protein (MRP2) and BCRP, for instance, are major efflux transporters that are responsible for limiting drug absorption. On the other hand, OATP1A2 and OATP2B1 have been suggested to participate in the intestinal absorption of drugs in human.[40] Further, peptide transporter (PEPT)1 is a major uptake transporter that facilitates absorption of peptide-like drugs in the systemic circulation such as β-lactam antibiotics.[38,41] Therefore, drug absorption in children will be highly dependent on the expression and activity of these intestinal transporters.

P-gp, BCRP, MRPs, OATP2B1 and PEPT1: In their review, Brouwer et al noted that ontogeny of intestinal transporters was mainly revealed by mRNA expression and localization data using immunohistochemistry.[14] P-gp and MRP2 mRNA expression levels in neonates and infants appeared to be comparable to adults.[17-24] Localization data suggested that BCRP and MRP1 distribution was similar in adult and fetal samples (5.5-28 weeks and 9-28 weeks of gestation, respectively).[24] In contrast to the other

Table 2. Human ontogeny data of membrane transporters in intestine, liver and kidney highlighted in this article.

Membrane transporter [protein name (gene name)]	Types of ontogeny data available	Reported ontogeny pattern	Reference
Intestinal transporters*			
P-gp (*ABCB1*)	Gene expression	mRNA level in neonates and infants was comparable to adults	17-23
BCRP (*ABCG2*)	Immunohistochemistry	BCRP distribution was similar in fetal (5.5-28 weeks of gestation) and adult samples	24
MRP1 (*ABCC1*)	Immunohistochemistry	MRP1 distribution was similar in adults and fetal samples (9-28 weeks of gestation)	24
MRP2 (*ABCC2*)	Gene expression	mRNA level was stable from neonates to adults	23
OATP2B1 (*SLCO2B1*)	Gene expression	mRNA level was higher in neonates than in adults	23
PEPT1 (*SLC15A1*)	Gene expression	mRNA was slightly lower in neonates than in older counterparts	25
	Immunohistochemistry	Tissue distribution was relatively stable from preterm neonates to adolescents	25
Liver transporters			
OCT1 (*SLC22A1*)	Gene expression	Transcript levels in pediatric livers was comparable to that in adults	26
	Western blot	Age-dependent increase in OCT1 protein expression from birth up to 8-12 years old	27
	Quantitative proteomics	Age-dependent increase in protein expression level; TM_{50} was approximately 6 months	28,29
OATP1B1 (*SLCO1B1*)	Gene expression	mRNA expression of OATP1B1 in fetal liver was 20-fold lower than that in adults. Neonates and infants have even lower levels than fetus (500-fold and 90-fold lower than adults, respectively)	23
	Quantitative proteomics	van Groen et al reported higher protein expression in fetal livers compared to that in term neonates. The protein expression in infants to adults were similar. Genetic polymorphism was not associated with expression levels in this study. Prasad et al reported that when all samples were considered, no age-dependent changes in the protein expression was found. Protein levels were higher in *1A/*1A >1-year-old cohort than the 0 to 12 months group	28,29
OATP1B3 (*SLCO1B3*)	Gene expression	mRNA levels in fetus, neonates and infants were lower than that in adults	23
	Quantitative proteomics	No age-dependent changes were found in van Groen et al; Age-dependent increase reported in Prasad et al with TM_{50} approximately 6-months	28,29
OATP2B1 (*SLCO2B1*)	Gene expression	mRNA level was significantly higher in adult livers compared to that in fetus (GA 18-23 weeks)	30
	Quantitative proteomics	Comparable protein expression levels in livers from fetus to adults	28,29,31

Table 2. Human ontogeny data of membrane transporters in intestine, liver and kidney highlighted in this article. (continued)

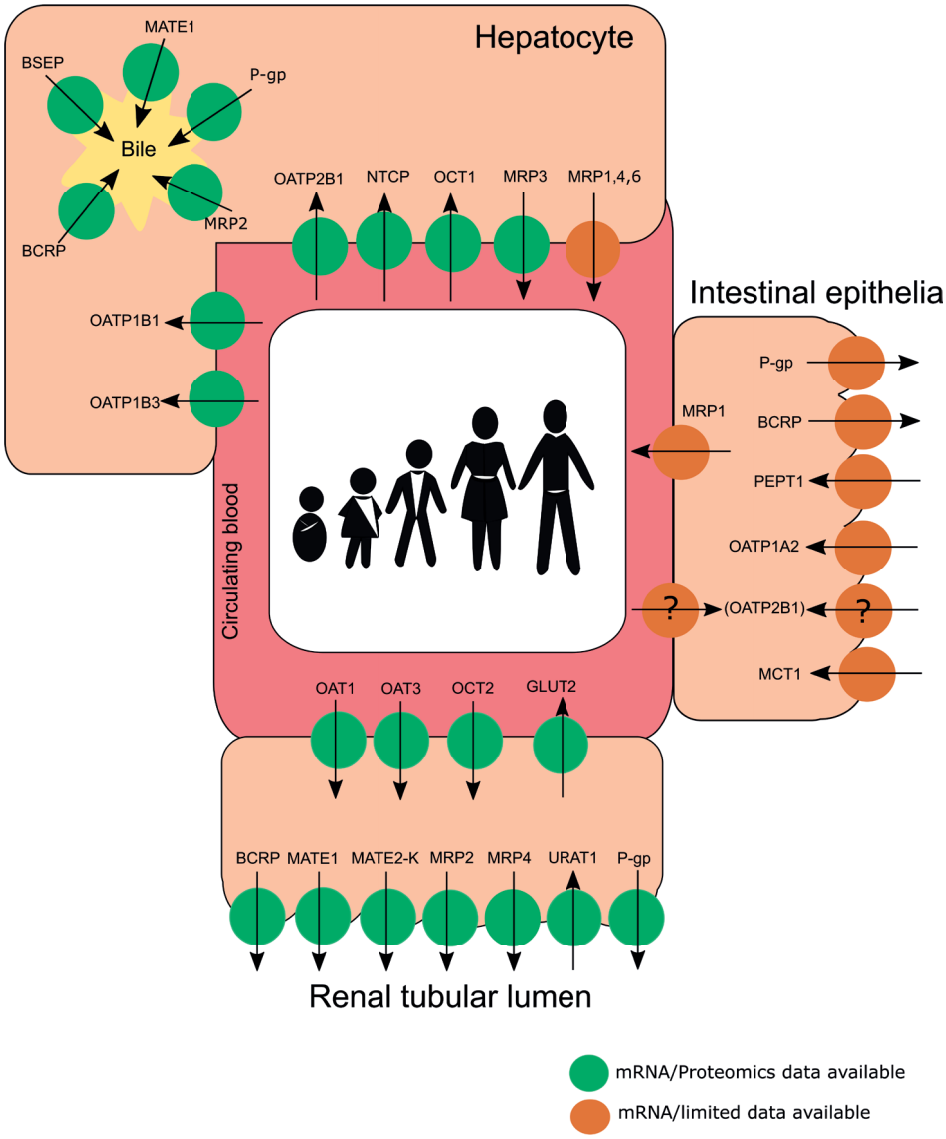
Membrane transporter [protein name (gene name)]	Types of ontogeny data available	Reported ontogeny pattern	Reference
NTCP (*SLC10A1*)	Gene expression	mRNA level was low in fetal liver compared to adults	30,32
	Western blot	Relative expression was stable in livers samples from neonates and adults	33
	Quantitative proteomics	Prasad et al reported stable protein expression from neonates to adults. van Groen showed that protein expression was significantly lower in fetuses than in term neonates, infants, children and adults	28,29
P-gp (*ABCB1*)	Gene expression	Detected in fetal liver; mRNA level increased rapidly during first 12 months of life in infants	23
	Western blot	No significant differences in the relative protein expression from 0.3 to 12 years old	34
	Quantitative proteomics	Protein level increase from fetus to adults with TM_{50} approximately 2.9 years old	28,29
MRP2 (*ABCC2*)	Gene expression	mRNA level increased; levels in fetal, neonatal and infant livers were substantially lower than that in older children up to 12 years old	23,35
	Quantitative proteomics	van Groen et al reported that MRP2 level was much lower in fetal and term newborn livers than that in adults. Prasad et al found no age-dependent changes	28,29
MRP3 (*ABCC3*)	Gene expression	mRNA level was lower in fetal livers than that in adults	30
	Quantitative proteomics	van Groen et al reported that protein abundance was lower in fetus and term neonates than in adults. In Prasad et al, lower protein abundance was found in infants and adolescents than adults.	28,29
MRP1 (*ABCC1*)	Quantitative proteomics	Protein levels were lower in fetus and term neonates than adults	28
MRP4 (*ABCC4*)	Gene expression	No age-dependent changes in mRNA level	30
MRP6 (*ABCC6*)	Gene expression	mRNA level increase from neonates to older children and adults	35
BCRP (*ABCG2*)	Immunohistochemistry	Detected in fetus as young as GA 5.5 weeks	24
	Gene expression	mRNA level was lower in fetal livers than in adults	30,35
	Quantitative proteomics	Stable across age groups from fetus to adults but age-dependent decrease observed in fetal and newborn cohorts	28,29
BSEP (*ABCB11*)	Quantitative proteomics	Significantly lower in fetal livers compared to that in adults; no age-dependent changes after birth	28,29
MATE1 (*SLC47A1*)	Gene expression	mRNA showed age-dependent increase	35
	Quantitative proteomics	No age-dependent changes in protein abundance	29
GLUT1 (*SLC2A1*)	Quantitative proteomics	Protein abundance was high in fetus and lower in other age groups	28
MCT1 (*SLC16A1*)	Quantitative proteomics	No age-dependent changes in protein abundance	28

Table 2. Human ontogeny data of membrane transporters in intestine, liver and kidney highlighted in this article. (continued)

Membrane transporter [protein name (gene name)]	Types of ontogeny data available	Reported ontogeny pattern	Reference
Kidney transporters			
BCRP (*ABCG2*)	Gene expression	mRNA level was higher in term neonates than older counterparts	36
	Quantitative proteomics	No age-dependent changes in protein abundance	36
MATE1 (*SLC47A1*)	Gene expression	No age-dependent changes in mRNA level	36
	Quantitative proteomics	No age-dependent changes in protein abundance	36
MATE2-K (*SLC47A2*)	Gene expression	mRNA level was lower in term newborns than adults	36
	Quantitative proteomics	No age-dependent changes in protein abundance	36
MRP2 (*ABCC2*)	Gene expression	No age-dependent changes in mRNA level	36
MRP4 (*ABCC4*)	Gene expression	No age-dependent changes in mRNA level	36
	Immunohistochemistry	Proper localization observed in renal cortical fetal sample as early as GA 27 weeks	36
URAT1 (*SLC22A12*)	Gene expression	mRNA levels increased with age from term newborn to adults	36
	Quantitative proteomics	Protein levels increased with age from term newborn to adults	36
P-gp (*ABCB1*)	Immunohistochemistry	Localization detected as early as end of first trimester of fetal life	22
	Gene expression	mRNA levels were lower in preterm newborn, term newborns and infants compared to older counterparts	36
	Quantitative proteomics	Protein levels increased with age with TM_{50} approximately 1 month	36
GLUT2 (*SLC2A2*)	Gene expression	No age-dependent changes in mRNA level	36
	Quantitative proteomics	No age-dependent changes in protein abundance	36
OAT1 (*SLC22A6*)	Gene expression	mRNA level increased with age	36
	Quantitative proteomics	Protein abundance increased with age. TM_{50} was approximately 5 months	36
OAT3 (*SLC22A8*)	Gene expression	mRNA level increased with age	36
	Quantitative proteomics	Protein abundance increased with age. TM_{50} was approximately 8 months	36
OCT2 (*SLC22A2*)	Gene expression	mRNA level increased with age	36
	Quantitative proteomics	Protein expression increased with age. TM_{50} was approximately 1 month	36

* *See Brouwer et al for more detailed review.*[14]

Figure 1 Summary of the human membrane transporters in the intestine, liver and kidneys that are mentioned in this review.



Transporters with only mRNA or limited data are depicted in brown circles; whereas those that have both gene expression and protein abundance data are depicted in green circles. (adapted/modified from Brouwer et al and Chu et al)[15,16] *Noteworthy, the localization of OATP2B1 remains questionable. Future investigation would also be needed to characterize if its localization is subject to developmental changes.*

intestinal transporters, OATP2B1 gene expression levels were much higher in neonates than in adults.[23] Noteworthy, the localization of OATP2B1 remains questionable; while two studies observed localization of the transporter to the apical membrane of human enterocytes, another research group, which studied mainly pediatric intestinal tissue samples, detected OATP2B1 in the basolateral membrane.[25,42-44] This basolateral localization was also reported by another independent group using six healthy human adult jejunal tissue samples.[45] Future studies are warranted to elucidate the localization of OATP2B1 and if it is subject to developmental changes. Using a total of 26 intestinal tissues samples, which included 19 preterm and term neonates, one infant 13.9 weeks old, two children and four adolescents, Mooij and colleagues studied the developmental changes in PEPT1 mRNA expression and localization.[25] While PEPT1 expression appeared to be slightly lower in neonates than in their older counterparts, the tissue distribution was relatively stable among all the samples studied.

While changes in the gene expression and localization of these transporters during development were stressed in various published studies, data on their protein expression levels are still missing. In addition, the ontogeny patterns of other human intestinal transporters, such as OATP1A2 and MCT1, remain uncertain. Since many drugs are administered orally, it is crucial to fill this knowledge gap in intestinal transporter ontogeny.

Ontogeny of liver transporters

In comparison to intestinal transporters, data on developmental changes in hepatic transporters have grown quite rapidly recently. Classic analytical approaches include quantitative real-time polymerase chain reaction (qRT-PCR), which measures gene expression levels, immunohistochemistry which visualizes localization and western blot which measures the relative protein expression. In addition, quantitative proteomics via liquid chromatography/tandem mass spectrometry (LC-MS/MS) has been increasingly utilized to measure the absolute protein abundance of these transporters, allowing the quantification of many transporters in only a small amount of tissue. Proteomics data generated from two independent laboratories complemented each other in terms of age range of the samples and provided a more complete picture of the developmental patterns of hepatic transporters with higher confidence than what was known previously.[28,29,46] In one study, the protein abundance of 11 hepatic transporters was measured in approximately 69 postmortem tissue samples that covered the whole pediatric age range (4 neonates, 19 infants, 32 children and 14 adolescents) and in 41 adult samples (> 16 years old).[29] In another study, the absolute protein expression of 13 liver transporters was quantified in a pediatric cohort with a focus on the fetus and newborn up to postnatal 18 weeks of age that consisted of 62 pediatric tissue samples

(36 fetuses, 12 premature newborns, 10 term newborns, 4 pediatric patients and 8 tissue samples from adults).[28] The findings in these two studies and other previous studies are discussed below.

OCT1: As previously reported, OCT1 mRNA levels in pediatric livers appeared to be comparable to that in adult livers.[14,26] Nonetheless, OCT1 protein levels have shown to undergo age-dependent increase.[27-29] This was supported by a recently published clinical study in neonates who were admitted to the neonatal intensive care unit where postmenstrual age as well as OCT1 genotype impacted the PK of the OCT1 substrate morphine.[47] Further, the age at which half of adult level is reached (TM_{50}) was also estimated using a sigmoidal Emax model and was reported to be about 6 months.

OATP1B1: mRNA expression of OATP1B1 in fetal liver was 20-fold lower than that in adults, and that in neonates and infants was even lower (500-fold and 90-fold, respectively).[14,23] Recent quantification of protein expression, nonetheless, revealed different findings. In their sample set, van Groen et al found that the OATP1B1 expression was significantly higher in the fetal livers compared to that term neonatal livers. The protein expressions in infants, children and adults were similar.[28] OATP1B1 is highly polymorphic. The impact of genetic variants on developmental changes in OATP1B1 expression was investigated in this cohort but no association was identified for the studied genotypes. When all tissue samples were considered, Prasad et al reported that OAPT1B1 did not show age-dependent changes in the protein expression.[29] Yet, when the analysis was performed on samples from donors with the OATP1B1 reference allele, *1A/*1A, samples from > 1 year old was found to have higher protein expression than the 0 to 12 months group. Notably, in the >1-year-old cohort, OATP1B1 expression was about 2.5-fold higher in samples from donors with *14/*1A than that with *15/*1A.

OATP1B3: Similar to OATP1B1, mRNA expression of OATP1B3 was reported to be much lower in fetuses, neonates and infants compared to adults.[14,23] While proteomics data in one study showed that OATP1B3 expression was not associated with age, the other illustrated that the expression of the transporter was subjected to age-dependent increase, and by 6 months of age, similar to OCT1, the protein expression would have reached 50% of the adult level.[28,29]

OATP2B1: mRNA levels of OAT2B1 was significantly higher in adult livers compared to that in livers from fetus at gestational age 18-23 weeks.[14,30] However, quantitative proteomics suggested that OATP2B1 expression in liver from fetus of median 23.4 (range 15.3-41.3) weeks was comparable to that from preterm neonates, term neonates,

children and adults.[28] This lack of correlation with age was supported by two other analyses.[29,31]

NTCP: Various studies suggested that maturation of NTCP starts during perinatal stage and the expression reaches adult levels at birth.[14,29,30,32,33] Protein expression of NTCP revealed similar trend where NTCP expression was significantly lower in fetuses than in term neonates, infants, children and adults and that in preterm neonates was lower than in adults.[28]

P-gp: Previously it has been reported that P-gp is subject to developmental changes in the mRNA expression.[14] The transcript level of P-gp was detected as early as 14 weeks gestational age and the level increased rapidly during the first 12 months of life in infants, which then reached a level comparable to adults.[22] Despite the developmental changes in gene expression, one study reported no age-related differences in the relative protein expression in patients from 0.3 to 12 years old.[34] Interestingly, however, the results from the two recent proteomic studies were in agreement with the mRNA data – P-gp protein expression was low in fetal liver tissues but increased with age.[28,29] Further, TM_{50} was also estimated to be 2.94 years old, suggesting that the P-gp expression continued to increase postnatally and would achieve adult level later on in children.[29]

MRP2: Using gene expression analysis, previous studies have shown that MRP2 mRNA levels were substantially lower in fetal, neonatal and infant livers compared to older children up to 12 years of age.[14,23,35] The result reported in one of the recent proteomic studies was in agreement with these findings, where MRP2 protein expression was approximately three-fold lower in fetal and term newborn livers compared to adults.[28] Yet, in another study, it was reported that MRP2 expression was not age-dependent in their cohort. [29]

MRP3: MRP3 mRNA was detected in fetal hepatocytes as early as 18 weeks gestational age, and was significantly lower than that found in adult livers.[30] Proteomic data from recent studies agree with this observation. The fetal MRP3 protein level was approximately 3-folder lower than the adult level.[28] Interestingly it was found in one study that the transporter expression appeared to be lower in adolescents compared to that in adults. [29]

MRP1, MRP4, MRP6: Developmental information on these three MRPs is scarce. In their study, van Groen et al showed that MRP1 levels in livers from fetus and term neonates were about two-fold lower than that in adults.[28] MRP4 mRNA did not change with age.[14,30] While MRP6 mRNA expression was shown to increase from neonates to older

children and adults, no proteomic data is currently available to determine if the actual protein expression shows similar age-dependent change.[35]

BCRP: Localization of BCRP in the hepatocytes was detected in fetus as young as 5.5 weeks gestational age.[24] BCRP mRNA expression was lower in fetal samples compared to adults.[30,35] BCRP protein levels appeared to be comparable in fetus and after birth in all age groups.[28,29] However, when data set was analyzed as continuous data by postnatal age and postmenstrual age within the fetal and newborn cohort, BCRP expression interestingly showed age-dependent decrease with a spearman correlation coefficients of -0.345 and -0.421, respectively.[28]

BSEP: Using sandwich-cultured fetal and adult hepatocytes, a functional study was conducted, which showed that the biliary excretion index for taurocholate, an endogenous BSEP substrate, was lower in the fetal liver cells compared to that in adults.[30] Results from quantitative proteomics studies coincide with this observation; the fetal liver tissues expressed significantly lower BSEP compared to term newborn and adults.[28] Maturation of BSEP appeared to occur mainly during perinatal period as no significant age-dependent changes were seen from neonates onwards.[28,29]

MATE1: In contrast to the age-dependent increase in mRNA reported previously, protein expression of MATE1 appeared to be independent of age.[14,28,29]

GLUT1: Developmental information for GLUT1 was previously lacking but recent proteomic study indicated that GLUT1 expression showed age-dependent decrease with fetal liver tissues expressing the highest protein abundance and lower expression in the other age groups.[28] This age-dependent decrease was more apparent when analyzing the expression levels in the youngest cohorts, fetus and newborn, based on the PNA and PMA with spearman correlation coefficient of -0.51 and -0.59, respectively.

MCT1: Similar to GLUT1, the ontogeny of MCT1 was missing. The absolute protein abundance of this transporter was found to be comparable in fetal liver and in other age groups after birth.[28]

Recent knowledge gain on liver transporters

Recent proteomics studies provided valuable ontogeny information for the liver transporters. Although gaps in the developmental changes in various liver transporters such as OAT2 and OAT7 still exist, the understanding in the association between transporter expression and age has been improved substantially, particularly for those

transporters that have been shown to be clinically important: BCRP, P-gp, MATE1 and OATP1B1/3.[13]

Ontogeny of renal transporters

The kidney is the major site for elimination of many drugs. Three major processes are involved in drug disposition: glomerular filtration, active secretion and reabsorption. Maturation of glomerular filtration has been studied quite extensively but information on ontogeny of renal membrane transporters, which are key players in the active secretion was relatively scarce.[14,48] Yet, information on the developmental changes in renal membrane transporters has emerged recently. Gene expression of 11 transporters was analyzed from a total of 184 frozen human renal cortical samples from preterm newborn to 75 years of age. The protein expression of 9 transporters and localization of MRP4 using immunohistochemistry were also studied using a subset of the kidney samples.[36]

BCRP: The mRNA level of BCRP was significantly higher in term neonates compared to other age groups but the protein abundance appeared to be comparable across all age groups from term neonates to adults. Further studies would be warranted to investigate this lack of gene-protein correlation as only one term neonate was included for the proteomic analysis in that study.[36]

MATE1 and **MATE2-K**: mRNA and protein levels of MATE1 were independent of age.[36] While transcript level of MATE2-K in term newborn was significantly lower than that in adults, the protein was found to be comparable across all the age groups studied from term newborn to adults. However, similar to BCRP, the cohort of term neonates for proteomic analysis would need to be expanded in order to better characterize the correlation between gene and protein expression.

MRP2 and **MRP4**: mRNA levels of MRP2 and MRP4 appeared to be stable in preterm newborn, term newborn, infants, children and their older counterparts. Interestingly, proper MRP4 localization was detected as early as GA 27 weeks, postnatal 9 day old. This result appeared to accompany the stable gene expression during development.[36]

URAT1: The mRNA and protein abundance of URAT1 increased with age from term newborn to adults.[36]

P-gp: Similar to the liver and intestine, the ontogeny of renal P-gp was studied relatively extensively. P-gp localization was detected as early as the end of first trimester of fetal life.[22] Results from gene expression analysis and quantitative proteomics expanded the

understanding of the developmental changes of P-gp in kidney. P-gp mRNA levels were significantly lower in preterm newborn, newborn and infants as compared to children, adolescents and adults. This observation appeared to be translated well to protein expressions. Sigmoidal Emax model described this age-dependent increase and the TM_{50} was approximately 1 month.[36]

GLUT2: An efficient carrier of glucose, GLUT2, did not show age-dependent changes in its mRNA expression and protein abundance.[36]

OAT1 and **OAT3:** The ontogeny of these two organic anion transporters were reflected in clinical data.[48] For instance, one study showed that the secretion capacity of *p*-aminohippurate (PAH), an OAT1/3 substrate, appeared to be about one-fifth of adult level at birth.[49-51] These observed age-related changes in pharmacokinetics of transporter substrates are likely due to a combination of maturation in both transporter expression and glomerular filtration. Yet, the changes in transcript levels and protein abundance aligned with the clinical observations. mRNA and protein expressions for both OAT1 and OAT3 increased with age with TM_{50} of approximately 5 months and 8 months, respectively. Further, inter-transporter correlation analysis also demonstrated that these two transporters were highly correlated in their gene and protein expression.[36]

OCT2: Similar to OATs and P-gp, the OCT2 mRNA levels and protein abundance are age-dependent with the levels in newborns being significantly lower compared to children and adults. Like P-gp, OCT2 would reach half of the adult level about one month after birth.[36]

Recent knowledge gain on renal transporters

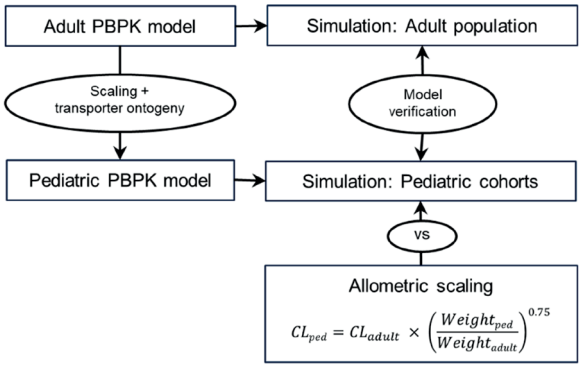
The data from gene expression analysis, quantitative proteomics and immunohistochemistry have painted a more complete picture for the ontogeny of renal membrane transporters. Within the six transporters that are clinically important and should be carefully considered in drug development, four of them, P-gp, OAT1, OAT3 and OCT2, showed age-dependent increase in their expression levels. This implies that drug substrates of these transporters would also be subject to age-dependent changes and might impact the elimination of these drugs in pediatric patients. Despite this increase in knowledge, more studies on term and preterm neonates would be needed to better capture the variability in the age-related changes of transporter expression, and also the interplay with maturation of glomerular filtration, during this rapid developmental phase of life.[52,53]

APPLICATION OF ONTOGENY OF TRANSPORTERS TO MIDD IN CHILDREN

Overall, there has been a recent surge in data on the ontogeny of membrane transporters, which will greatly enhance our understanding in not only the disposition of drug substrates but the involvement of these transporters in developmental physiology in children. While scarce data in the ontogeny of intestinal transporters still limits their application to modeling and simulation of oral drugs, the wealth of data in the domain of hepatic and renal transporter ontogeny present an opportunity to be leveraged for pediatric PBPK modeling, especially for intravenous administrated drugs, to assist the prediction in drug disposition and clearance in children.

The workflow of pediatric PBPK model development has been previously described (see Figure 2).[54-57] In most cases, an adult PBPK model is first established, verified and refined. This model is comprised of the drug profile as well as the virtual adult population in

Figure 2 Workflow of the pediatric physiologically-based pharmacokinetic model (PBPK) establishment to simulate drug exposure in children.



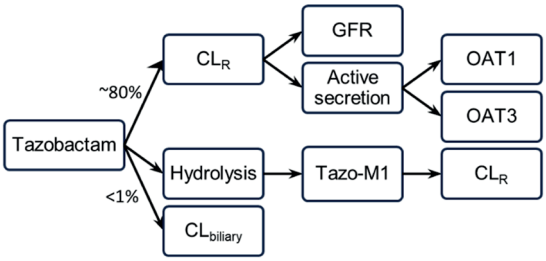
An adult PBPK model was first established and verified by comparing the output from the simulations to that in observed data. After ensuring that adult model was robust, the pediatric model was generated by scaling the anatomical and physiological parameters using default age-dependent algorithms, and incorporating ontogeny information for the transporters that are pertinent to this study. The pediatric PBPK model was verified, once again, by comparing the output from the simulation input with observed data from literature. Predictive performance of allometry and PBPK in estimating the clearance in children was also evaluated.

which transporter protein abundance data and kinetics parameters, such as Km and Vmax, can be incorporated to predict the organ-specific clearance (CL).[58] Following the finalization and verification of the robust adult PBPK model, pediatric models could be generated by modifying the population-specific inputs (e.g. blood flow to organs, organ weights and protein abundance of drug metabolizing enzymes and transporters) using algorithms or parameters such as ontogeny scaling factor for transporter abundance or

intrinsic clearance. These are expressed as a function of age and can be derived from the developmental changes in the expression data described in previous section. Of note, while pediatric PBPK models can also be established based on drug physiological properties and preclinical data alone, this approach could lead to a lower confidence in the prediction compared to a model that is verified with adult clinical data.

The success and confidence of PBPK modeling and simulation that involves transporter-mediated disposition using bottom-up approach are critically dependent on factors such as the quality and availability of transporter kinetic data and understanding in in vitro-in vivo correlation. The following case study illustrates that the utility of leveraging transporter ontogeny data in PBPK modeling, with sufficient information gathered, can be useful to simulate drug exposure in pediatric patients.[59]

Figure 3 The elimination pathway of tazobactam.



After intravenous administration, approximately 80% of tazobactam would be cleared renally by glomerular filtration and active secretion via OAT1 and OAT3. Majority of the rest of tazobactam would undergo hydrolysis to form the inactive metabolite, tazo-M1, which, similar to the parent drug, will be eliminated renally. A small amount (<1%) of tazobactam would undergo biliary excretion.

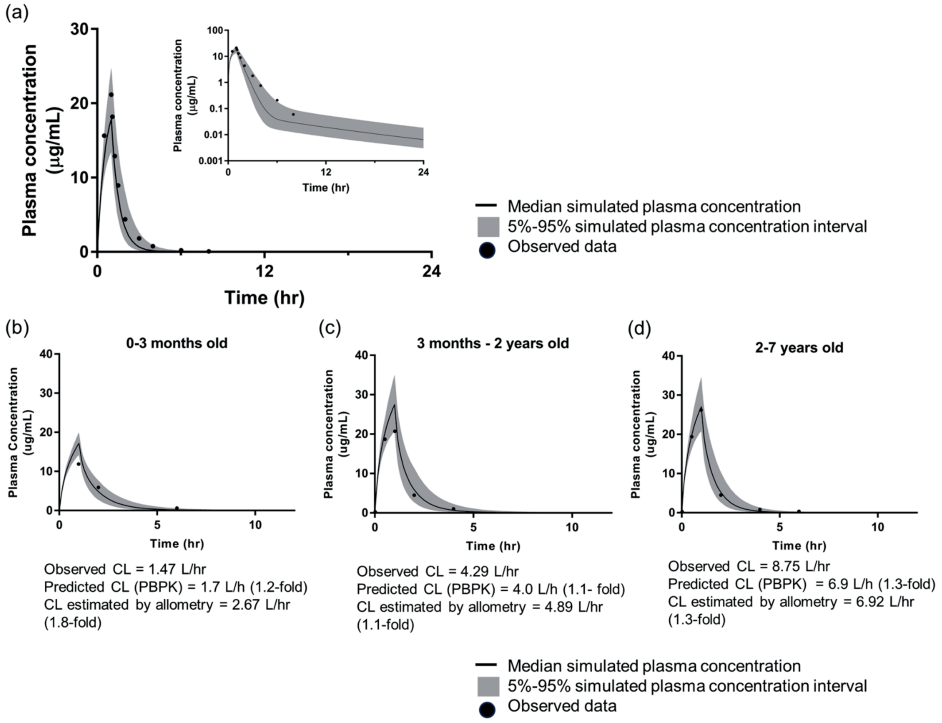
PBPK modeling with integrated transporter ontogeny reasonably predicted the exposure of an actively renally secreted drug in children

Tazobactam is a beta-lactamase inhibitor. Currently, it is formulated as an intravenously-administered combination product with either piperacillin, a beta-lactam, or ceftolozane, a cephalosporin, as a broad-spectrum antibiotic.[60,61] Tazobactam is prescribed for infections that could potentially be life threatening when left untreated. Hence sufficient exposure is needed to assure therapeutic action without adverse events. As much as 80% of tazobactam is renally cleared in adults.[61,62] In addition to glomerular filtration, tazobactam undergoes active tubular secretion that is mediated by OAT1 and OAT3.[62,63] The remaining tazobactam is either converted to the inactive metabolite, M1, via hydrolysis and then eliminated renally or undergoes biliary excretion (Figure 3).[62,64]

The workflow of the adult and pediatric PBPK model establishment is summarized in Figure 2. OAT1/ 3 protein abundance and transporter kinetics from *in vitro* studies are

obtained from literature.[36,63] The ontogeny scaling factors, which are the sigmoidal Emax functions, of OAT1/3 were incorporated.[36] To address the argument on whether PBPK modeling is preferred over an allometric scaling approach in predicting the PK for pediatric patients < 2 years old, the clearance in the pediatric cohorts were also estimated based on allometry and compared to that predicted using PBPK models.[9]

Figure 4 Simulation of tazobactam exposure in adult population (a) and three pediatric cohorts (b – d) using PBPK (PK-Sim v7.3).



Following 500mg x 60min infusion in the virtual adult population, the predicted Cmax, AUC and CL were between 1.02-1.2-fold of the observed data.[65] Three pediatric cohorts were generated: 0-3 months old (b), 3 months-2 years old (c), and 2-7 years old (d). By taking into account the physiological and anatomical changes during development, and the ontogeny of transporters that are pertinent to the disposition of tazobactam, the tazobactam exposure was predicted reasonably well with Cmax, AUC and CL were all within 1.5-fold of observed data.[66] Allometric scaling approach resulted in CL estimation that were comparable to that predicted using PBPK. However, for the youngest age group, 0-3 months old (b), PBPK model performed slightly better as allometry slightly overpredicted the CL (1.2-fold vs 1.8-fold of observed CL).

The PBPK model captured the exposure of tazobactam after 500mg x 60min IV infusion in adults well with the predicted maximal concentration (C_{max}), area under the curve (AUC) and clearance (CL) between 1.02- to-1.2-fold of the observed data (Fig 4a).[65] After verifying and ensuring that the adult model was robust, three virtual pediatric populations were generated using the approach as outlined above: 0 to 3 months, 3 months to 2 years old, 2 to 7 years old. C_{max}, AUC and CL were all within 1.5-fold of observed data when the simulation was performed in these three cohorts, suggesting that the pediatric PBPK model predicted the exposure of tazobactam adequately in neonates, infants and children (Fig 4b).[66] Significantly, allometric scaling approach resulted in CL estimations that were comparable to that predicted using PBPK. However, for the youngest age group, 0-3 months old, PBPK model performed slightly better as allometry slightly overpredicted the CL (1.2-fold vs 1.8-fold of observed CL).

This case study illustrated the utility of a pediatric PBPK model with integrated renal transporter ontogeny function in simulating exposure of a drug that is actively renally secreted in pediatric patients. It exemplifies how this approach could be applied in pediatric drug development to support decision-making on dosing to limit unnecessary exposure in pediatric patients. Further, it also highlighted how a PBPK model can be used to complement allometric scaling approach by predicting the whole PK (concentration-time) profile, rather than just the drug clearance.

CONSIDERATIONS

The recent emergence of quantitative proteomics data on the expression and ontogeny of transporters substantially improves the predictive power of pediatric PBPK models for drug substrates. Nonetheless, there are important factors that need to be considered when incorporating protein abundance into PBPK models. While LC-MS/MS based quantitative proteomics is a powerful tool by using peptide sequences to measure the absolute abundance of transporter proteins, it could not acknowledge if the transporters are successfully localized to the membrane, nor could it distinguish truncated protein and splice variants from properly formed proteins, and glycosylated and not-glycosylated protein.[67] Consequently, these would undermine the assumed correlation between transporter expression and activity. Further, scaling from the protein abundance data per *crude membrane protein* or *per gram tissue* level to per *organ* level is the first step when integrating such data into PBPK. This should be done carefully as parameters that are used to scale, such as membrane protein yield per gram of tissue could also subject to age-related changes.[28] Lastly, it is important to reiterate that the success of PBPK simulation in children using bottom-up approach depends highly on the

knowledge in the drug disposition pathway and the data available for the ontogeny of the metabolic enzymes and transporter involved. For instance, with good understanding in the maturational differences between UGT enzymes and sulfotransferase, one study successfully predicted the exposure, as well as metabolic formation and elimination of acetaminophen, which is mainly glucuronidated in adults but almost exclusively sulfated in newborn due to age-dependent changes in the UGT enzymes expression and activity, in various pediatric age groups using PBPK.[68] Nonetheless, for drugs that are substrates of certain metabolic enzymes and transporters of which the developmental changes are not fully understood, results from the simulation should be interpreted carefully.

CONCLUSION/FUTURE DIRECTION

Collectively, international collaborative efforts have greatly improved the understanding of the role of transporters in drug PK, PD, safety and efficacy not only in adults but also in specific populations such as pediatrics. This understanding is supported by the expansion of knowledge in the ontogeny of membrane transporters, especially those in the liver and kidney. This increased knowledge has significant implications for PBPK modeling for drug substrates and therefore is of great importance for pediatric drug development. However, knowledge gaps in the ontogeny of transporters in the intestine and other important barrier tissues such as the blood-brain barrier remain, and are awaiting to be addressed through future collaborative work. Further investigation would also be required to elucidate how gene and protein expression relate to transporter activity. Lastly, as illustrated in the case study, in addition to ontogeny, a thorough understanding of the disposition of drugs and their interplay are critical in the application of PBPK to adequately predict drug exposure in children.

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