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HUMAN ONTOGENY OF DRUG TRANSPORTERS: REVIEW AND RECOMMENDATIONS OF THE PEDIATRIC TRANSPORTER WORKING GROUP

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INTRODUCTION

The critical importance of membrane-bound transporters in pharmacotherapy is widely recognized, but little is known about drug transporter activity in children. In this white paper, the Pediatric Transporter Working Group presents a systematic review of clinically relevant membrane transporters (e.g., SLC, ABC superfamilies) in intestine, liver and kidney. Different developmental patterns for individual transporters emerge, but much remains unknown. Recommendations to increase our understanding of membrane transporters in pediatric pharmacotherapy are presented.

SECTION I. OVERVIEW OF TRANSPORTERS IN PEDIATRICS

Transporters are membrane-bound proteins that are present in many tissues throughout the body, notably in the apical and basolateral membranes of organs involved in absorption and excretion such as the gastrointestinal tract, liver and kidney (Figure 1) (1). The biological role of transporters is to facilitate movement of important compounds across membranes; in addition to their physiological substrates, many transporters also have the capacity to transport drugs. It is now clear that drug transporters are critical determinants of tissue and cellular drug disposition, not only for the organs noted above, but also for sanctuary sites such as the brain (2). Moreover, the sodium taurocholate co-transporting polypeptide (NTCP), a hepatic bile acid transporter with affinity for some drug substrates, can act as a viral entry receptor for the hepatitis B virus (3). A considerable body of *in vitro* and animal work, and a growing body of *in vivo* adult data, have demonstrated that variations in the activity of drug transporters – whether on the basis of genetic differences, drug-drug interactions or environmental influences such as diet – impact the disposition of drugs and may influence drug efficacy and/or safety (2, 4). One important example is the statins, which are among the most commonly used medications in developed countries. It is clear that variations in transporter activity (e.g., OATP1B1) are key clinical determinants of statin-related myopathy. OATP1B1 activity can be modulated by polymorphisms in the *SLCO1B1* gene. In fact, this has led to changes in the product label by drug regulatory agencies with respect to the maximum dosages of some statins in specific ethnic populations (5, 6). This point is further highlighted by the Clinical Pharmacogenetics Implementation Consortium Guidelines, which recommend the use of haplotypes for dosing decisions of simvastatin (7). While there are a growing number of examples of the importance of drug transporters in adult medicine, there has been relatively little work in this area with respect to children, or the potential impact of ontogeny of transport function on clinically relevant outcomes.

Recent data suggest that variability in drug transporter activity may be critically important for safe and effective anti-leukemia drug therapy in children. A high-dose methotrexate study performed in 1,883 acute lymphoblastic leukemia patients enrolled in a multicenter Children's Oncology Group clinical trial revealed a relationship between multiple SNP variants in the OATP1B1 gene *SLCO1B1* and high-dose methotrexate toxicity (8). The study demonstrated that methotrexate clearance was lower in 1,279 patients carrying one of several loss-of-function SNP variants of *SLCO1B1*. Significant correlations also were established between lower clearance rates after high-dose methotrexate administration in

older children, girls, and in patients receiving delayed infusion of the drug (8). Lower doses, increased hydration and/or altered urine alkalinization were recommended, although the extent to which these adjustments would alleviate methotrexate toxicity was not clear. The impact of these *SLCO1B1* variants on the clearance of other drug substrates in children remains to be determined. Polymorphisms in the MRP2 gene *ABCC2* also have been associated with variability in methotrexate pharmacokinetics and an increased risk for methotrexate toxicity including leukopenia, thrombocytopenia, anemia, oral mucositis, and vomiting in children with acute lymphoblastic leukemia (9, 10).

The clinical relevance of transporter genetic variability and pediatric drug therapy extends beyond childhood leukemia. As an example, combined polymorphisms in *ABCC2* and the *UGT1A9* and *UGT2B7* genes can be important predictors of inter-individual variability in mycophenolic acid exposure, and have been associated with higher AUC in pediatric kidney transplant recipients (11). Lower morphine clearance was reported in children with defective OCT1 variants undergoing outpatient adenotonsillectomy. These findings are consistent with the role of OCT1 in the hepatic uptake of morphine (12). Racial differences in the allelic frequency of these variants may explain, in part, the higher incidence of morphine-related adverse effects in Caucasian compared with African-American children. Examples continue to emerge demonstrating the importance of transport proteins in determining drug efficacy and toxicity in the pediatric population.

SECTION II. METHODS TO STUDY DRUG TRANSPORTERS IN PEDIATRICS

Researchers have used primarily two approaches to characterize the ontogeny of transporters in the intestines, liver, and kidneys. The first approach is the quantification of transporter mRNA or protein expression in surgical or postmortem samples from humans at different ages. Typically, these studies are small in number and are not able to control for factors such as race/ethnicity, sex, environmental exposures, co-morbidities and other potentially important variables due to the limited sample size. The second approach is the assessment of transporter mRNA and/or protein levels, and to a lesser extent functional changes, in animal models such as rats and mice at various prenatal and postnatal ages.

Transport activity has been related to protein expression levels in adult tissue samples assuming that the transporter is localized predominantly in the plasma membrane, and that there is a correlation between the protein expression level and transporter function. Given that transporter expression at the mRNA and protein level don’t always correlate well, it becomes important to understand transporter ontogeny at the protein level. Protein expression levels of membrane transporters have been estimated traditionally by Western blot analysis using highly sensitive and specific, antibody-based methods. However, mass spectrometry-based targeted proteomics is being used increasingly to provide a quantitative assessment of protein expression levels.

SECTION III. ONTOGENY OF TRANSPORTERS

Key transport proteins relevant to drug disposition in the intestine, liver and kidney were selected based on recent reviews by the International Transporter Consortium (see Figure 1 for protein and gene nomenclature and localization) (1, 13). A systematic search of the

PubMed and EMBASE databases was performed to identify relevant published studies of drug transporters within these three organs. The search strategies were adapted to accommodate the unique searching features of each database, including database-specific MeSH and Emtree controlled vocabulary terms. Search queries for each transporter were combined with matching queries for the gastrointestinal tract (hereafter referred to as the intestine), the liver or the kidney. Searches were limited to the pediatric population but were not limited by date, language or publication status (see Appendix I for a detailed summary of the search strategies). The literature was reviewed, by organ, to evaluate changes in transporter expression with age, and the role that individual transporters may play from a developmental biology perspective. In addition, the age at which transporters reach adult levels and children "functionally" become adults with respect to transporter activity was evaluated, where possible, for each transporter. The following sections and accompanying tables summarize the relevant pediatric transporter data recovered from the literature. Transport proteins depicted in Figure 1 were excluded from discussion for a particular organ if no pediatric data were available, or if the data were so limited that a broader discussion of the temporal or spatial expression, or activity as a function of development was not possible. In all cases, human proteins are denoted by upper case text and rodent proteins by lower case text, with gene nomenclature in italics.

INTESTINE

We searched for human and animal studies exploring the developmental changes of the following intestinal transporters: MDR1 P-glycoprotein (P-gp), BCRP, MRP1, MRP2, MRP3, OATP2B1, OATP1A2, OCT1, MCT1 and PEPT1. Only original, peer-reviewed research publications that explicitly presented fetal and/or pediatric data on transporter expression and/or localization were included. The focus was on human data, but in the absence of convincing human data, relevant animal data are presented as they may provide an indication of the expected developmental changes in humans (Table 1). When reported, the intestinal localization of the protein is denoted. For the fetal studies, no precise localization patterns are available due to intestinal immaturity.

P-gp—P-gp was the intestinal transport protein for which there is the most data relevant to pediatrics. Numerous studies were evaluated to determine developmental differences in expression or localization of P-gp in the intestine. Collectively samples from 55 fetuses, ranging from 5.5 to 28 weeks of gestation, and 302 additional samples across a pediatric age range, were investigated in this group of studies (14–21). The majority of mRNA samples (upper jejunum) originated from one study in pediatric liver transplant patients, which may present confounding variation based on hepatic disorders (14). In most studies, P-gp mRNA expression was determined; localization with immunohistochemistry was only conducted in a small number of samples. All of the studies demonstrated large inter-individual variation. The overall developmental pattern of P-gp expression revealed a consistently emerging change from undetectable expression in the first trimester of fetal life to present and apparently stable P-gp expression from approximately 12 weeks of gestation onwards. Very limited data suggest that P-gp expression increased slowly to reach adult levels at or very shortly after birth. Interestingly, two studies reported higher intestinal P-gp expression in children with treated Crohn's and celiac disease compared to healthy controls (15).

BCRP—The localization of BCRP staining appeared stable from 5.5 to 28 weeks of gestation in humans (21).

MRP1—In 35 human fetal samples from 5.5 to 28 weeks of gestation, MRP1 staining was visualized in all samples using immunohistochemistry (21). The intensity was weak during early gestation, but appeared to mature to an adult distribution pattern from 7 weeks of gestation onwards.

MRP2—Analogous to P-gp, the mRNA expression of MRP2 appeared stable from neonates to adults in ileal and jejunal surgical samples (20).

OATP2B1—In contrast to MRP2, OATP2B1 mRNA expression was significantly higher in the samples from neonates compared to adults (20).

Other intestinal transport proteins—Human data for MRP3 and PEPT1 appear to be missing, but animal data are available. In rabbits, Mrp3 mRNA expression was lowest in newborns and subsequently increased in weanlings until reaching the highest levels in adults (22). The four available animal studies (three in rat; one in turkey) on PepT1 all support a similar developmental pattern of significant prenatal expression that reached maximal levels in the postnatal period, and then decreased to adult levels from weaning onwards (23–26). This finding seems consistent with the role of PEPT1 in the absorption of di- and tripeptides, which constitutes an important part of infant nutrition. Intestinal temporal and spatial expression data during development for OATP1A2, OCT1 and MCT1 appear to be lacking.

It is clear from the data in Table 1 that no single developmental pattern can be identified for all of the intestinal transporters. Different patterns are apparent based on the available, albeit limited human data: 1) low in the embryo and then stable from neonate to adult (e.g. P-gp, MRP2); 2) high at birth and decreasing in the first months of life (OATP2B1).

LIVER

For the liver, all transport proteins designated by the International Transporter Consortium as important for disposition of drugs and endogenous substances were reviewed (1, 13). These include the efflux transporters P-gp, BCRP, MRP2, MRP3, MRP4, MRP6, MATE1, BSEP, the uptake transporters NTCP, OATP1B1, OATP1B3, OATP2B1, OAT2, OCT1, and the bidirectional transporters OAT7 and OSTα/β. Similar to the other organs, this review on hepatic transporters exclusively focuses on human data, if available, but resorts to animal data in the absence of any relevant human information (Table 2). Similar to intestinal transporters, most published data on the ontogeny of hepatic transport proteins is available for efflux transporters, particularly P-gp.

P-gp—Hepatic P-gp was already detectable in the wall of bile canaliculi in early fetal life at 14 weeks by immunohistochemistry and at the mRNA level (18). Although expression was low initially, it seemed to increase throughout fetal development and was considered moderate at the protein level by fetal week 19 (16, 27). mRNA expression for P-gp increased throughout childhood development. In a study with 61 liver specimens, mRNA expression levels were 20- to 30-fold lower in the fetal and neonatal age group compared to

adults. mRNA expression, however, rapidly increased in the early months of life: in infants (1–12 months), mRNA was only 5-fold lower compared to adults, and in children and adolescents it was indistinguishable from adult expression (20). These observations are supported by less extensive studies from others (28), and also revealed a similarly high inter-individual variability in expression in children compared to adults (20, 29). The limited results on P-gp protein expression, however, do not corroborate the reported age-dependent P-gp mRNA expression. P-gp protein was detectable in samples as early as 1 month (29), and relative protein expression was not significantly different in 65 liver specimens from age groups 0.3–0.7, 0.7–2, 2–5, and 5–12 years (30). This observation may not be surprising in light of the observed lack of correlation between mRNA and protein levels for P-gp in adult liver (31). P-gp protein expression quantified by LC-MS/MS was not associated with age in 64 liver specimens in the age range of 7–70 years (32). Thus, further studies are needed to clarify whether the age-dependent differences in mRNA expression translate into differences at the protein and ultimately the functional level for P-gp in the liver.

BCRP—BCRP was detectable by immunohistochemistry in fetal liver specimens from 5.5 to 28 weeks (21), and mRNA expression increased from 18–22 week fetal samples to adults (33). A comparison of relative protein expression between 5 neonates and 5 adult livers by Western blot analysis indicated no detectable difference (34), although mRNA expression seemed to increase between neonates and older children (28). In 50 livers from age 7 to 70 years, BCRP protein expression was correlated neither with age nor with mRNA expression (35). These results together suggest that hepatic BCRP is expressed early during human development and does not undergo relevant developmental changes after term birth.

MRP2—Similar to P-gp, MRP2 has been detected by immunohistochemistry in the bile canalicular membranes of 14-week old fetuses, with a tendency for higher expression levels in older fetal liver (27) and adults (33, 36). The differences in age-dependent expression seem to be similar to P-gp in fetal liver (30-fold lower mRNA expression), but were substantially more pronounced in neonates (200-fold lower mRNA expression) and infants (1–12 months) (100-fold lower mRNA expression) compared to adults (20, 28). These substantial differences at the transcriptional level seem to translate into developmental differences in MRP2 protein expression: MRP2 protein levels determined by Western blot were significantly lower in liver specimens from children younger than 8 months (n=24) compared to older children up to 12 years (n=59) (30). Later in childhood development, however, MRP2 protein expression assessed by mass spectrometry was independent of age in 51 liver specimens in the age range of 7–63 years (37).

BSEP—At gestational weeks 14–20, BSEP was detectable by immunohistochemistry (36), and mRNA expression increased from neonates to older children (28) and adults (33). Functional studies in isolated sandwich-cultured fetal and adult hepatocytes suggest that the biliary excretion index for taurocholate is substantially higher in adults compared to fetal hepatocytes. This higher functional activity for the BSEP substrate taurocholate could be explained by a higher expression level of BSEP in adult cells, assuming that there is a correlation between BSEP mRNA and protein expression (33).

NTCP—NTCP was detectable by immunohistochemistry in fetal liver specimens at 14–20 weeks of gestation (36), and mRNA expression was reduced substantially to 4% of adult values in fetal livers of 18–22 weeks of gestational age (33). At the protein level postpartum, NTCP expression in neonates was comparable to that in adults (34). Thus, there also seems to be no developmental maturation of NTCP expression after birth in humans. Rodent data suggest, however, that acquisition of functional transporter activity lags behind the developmental trajectories of mRNA and immunoreactive protein, and is not present until glycosylation is mature (38).

OATPs—mRNA for the OATP isoforms OATP1B1, OATP1B3, and OATP2B1 was detectable in fetal hepatocytes by gestational weeks 18–23, and was significantly higher in adults compared to fetal livers for OATP1B1 and OATP2B1 (33). In a limited number of neonatal and adult liver specimens (n=5 each), no relevant difference was observed in OATP1B1 or OATP1B3 expression as determined by Western blot analysis (34). mRNA expression in 45 liver specimens, however, was found to be highly age-dependent. For OATP1B1, mRNA expression was 20-fold lower in fetal liver, 500-fold lower in neonates, and 90-fold lower in infants compared to adults (20). For OATP1B3, mRNA expression was 30-fold lower in fetuses, 600-fold lower in neonates, and 100-fold lower in infants (1–12 months) than in adults (20). These data are supported by Western blot analyses based on relative protein quantification in 78 liver samples that suggest a low expression from birth to age 6 years with increased expression thereafter for OATP1B1, and high expression for OATP1B3 at birth which declines over the first month of life, and then rises again by age 6 years OATP1B2 (39). In 64 livers from age 7 to 70 years, relative protein expression of OATP1B1, OATP1B3, and OATP2B1 as assessed by mass spectrometry did not correlate with age (32). **OCT1.** There is only very limited human data on the ontogeny of hepatic OCT1. In human hepatocytes from pediatric and adult livers, there was no significant difference in the mRNA expression of OCT1, but OCT1-mediated transport seemed lower in pediatric compared to adult hepatocytes (40).

Other liver transport proteins—For MRP3, MRP4, MRP6, MATE1, OAT2, OAT7, and OSTα/β, there are no or only very limited data available on the human ontogeny of these transporters. Thus, observations from rodent species also are provided in the following section, although there is so far no indication that rodent protein, and especially mRNA expression profiles, are in any way predictive of human transporter ontogeny.

MRP3—In humans, MRP3 mRNA expression was significantly lower in fetal hepatocytes by gestational weeks 18–23 compared to adults (33). Similarly, Mrp3 in fetal rat liver progressively increased from about 10% to over 30% of the maternal mRNA levels from day 15 of gestation to day 20, and increased to near 90% of the maternal level at day 21 and after birth (41). This is consistent with increased mRNA expression observed from neonates to older children and adults in a small set of human specimens (perinatal n=6, children n=8, adult n=6) (28).

MRP4—In humans, mRNA expression for MRP4 did not show any significant differences in fetal hepatocytes by gestational week 18–23 compared to adults (33), or when comparing

neonates to older children and adults (28). These observations are supported by mRNA expression in mice (42, 43).

MRP6—Similar to MRP3, MRP6 mRNA expression increased in humans from neonates to older children and adults in a small set of subjects (28). In rat liver, Mrp6 mRNA expression was detectable on embryonic day 16 at 5% of adult levels, and increased to 40% at birth, but did not reach adult levels until postnatal day 29 (44).

MATE1—mRNA expression for MATE1 increased in humans from neonates to older children and adults in a very small set of subjects (28). Mate1 mRNA expression was absent on embryonic day 7.5 in mice (45).

OAT2—Similar to MRP3 and MRP6, OAT2 mRNA expression increased from neonates to older children and adults in a small set of human subjects (28).

OAT7—No information could be found on the ontogeny of OAT7 in humans or rodents.

OSTα/β—mRNA expression was detectable for OSTα and OSTβ in pediatric liver with an age around 1 year (46). Although Ostα mRNA is expressed at low levels in liver throughout development from day -2 to day 45 in mice, Ostβ mRNA markedly increased to 4.5-fold of prenatal levels with a peak around 1 day after birth (47).

Overall, there are limited data available on the human ontogeny of hepatic transport proteins. The emerging picture, however, suggests that there may be substantial differences between transporters in the time course of development and expression. Some transport proteins such as P-gp, BCRP, and NTCP are expressed early in childhood development, while others such as OATP1B1, BSEP and MRP2 seem to exhibit delayed maturation and reduced expression levels compared to adults during at least the first months of life. In general, differences seem to be absent between older children and adults. This conclusion is supported by the notion that localization of the canalicular transporters (BSEP, P-gp, MRP2) in pediatric liver (6–17 months) had reached a similar level and pattern as adult liver indicating that the pediatric liver around 1 year of age has obtained a mature canalicular structure (46).

KIDNEY

The drug transporters generally considered in the context of kidney development were: P-gp, BCRP, OAT1, OAT3, OCT2 and MATEs. Data on MRP2, MRP4, OATPs, OAT2, OCT1, OCTNs and URAT1 (Rst) also were evaluated when presented in literature regarding the aforementioned transporters. Currently, there is relatively little information regarding drug transporter expression, at either the mRNA or protein level, or function in the human developing kidney. Given the paucity of human data, the studies described in Table 3 and Appendix 2 also include the considerable data on the ontogeny of drug transporters in the developing rodent (rat, mouse) kidney. In a limited number of instances, there are also data on postnatal developmental function of particular transporters, for instance, in a knockout mouse model or a study on the developmental clearance of a drug (digoxin) or probe substrate (p-aminohippurate, PAH) in rodents. Although there are differences in individual

transporter expression patterns (eg. P-gp, Bcrp, Mrp2, Mrp4, Mate1, Oat1, Oat3, Oct2, Octn1, Octn2, Urat1), these rodent studies generally indicate low expression of various transporters in the late stages of kidney development, followed by a rapid rise in expression after birth, and a further increase in expression (and function) during postnatal maturation (48–50). Please see Table 3 and Appendix 2 for additional rodent information.

P-gp—In humans, P-gp transcript and protein levels have been analyzed in fetal and adult kidney (17, 18). P-gp expression is detectable as early as 11 weeks of gestation (18). In the fetal kidney, RT-PCR of tissue obtained by laser capture microdissection revealed transcripts in the renal tubule (17).

It is important to note that developmental biology studies suggest that there may be some significant differences in postnatal nephron development and maturation between mice, rats and humans, as well as between sexes. Of note, recent mRNA data regarding the developmental expression of renal transporters in humans (51), presented as an abstract, appears largely consistent with patterns reported in rats (52).

In summary, information on the ontogeny of human drug transporters is scarce in the case of the kidney. A major knowledge gap exists regarding gene expression, protein abundance and actual transporter activity in humans. This information is essential to understand how maturational changes impact the role that these transporters play in normal growth and development, and to accurately predict the impact of changes due to pathophysiological conditions on drug disposition, efficacy and toxicity of medications. This knowledge is requisite to the development of personalized drug therapy in children.

SECTION IV. COMPARATIVE DEVELOPMENT OF ORGAN FUNCTIONS

The majority of the data regarding transporter ontogeny has been obtained from developmental studies in rodents, although some literature does exist for larger species. The ability to extrapolate across species, namely rodents to larger species (e.g. primates) and humans, can be limited, however, by variation in the developmental timing of key anatomical, physiological, biochemical, and physicochemical events, as well as significant functional variance in isoforms. These differences arise from comparative differences in the gestational length and the timing of parturition between the different species and humans. The conventional approach is to compare developmental milestones relative to birth (prenatal versus postnatal); however, this may not always be appropriate. For example, in contrast to humans and porcine models, newborn rodents exhibit relatively immature intestines with few villi and little evidence of crypt formation (53, 54). The timing of tissue maturation between rodent species is also dependent upon the organ. As an example, nephrogenesis is largely complete prior to birth in mice, which is similar to humans, but continues in rats during the postnatal period (55). For this reason, the study of transporter ontogeny would be improved by reporting perinatal findings as days post-conception, rather than days relative to parturition. By viewing development as a continuum instead of arbitrary categories (such as neonatal, infant, etc.), there will be greater potential to translate rodent and other mammalian studies to humans. One caveat to this approach is that some

transporters increase in expression upon commencement of feeding as has been observed for Ntcp, Bsep, and Mrp4 in the neonatal livers of mice (42).

It should be noted that another considerable limitation occurs when contrasting mRNA expression data, because linearity in protein transcription and inferred function cannot be assumed. As mentioned in the Methods section, advances in quantitative proteomics using LC-MS/MS have led to a significant increase in our ability to quantify drug transporter protein abundance in adult samples. While a number of technical challenges still exist with this methodology, the application of quantitative proteomic approaches to ontogenic studies with pediatric tissues will yield more useful data for establishing predictive developmental PBPK models. One major limitation is that the availability of human pediatric tissue specimens is limited, and shared pediatric biobanks need to be established. The application of quantitative proteomics in animal tissue developmental studies would provide greater insight into the utility of scaling across species to predict the function of drug transporter activity in pediatric patients.

There is also the potential for significant confounding variables to limit the ability of cross species comparisons with humans, particularly with respect to pediatric populations. Of considerable concern is the health of the patients in which the specimens were obtained. A significant portion of the human pediatric tissue biopsied specimens originate from patients who suffer from co-morbidity or are collected postmortem. Collection procedures and timing also can impact tissue quality and subsequent expression data. Most animal studies are conducted under controlled conditions, whereas human tissue specimens are collected from patients who will have varying xenobiotic and dietary exposures. These confounding factors cannot be readily normalized for in many cases. Another emerging issue is that mRNA expression of housekeeper genes can vary significantly, thus calling into question quantitative data from qRT-PCR studies normalized to one control (56, 57). These concerns may be alleviated by use of RNA-sequencing to quantify human transporter ontogeny, as has been performed in rodents (47).

There has been limited use of cell-based systems and mathematical models to describe the ontogeny of transporters. However, there may be the potential to complement *in vivo* rodent, other species, and human biopsy studies with human embryonic or induced pluripotent stem cells undergoing differentiation to hepatocytes, enterocytes, and renal tubule cells. While these cellular systems are artificial and lack the holistic development of an organism, they may be a mechanistic tool to probe the effects of exogenous factors such as hormones, drugs, and exposures on the sequence and timing of transporter expression. Initial studies have begun to profile the expression and activity of transporters in hepatocyte-like cells derived from human embryonic and induced pluripotent stem cells (58), although comparisons to juvenile human livers are needed. In addition, there is a need to develop mathematical and statistical modeling approaches that integrate transporter ontogeny with the maturation of physiological processes, such as tubule reabsorption or intestinal secretion. This effort would provide researchers with the ability to ‘translate time’ between species by developing algorithms that compare and predict development (59, 60). Moreover, this approach can integrate the ontogeny of multiple tissues in parallel and provide a more global view on whole organism development. A computational and systems biology approach

could be used to integrate genomic, epigenetic, proteomic, and pharmacokinetic endpoints (e.g., changes in pH, plasma membrane composition, expression of drug metabolizing enzymes) to better assess the ontogeny of transport systems (61). The utility of data driven, physiologically-based pharmacokinetic models generated in this fashion would help to improve new pediatric drug translation from discovery to the clinical stages (62).

SECTION V. EMERGING AREAS AND MAJOR CHALLENGES IN STUDYING PEDIATRIC DRUG TRANSPORT

Developmental programming and regulation of transporters

Little is known about the factors that govern the regulation of transporter expression and activity (e.g., induction and inhibition of transporters as a function of gestational age) during growth and development. The age-related variation in mRNA expression of the transcription factor PXR correlated with P-gp expression in a small number of fetal, neonatal, younger and older adult samples of human liver, kidney and intestine (17). This finding suggests a role for transcription factor-mediated regulation of age-related transporter expression. Moreover, the mechanism of age-related changes in transcription factors may be related to DNA methylation; in fetal liver, hypermethylation of important cytochrome P450 (CYP) 3A4 transcription factor binding sites has been observed, consistent with low CYP3A4 expression before birth (63, 64). Endocrine changes in adolescents may impact drug transporter expression through hormonal and growth factor regulation of relevant transcription factors To date, studies focused on the endocrine regulation of transporters have largely evaluated adult rodents (65, 66) and serve as a basis for future work that should be expanded to hormonal fluctuations during human development. Clearly, considerable work is needed to understand the factors that regulate drug transporters during human growth and development.

Pharmacogenomics

Genetic variation may add to the age-related variation in drug transporter expression and function. In contrast to adults, pharmacogenomic drug transporter studies in children are rare. The available studies have been performed primarily in age ranges at which full maturation of transporters can be expected, and results in general are similar to adults (12, 67). Further studies in children are needed to elucidate the interplay of age and genetic variation. Decreased transporter expression due to genetic variation may not become apparent until expression is at least at adult levels (68).

Impact of disease, drug interactions and/or environmental exposure

Other factors impacting drug transporter activity are disease, drug-gene interactions, drug-drug interactions (DDIs), food-drug interactions as well as exposures to environmental chemicals. In adults, the impact of liver disease has been investigated, but the findings may not be applicable to children, as the underlying disease may be very different: e.g., alcohol-induced liver steatosis is not a pediatric disease, while biliary atresia is the most prevalent disease in children who receive a liver transplant. As underlying liver disease may impact transporter expression (69, 70), these pathophysiological differences mandate studies in

pediatric patients. In one study, MRP2, BSEP and MDR3 expression in livers from patients with pediatric biliary atresia taken at post-natal age 1–2 months was much higher than both fetal and adult expression levels, but unfortunately no age-matched controls were studied (36). Hence, it is unclear whether the observed differences can be attributed solely to the disease or whether age-related changes also played a role. DDIs also may be different in children due to developmental changes in drug disposition pathways; furthermore, the potential for a specific DDI may change during growth and development (71). Finally, as nutrition changes during childhood, the impact of frequent milk or different types of formula feedings and fruit juice on drug transporter expression and function must be considered.

Development of human-relevant cell/*in vitro* and preclinical/*in vivo* transport models that are representative of the pediatric population in health and disease

Once transport protein expression and function have been characterized across the pediatric age spectrum in healthy and diseased tissue, an important next step will be to develop human-relevant *in vitro* models, such as cell lines, embryonic stem cells, or modified primary cells, that mimic transporter expression and function at various ages. Such systems could be useful to predict drug disposition and DDIs in pediatrics. The utility of preclinical, *in vivo* transporter models as a predictive tool is less clear due to significant species differences in the expression and function of some transport proteins and regulatory machinery, and lack of established correlations between transporter data from animal models and humans.

Development of systems-based, mechanistic modeling approaches to integrate *in vitro* data and physiological processes to predict transporter-mediated changes in drug disposition as a function of age and disease

Physiologically-based pharmacokinetic models (PBPK) link information about the anatomical and physiological structure of the body with the physicochemical and biopharmaceutical properties of the drug to predict drug disposition in the body. PBPK models are established tools for predicting human pharmacokinetics based on preclinical data from animals and *in vitro* studies (72, 73). Successful applications include scaling the human pharmacokinetics from healthy volunteers to patient populations (e.g., liver cirrhosis) by accounting for changes in physiology (74, 75). PBPK models are being applied increasingly in scaling adult pharmacokinetics to pediatric populations. This has been demonstrated for compounds eliminated primarily by metabolism, where the ontogeny has been better characterized than for some transporters, (76, 77). The use of modeling and simulation methods in pediatric drug development was endorsed at a recent United States Food and Drug Administration (FDA) Clinical Pharmacology Advisory Committee meeting (78), and also is recognized by the agency as a potentially useful tool in the design of pediatric clinical trials and helping to expedite pediatric drug development (79). Between the years 2008 and 2012, the FDA's Office of Clinical Pharmacology received 33 New Drug Application/Investigational New Drug submissions containing PBPK models; six of them were pediatric submissions (80). The main applications of pediatric PBPK models in these submissions included dose selection, study design, informing enzyme ontogeny using benchmark drugs, and facilitating covariate analysis. The applications in pediatric drug development generally start with development and validation of an adult model (frequently

utilizing *in vitro* characterization of the drug's interaction with enzymes and/or transporters) followed by scaling to pediatric populations by accounting for ontogeny of relevant physiological processes across the age continuum from neonates (including premature neonates) to adults. However, currently there is limited availability of transporter information for modeling and simulation (e.g., PBPK modeling) of drug disposition, tissue exposure, and pharmacodynamic response in pediatrics. A possible approach to circumvent this issue is to utilize available pediatric clinical data for a variety of drugs to estimate the ontogeny of relevant enzymes and transporters. In one example, the ontogeny of renal transport was estimated from age-dependent renal clearance of a model compound known to be a substrate for the same renal transporter as the investigated drug (81). In another example, a pediatric population model for zidovudine was constructed by utilizing a previously derived pediatric covariate model for morphine glucuronidation (82). Finally, it is worth noting that systems biology approaches, as well as methods for integration of "omics" data from multiple levels of analysis (e.g., genomics, transcriptomics, proteomics, metabolomics) are advancing rapidly, and there is an ongoing effort to apply these approaches to the adult and developmental contexts (83). While distinct from the usual methods used in PBPK, it is anticipated that these approaches may begin to converge in the near future, providing a deeper understanding of pediatric drug disposition.

Limited availability of quality pediatric tissue (all age groups) for protein quantification and assessment of transporter function

One of the major roadblocks to drug transporter research during human growth and development is the dearth of quality pediatric tissue. Current tissue sources include left-over tissue from surgery and biopsies, as well as postmortem tissue from organ transplants and autopsies. Collaboration between clinicians with access to these tissue sources and researchers in need of tissue appears to be a major obstacle. In order to overcome these logistical challenges, a clear understanding and commitment on both sides regarding the respective challenges and solutions may be the first step to increase the availability of quality pediatric tissue. For example, the logistics of collecting a sample, including asking for informed consent, retrieving a dry ice container, transporting tissue to a storage facility, and collecting clinical data seems relatively straightforward, but is challenging when success depends on busy clinical staff to organize all these details. Also, a few inches of residual intestine may be very reasonable to obtain in adults, but cannot be considered 'leftover' in neonates with a considerably shorter intestinal length. Timely handling of postmortem pediatric tissue is particularly challenging due to parents, who may need time to say goodbye when a child passes away, and the availability of autopsies only during the day. Furthermore, the availability of tissue may be limited compared to adults because the death rate among children is much lower than among adults, there may be reluctance on the part of parents to give consent for autopsy, and there are relatively few liver transplants from pediatric donors.

Ethical and practical challenges with performing non-therapeutic studies in minors

Research that does not potentially benefit the participating child ('non-therapeutic') is subject to several limitations (84), most importantly, the restrictions of minimal risk (a slight increase over what is ordinarily encountered in daily life) and minimal burden. These ethical

limitations, which are intended to protect the individual child, limit the possibility of performing non-therapeutic pharmacokinetics and/or DDI studies related to drug transporters in minors. Giving a child a therapeutic dose of a drug solely for these purposes will, in many places, not be considered minimal risk. However, in some centers this approach is acceptable when the child will receive the drug later for therapeutic reasons. One solution is to study the disposition and effect of the drug in the context of clinical drug treatment. This may introduce unwanted complexity to studies, such as variation due to underlying disease and/or co-medication, and the need for more sophisticated pharmacokinetic analyses. Microdosing may overcome these limitations, but can only be used for drugs that exhibit dose-linearity (85). Other challenges include the need for repeated blood sampling as well as limitations with blood volumes. These can be overcome by using indwelling catheters already in place for clinical care, low volume drug assays, and population pharmacokinetic analyses.

Lack of transporter-specific probes to assess *in vivo* function

In vivo probes for individual drug metabolizing enzymes (e.g., midazolam for CYP3A4, dextromethorphan for CYP2D6/CYP3A4 and caffeine for CYP1A2) have facilitated investigations about the impact of growth and development, and the effects of pharmacogenomics and disease, on drug metabolism. These critical tools have led to new knowledge. A similar approach is more challenging for drug transporters, as specific probes for individual transporters are lacking. Most drugs are substrates for multiple transporters, which enable alternate transporter pathways to compensate in case the primary transporter is absent or has reduced activity. Nevertheless, pharmacogenomic studies on individual transporters have elucidated differences in drug disposition, efficacy and safety in adults. This approach may aid in studying the developmental changes of these transporters *in vivo*. The use of microdosing in pediatric patients has been explored and may provide a basis for developing a better understanding of drug disposition and metabolomic profiling. While it is important to continue to analyze mRNA and protein expression levels of transporters in developing organs like the liver, kidney and intestine, ultimately this must be related to physiological processes mediated by these particular transporters. Because of unique safety concerns about using exogenous compounds as functional probes in the pediatric population, continued exploration of endogenous metabolites and other markers that can serve as surrogates for assessing transporter function during organ development and maturation is needed. The relevant sets of endogenous metabolites may be specific to each organ, and to particular developmental points, reflecting unique patterns of transporter expression and aspects of organ-specific physiology.

SECTION VI. CONCLUSIONS

Many fundamental and clinically relevant questions remain unanswered about the human ontogeny of drug transporters. As information highlighting the importance of drug transporters in adult medicine continues to emerge, this critical knowledge gap in the pediatric population becomes even more evident. In order to achieve safe and effective drug therapy for children, it is imperative that developmental patterns of transporter gene expression and protein abundance are elucidated, and that drug transporter function is

defined across the age spectrum. Recommendations are provided (Table 4) to address some of the major challenges in obtaining this information. Fundamental and applied knowledge about the human ontogeny of drug transporters is absolutely essential to ultimately achieve personalized pharmacotherapy in pediatric patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

Abbreviations and notations used throughout the text, tables and figures are defined as follows

ABC	ATP-binding cassette
AUC	area under the curve
BCRP (*ABCG2*)	breast cancer resistance protein
BSEP (*ABCB11*)	bile salt export pump
CYP	Cytochrome P450
DDI	drug-drug interaction
IHC	immunohistochemistry
MATE (*SLC47A*)	multidrug and toxin extrusion protein
MDR1 P-glycoprotein (P-gp *ABCB1)*	multi-drug resistance 1 P-glycoprotein
MRP (ABCC)	multidrug resistance-associated protein
NTCP (*SLC10A1)*	Na^+-taurocholate co-transporting polypeptide
OAT (*SLC22A*)	organic anion transporter
OATP (SLCO)	organic anion transporting polypeptide
OCTN (*SLC22A*)	organic cation/ergothioneine transporter

OCT (*SLC22A*)	organic cation transporter
OSTα/β	organic solute transporter
PAH	p-aminohippurate
PEPT (*SLC15A*)	peptide transporter
PXR	pregnane X receptor
SLC	Solute carrier
PBPK	physiologically based pharmacokinetic
UGT	UDP-glucuronosyltransferase
URAT1	urate transporter 1 (*SCL22A12*)

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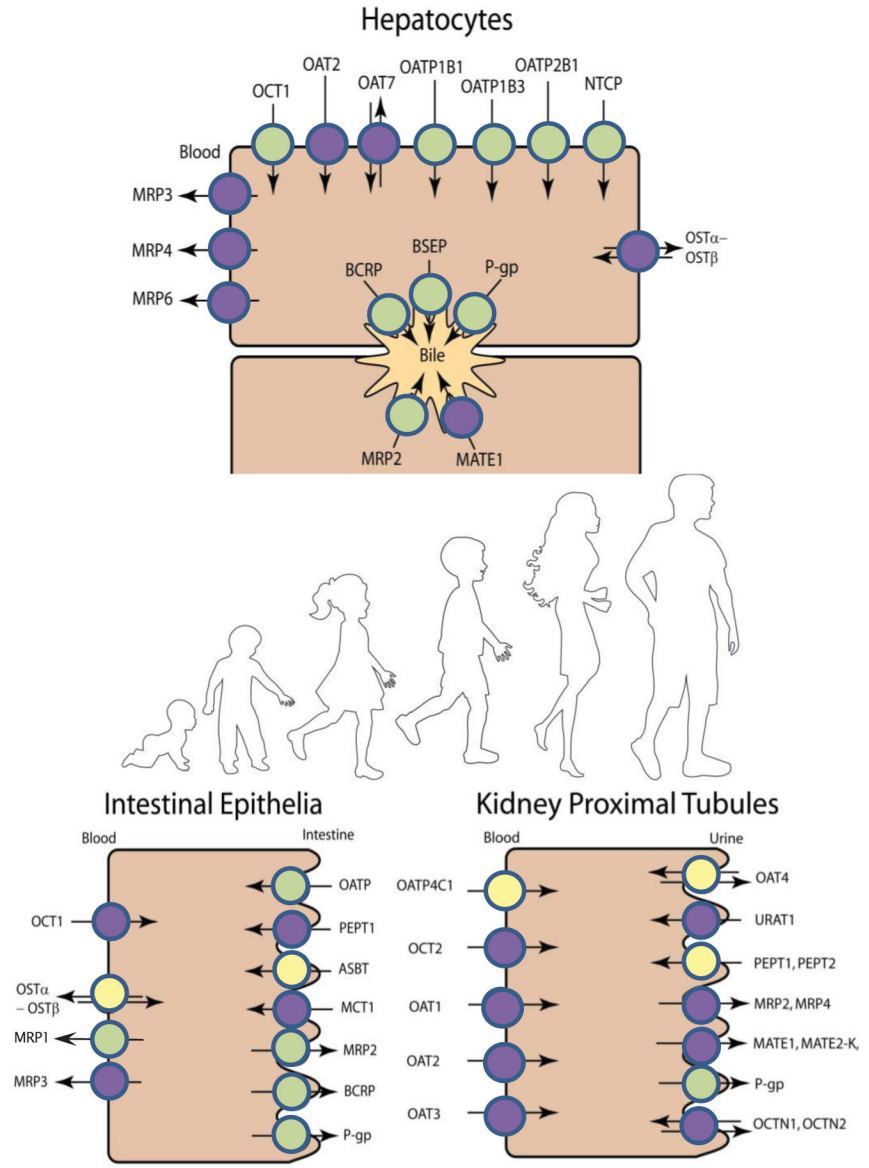

Figure 1. Human Transport Proteins for Drugs and Endogenous Substances

Schemes depict localization of transporters [protein (*gene*) nomenclature] on the apical (luminal) and basolateral membrane of human intestinal epithelia, hepatocytes, and kidney proximal tubule cells. Developmental changes have been reported for some human transporters (green circles), but no information, or only limited data are available for other transporters (purple circles). Yellow circles depict other drug and/or endogenous substrate transporters that were not included in the present literature search. Transporters recommended for evaluation in the 2012 FDA Draft Drug Interaction Guidance include: MDR1 P-glycoprotein (P-gp; *ABCB1*), breast cancer resistance protein (BCRP; *ABCG2*); two members of the organic anion transporting polypeptide (OATP) family [OATP1B1 (*SLCO1B1*), OATP1B3 (*SLCO1B3*)]; two members of the organic anion transporter (OAT) family [OAT1 (*SLC22A6*), OAT3 (*SLC22A8*)]; and organic cation transporter 2 [OCT2

(*SLC22A2*)]. Transporters proposed for prospective investigation in drug development include: multidrug and toxin extrusion protein 1 and 2 [MATE1 (*SLC47A1*) and MATE2-K (*SLC47A2*)]. Transporters recommended for retrospective inhibition studies based on preclinical and clinical observations include: multidrug resistance-associated protein 2 [MRP2 (*ABCC2*)] and bile salt export pump [BSEP (*ABCB11*)]. Other transporters that are of importance include: peptide transporter 1 and 2 [PEPT1 (*SLC15A1*) and PEPT2 (*SLC15A2*)]; ileal apical sodium/bile acid co-transporter [ASBT (*SLC10A2*)]; monocarboxylic acid transporter 1 [MCT1 (*SLC16A1*)]; OCT1 (*SLC22A1*); heteromeric organic solute transporter (OSTα–OSTβ); sodium/taurocholate co-transporting polypeptide [NTCP (*SLC10A1*)]; OATP2B1 (*SLCO2B1*)); OAT2 (*SLC22A7*); OAT7 (*SLC22A9*); MRP3 (*ABCC3*); MRP4 (*ABCC4*); MRP6 (*ABCC6*); OAT4 (*SLC22A11*); urate transporter 1 [URAT1 (*SCL22A12*)]; organic cation/ergothioneine transporter 1 and 2 [OCTN1 (*SLC22A4*)] and OCTN2 (*SLC22A5*)]; OATP4C1 (*SLCO4C1*); and OAT3 (*SLC22A8*). Adapted from Giacomini et al. (1) and Zamek-Gliszczynski et al. (13).

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Table 1

Protein	Human/ animal	Age range and number of samples	Localization	Methods used	Major results	Reference First author
P-gp BCRP MRP1	Human Postmortem	N=35 homogenously distributed from the 5.5th – 28th week of intrauterine development (IUD)	Small intestine, not further specified	Immunohistochemistry (IHC)	A positive reaction for P-gp was not present in the early stages of gestation. From the 12th week of IUD onwards, P-gp was clearly present on the epithelial membrane. P-gp was localized on the apical membrane of enterocytes. BCRP was detected in both enterocyte membranes and in the cytoplasm of the epithelium at all gestational ages studied. MRP1 showed very weak staining around the 6th week of gestation. The staining of nuclei in enterocytes began to occur later and up to the 9th week of IUD, there was irregular localization of positive nuclei. Cell staining shifted to the apex of villi with the development of simple columnar epithelium.	[21] Konieczna
P-gp OATP2B1 MRP2	Human Surgical	surgical small bowel samples (neonates n=15, infants n=3, adults n=14)	Ileum and jejunum	mRNA (RT-PCR)	Neonatal intestinal expression of P-gp was comparable to adult. Intestinal OATP2B1 expression in neonates was significantly higher than in adults. Neonatal intestinal MRP2 mRNA expression was comparable to adults.	[20] Mooij
P-gp	Human Liver transplant recipients	N=206 median age (range), 1.27 years (62 days 18.9 years)	Jejunum from part of the Roux-en Y limb for biliary reconstruction	mRNA (RT-PCR)	Intestinal P-gp mRNA reached adult levels shortly after birth. Large inter-individual variability in intestinal P-gp expression was observed across all ages.	[14] Mizuno
P-gp	Human Healthy	N=59: aged 1 month to 1 year (n = 19); 1 to 6 years (n = 16); patients > 6 years of age (n=24).	Duodenum	mRNA (RT-PCR)	P-gp mRNA was detected in all the samples. Expression was highly variable between samples, with the P-gp/villin ratio ranging from 0.005 to 4, with nine patients having a ratio >1.3. No significant relation was found between P-gp mRNA expression and age (p = 0.195). 18S rRNA and villin mRNA levels were not different among the three groups.	[19] Fakhoury
P-gp	Human Postmortem	N=3 Prenatal: 1 fetus 15 wks and 1 fetus 27 wks. Postnatal: 1 term 42 wk	Duodenum	mRNA (RT-PCR)	The P-gp:actin ratio increased from 0.00529 (15 wk) and 0.0335 (27 wk) to 0.0153 at term.	[16] Fakhoury
P-gp	Human Postmortem	Fetus, n = 8 Neonatal, n = 4 Young adults (15–38 yrs), n = 8 Middle age (45–65 yrs), n = 8 Elderly (67–85 yrs), n = 8	Small and large intestine	mRNA (RT-PCR)	P-gp expression was relatively low in small and large intestine from fetus to elderly with the exception of the small intestine in the young group.	[17] Miki
P-gp	Human Postmortem	N=11 Prenatal: Fetal 7–20 wks, n=9 Postnatal: Newborn: 25 wks	Not specified	IHC mRNA (RNAse protection assay)	In five fetal samples (after 11, 13, 14, 16, and 20 wks of gestation) no or only weak staining was detected. Strong heterogeneous staining was observed in the stomach and the colon of a 7-day old premature born	[18] van Kalken

Protein	Human/ animal	Age range and number of samples	Localization	Methods used	Major results	Reference First author
		4 month old, n=1 Adult, n=1			neonate, but evaluation of all samples was difficult as strong background staining of goblet cells interfered. No or very weak expression of P-gp mRNA was seen in intestinal specimens after 11, 13, 14 weeks, whereas a clear P-gp mRNA expression was observed in fetal intestinal specimens at a later stage of fetal development (16–20 weeks). Tissue distribution differed between fetus and adult.	
P-gp	Human Celiac disease: treated and untreated; Healthy controls	N=25 Untreated: 6.7 (3.9–13.9) yrs Treated 6.7 (4.9–12.7) yrs Controls 8 (1.7–13) yrs	Duodenal biopsies	mRNA (RT-PCR)	The mRNA expression of P-gp was increased in children with treated celiac disease compared to controls.	[86]* Vannay
P-gp	Human Control and Crohn's disease biopsies	Controls n=19 Crohn's disease n=19 Postnatal age 1 month -17 yr	Duodenum	mRNA (RT-PCR)	P-gp mRNA was detected in all of the samples and highly variable between samples, with P-gp/Villin ratio ranging from 0.009 to 1.421 in the control group (266-fold variation) and from 0.047 to 4.5 in the Crohn's disease group (121-fold variation). P-gp expression was significantly higher in the Crohn's disease group than in the control group ($P<0.0001$).	[15] Fakhoury
Mrp3	Rabbit Postmortem	Newborn: 5–8 day, Weanling: 25–28 day Adult: >60 day	Distal ileum and colon	mRNA (RT-PCR) and western blotting	Mrp3 RNA and protein expression in colon highest in adult followed by weanling and then newborn animal. In adult animals, more Mrp3 RNA was observed in the colon than in the ileum; protein was almost unmeasurable in the ileum.	[22] Weihrauch
Oct1	Mouse	Embryos (embryonic days 11.5–16.5), Neonatal mice (day 5)	Small intestine and colon	*In situ* hybridization, Northern blot analysis	No obvious Oct1 transcripts were detected in fetal and newborn small intestine or colon (day embryonic 14 - day 5 postpartum).	[48] Pavlova
PepT1	Rat	Fetuses [embryonic day 18] Term neonates (21 days of gestation; 12h postnatal) Weaning (21 days old postnatal) Adult rat (3–4 months)	Duodenum: proximal small intestine immediately distal to the pyloric antrum	IHC	Duodenal PepT1 expression varies at different stages of pre- and postnatal development. At embryonic day 18, there is immunostaining for PepT1 at the epithelial brush border, but less prominent than in the adult and more variable from cell to cell. Directly after birth there is pronounced PepT1 immunoreactivity, consistently in the brush border but surprisingly also elsewhere in the epithelium. At day 21 postnatal (weaning) the distribution of PepT1 protein is similar to that seen in the adult: in the villus brush border.	[23] Hussain
	Rat	Day 17, day 20 of gestation, at days 1, 3, 5, 7, 14, 21, 24, 28, 60 and 75 (adult) after birth	Intestinal segments; duodenum, jejunum, ileum, colon	Northern blot (mRNA) and Southern blot (protein)	Rapid increase at birth in the duodenum, jejunum, and ileum, to a maximum by days 3–5, followed by a rapid decrease to 11–13% of maximal expression by day 14, and then an increase to 23–58% of maximal expression by day 24 (time of weaning). A similar pattern of expression was observed for PEPT1 protein. In the colon, significant expression was observed postpartum, but levels were undetectable from 1 week onwards.	[24] Shen

Protein	Human/ animal	Age range and number of samples	Localization	Methods used	Major results	Reference First author
	Rat	Mothers, weanling and adult	Small intestine	mRNA (RT-PCR)	Level of intestinal PepTl mRNA was markedly increased in 10- day-old rats, and then decreased reaching adult levels by day 28 after birth.	[25] Miyamoto
	Turkey	Embryonic day 23 to birth	Not specified	mRNA (RT-PCR)	A quadratic increase (P< 0.001) in PepT1 mRNA abundance with age in turkey embryonic small intestinal tissue from embryonic day 23 to birth. PepT1 mRNA was barely detectable in turkey embryonic small intestine at embryonic day 23 but increased 3.2-fold embryonic day 23 to birth.	[26] Van
OCT3 OCTN1	No relevant human or animal ontogeny data					

* References 1–85 are located in the published manuscript and references 86–103 are located in Appendix 3.

IUD: intrauterine development

IHC, immunohistochemistry

Table 2

Protein	Human/ animal	Age range and number of samples	Methods used	Major results	Reference First author
P-gp	Human Postmortem	N=35 Fetal: 14–28 weeks of gestation	Immunohistochemistry (IHC), mRNA (RNAse protection assay)	Staining for P-gp in bile canaliculi already at 14 weeks of gestation. No apparent difference in staining intensity in different fetal stages. Presence of mRNA confirmed in one specimen (16 weeks).	[18] Van Kalken
P-gp MRP2	Human Postmortem	N not determined Fetal: 14 and 19 weeks of gestation	IHC	Immunopositivity of P-gp and MRP2 was localized to the canalicular membrane of differentiating and mature hepatocytes. MPR2 was detectable in liver of 14-week old fetuses, but had strong expression at 19 weeks. P-gp was detectable at very low levels at 14 weeks, and was moderately expressed at 19 weeks.	[27] Cizkova
P-gp	Human Postmortem	N=3 Fetal: 15, 27 and 42 weeks of gestation	mRNA (RT-PCR)	Expression of P-gp was detectable in the 15 week samples, and gradually increased with gestational age until 42 weeks.	[16] Fakhoury
P-gp MRP2 OATP1B1 OATP1B3	Human Postmortem	N=72 Fetal: n=9 (8–23 wks) Pediatric: n=52 (0–17 yrs) Adult: n=11	mRNA (RT-PCR)	mRNA for P-gp expression in fetal and neonatal groups was 20–30 fold lower than in adults. Expression in infants was slightly lower than in adults, with no difference between children 1–7 years and adults. MRP2 mRNA was 30-fold lower in fetal, 200-fold lower in neonatal, and 100-fold lower in infant liver compared to adults. OATP1B1 expression was 20-fold lower in fetal, 500-fold lower in neonatal, and 90-fold lower in infant liver compared to adults. OATP1B3 mRNA was 30-fold lower in fetal, 600-fold lower in neonatal and 100-fold lower in infant liver compared to adults.	[20] Mooji
P-gp MATE1 BCRP BSEP MRP2 MRP3 MRP4 MRP6 OATP1B1 OATP1B3 OAT2 OCT1	Human	Perinatal (0–30 days): N=6 0–4 years: N=8 >7 years: N=6	mRNA	Gradual increase in mRNA expression from neonates to older children was observed for P-gp, MRP2, MRP3, MRP6, NTCP, OAT2, OATP1B1, OATP1B3, OCT1, BCRP, BSEP, and MATE1. MRP4 expression was high in neonates, but lower in older children.	[28] Klaassen
P-gp	Human Postmortem	N=12 1–6 months	mRNA (Northern) Protein (Western)	P-gp expression is regulated developmentally. P-gp mRNA and protein were present at 1 month postnatal.	[29] Schuetz
P-gp MRP2	Human Living and Postmortem	N=65 0.3–0.7 yrs: n=6 0.7–2 yrs: n=13 2–5 yrs: n=13 5–12 yrs: n=33	Protein (Western)	For P-gp, relative protein expression was not significantly different among the studied age groups. For MRP2, protein levels were significantly lower in infants under 8 months compared to older children.	[30] Tang
P-gp OATP1B1 OATP1B3	Human Living	N=64 7–70 yrs	Protein (LC-MS/MS)	P-gp, OATP1B1 and OATP1B3 protein expression was not associated with age in the studied age range.	[32] Prasad

Protein	Human/ animal	Age range and number of samples	Methods used	Major results	Reference First author
P-gp MRP2 MRP3 MRP4 BCRP BSEP NTCP OATP1B1 OATP1B3	Human Postmortem	N=20 Fetal: 18–23 weeks of gestation (n=8) Adult (n=12)	mRNA (RT-PCR)	All investigated transporters except for MRP4 were expressed, albeit at lower mRNA levels, in fetal hepatocytes compared to hepatocytes from adults.	[33] Sharma
BCRP	Human	N=35 5.5 to 28 weeks of gestational age	IHC	BCRP was detected in all stages of intrauterine development under study.	[21] Konieczna
BCRP	Human Living	N=65 7–70 yrs	Protein (LC-MS/MS)	BCRP protein expression was not associated with age in the studied age range.	[35] Prasad
BCRP MRP3 NTCP OATP1B1 OATP1B3	Human	N=10 Neonates (n=5) Adults (n=5)	Protein (Western)	The relative protein expression was similar between neonates and adults for BCRP, MRP3, BSEP, NTCP, OATP1B1 and OATP1B3.	[34] Yanni
MRP2	Human Living	N=51 7–63 yrs	Protein (LC-MS/MS)	MRP2 protein expression was not associated with age in the studied age range.	[37] Deo
MRP2 BSEP NTCP	Human Living and Postmortem	N=15 Fetal: 14–20 weeks of gestation (n=10) Adult (n=5)	mRNA (RT-PCR)	NTCP mRNA was 1.8% of adult expression in fetal liver. BSEP and MRP2 were 30–50% of adult expression.	[36] Chen
Ntcp	Rat	Fetal: 18, 20, 21 days of gestation Neonatal: day 1 Suckling: week 1, 2, 3, 4	mRNA (Northern) Protein (Western)	mRNA reached adult levels at day 7, but transport activity was only 25% of the adult value. Immunoreactive protein expression was near adult levels at birth, but molecular mass was substantially lower until 4 weeks of age due to incomplete glycosylation.	[38] Hardikar
OATP1B1 OATP1B3	Human Living and Postmortem	N=78 0–0.7 yrs: n=35 0.7–3 yrs: n=8 3–6 yrs: n=13 6–12 yrs: n=22	Protein (Western)	OATP1B1 has low expression from birth until 6 years with increased expression thereafter. OATP1B3 exhibited high expression at birth, which declined over the first months of life, and then increased again in the pre-adolescent period.	[39] Thomson
OATP1B1 OATP1B3 OAT2 OCT1	Human	Hepatocytes from pediatric and adult donors	mRNA Transport function	No difference in gene expression for all four transporters. Uptake activity of OATP1B1 and OCT1 was significantly lower in pediatric relative to adult hepatocytes. OATP1B3 was significantly higher in pediatric hepatocytes.	[40] Hayashi
Mrp3	Rat	Embryonic days 15.5, 17, 19, 20, 22 and newborn (22 days gestational age)	mRNA (RT-PCR)	Mrp3 expression progressively increased from ~10% to over 30% from day 15 to day 20 of gestation, and increased to near 90% of maternal expression at day 21 and after birth.	[41] St-Pierre
Mrp4	Mouse	Postnatal days −2, 0, 1, 3, 5, 10, 15, 20, 30, 45	mRNA (bDNA assay)	mRNA expression for Mrp4 was consistent from 2 days before birth to 45 days of age, except for a moderate increase on day 1 of age.	[42] Cui
Mrp4	Mouse	Postnatal days −2, 0, 5, 10, 15, 23, 30, 35, 40, 45	mRNA (bDNA assay)	Mrp4 mRNA expression was maximal at birth and decreased ~70% by 2 weeks of age, but was relatively constant thereafter.	[43] Maher

Protein	Human/ animal	Age range and number of samples	Methods used	Major results	Reference First author
Mrp6	Rat	Embryonic days 16 and 20; Postnatal days 0, 5, 12, 20, 29 and adult	mRNA (RT-PCR)	Mrp6 mRNA expression was detectable at low levels by embryonic day 16, increased to 40% of adult on postnatal day 0, and reached adult levels by day 29.	[44] Gao
Mate1	Mouse	Embryonic day 7.5 and adult	mRNA (RT-PCR)	Mate1 mRNA was barely detectable in embryos and was 200-fold less than that expressed in adult liver.	[45] Lee
OSTα/β	Human Living	N=8 6–17 months	mRNA (RT-PCR) IHC	OSTα and OSTβ were expressed in the specimens investigated in this study.	[46] Chen
Ostα/β	Mouse	Postnatal days −2, 0, 1, 3, 5, 10, 15, 20, 25, 30, 45, 60	mRNA (bDNA assay)	Ostα mRNA was expressed at low levels throughout development. Ostβ mRNA expression increased rapidly after birth with peak expression at day 1 (4.5-fold increase from prenatal), and decreased to adult levels between days 5 and 10.	[47] Cui

IHC, Immunohistochemistry

Table 3

Protein	Human/ Animal	Age range and number of samples	Methods used	Major results	Reference First author
P-gp	Human	N ≥12 Fetal (weeks 7, 11, 13, 15, 16, 20, 25, 28, 38) to Adult	mRNA Protein (IHC)	P-gp was expressed as early as week 11 of gestation. Staining was observed in the developing renal tubule.	[18] van Kalken
P-gp	Human	N=8 Fetal (mean: 18 weeks) N=4 Neonatal (1–24 days after birth) N=24 Adult	mRNA (PCR, RT-PCR of laser capture microdissected tissue)	P-gp was expressed in fetal, neonatal, young adult, middle age and elderly kidney. Adult kidney tended to have higher expression. By RT-PCR, transcript was found in laser capture microdissected tubules.	[17] Miki
Mdr1a Mdr1b	Mouse	N=56 Postnatal: 0, 7, 14, 21, 28, 45 days after birth	mRNA (RT-PCR)	Mdr1a and Mdr1b are marginally expressed in newborns and after 1 week of maturation. Mdr1b expression at day 21 was found to be higher than that seen on either day 14 or day 28.	[87]* Pinto
Mdr1a Mdr1b Bcrp Mrp2 Mrp4 Mate1 Oat1 Oat3 Oct1 Oct2 Octn1 Octn2 Urat1	Rat Mouse	Rat: N=45 Fetal: 13–21 days of gestation; Postnatal: days 1, 7 and 28 after birth Adult: 8 weeks	mRNA (microarray)	An overall increase in the mRNA levels of various transporters was observed between the fetal, compared to the neonatal kidney. Large increases in transporter expression also were evident in the postnatal period.	[52] Sweeney
Mdr1 Bcrp Mrp1 Mrp2 Mrp3 Oatp1a4 Oat1 Oat2 Oat3 Oct1 Oct2	Rat	N=80 Fetal: gestational day 21 Postnatal: 1, 4, 7, 11, 15, 18, 21, 26 and 42 days after birth	mRNA (RT-PCR)	Mrp1, Mrp3 and Oatp1a4 were most highly expressed at birth; Mrp2 and Bcrp showed constant expression during development, while Mdr1, Oct1, Oct2, Oat1, Oat2 and Oat3 expression was highest between postnatal days 11 and 26 with only limited expression at birth. There also were some differences in the expression of the transporters between males and females.	[49] de Zwart
Mate1	Mouse	N=100 Fetal: Day 18 of gestation Postnatal: 0, 5, 10, 15, 23, 30, 35, 40, and 45 days after birth	mRNA (RT-PCR; bDNA)	Mate1 expression steadily increased during late prenatal and postnatal development with a gender difference becoming apparent at 30 days of age.	[88]* Licktieg
Mate1 Mate2 Oct1 Oct2	Rat	N=8 Fetal: 18 and 21 days of gestation	mRNA (RT-PCR)	Expression of Mate1, Mate2, Oct1 and Oct2 in the developing kidney significantly increased between 18 and 21 days of gestation.	[89]* Ahmadimoghaddam

Protein	Human/ Animal	Age range and number of samples	Methods used	Major results	Reference First author
Mrp2 Oatp1a1 Oatp3a1 Oat3 Oct1 Oct2	Mouse Rat	Fetal Adult	mRNA (microarray)	Focused analysis of previously published microarray data during development revealed changes in many transporters.	[90]* Martovetsky
OATPs	Human	Fetal and adult (ages not stated)	mRNA (RT-PCR)	OATP-B (OATP2B1) and OATP-D (OATP3A1) are expressed in the human fetal kidney.	[91]* Tamai
Oatp1a1 Oatp1a6 Oatp3a1	Mouse	N=100 Fetal: Day 18 of gestation Postnatal: 0, 5, 10, 15, 22, 30, 35, 40, and 45 days after birth	mRNA (RT-PCR; bDNA)	Oatp1a1, Oatp1a6 and Oatp3a1 display lower expression at birth compared to 6 weeks later, while other Oatps had similar levels of expression at both time points.	[92]* Cheng
Oat1 Oat3 Mrp2 Mrp4	Rat	N=63 Postnatal: 1, 2, 4, 6, 14, 21 and 28 days after birth (N=5–8 mRNA; N=4–6 protein)	mRNA (RT-PCR) Protein (western blot and IHC)	Levels of Oat1 and Oat3 mRNA and protein increased significantly from postnatal days 1 to 28. Neither Mrp4 mRNA nor protein changed significantly over the same time frame. Mrp2 protein increased from postnatal days 14 to 21.	[93]* Nomura
Oats	Rat	Engineered kidney tissue from embryonic rudiments	Protein function (functional uptake assay)	Differentiating tubules of engineered recombined embryonic kidney tissue are functionally capable of organic anion transport.	[94]* Rosines
Oat1	Rat	Fetal: 17–20 days of gestation Postnatal: 0, 1, 2, and 6 days of age Adult: 8–10 weeks	mRNA (Northern blot, in situ hybridization) Protein (Western blot, and IHC)	Oat1 mRNA is detectable at day 18 of gestation, and Oat1 protein is detectable at day 20 of gestation. The expression of both Oat1 mRNA and Oat1 protein increase markedly after birth.	[95]* Nakajima
Oat1	Mouse	Fetal: 15–19 days of gestation Adult: 8–10 weeks	mRNA (Northern blot)	Oat1 mRNA expression by Northern blot was found at day 18 of gestation and continued to increase before birth.	[96]* Lopez-Nieto
Oat1	Mouse	N=44 Fetal: 13.5–18.5 days of gestation Postnatal: 1, 7, 21, 28 days after birth Adult: 8–10 weeks	mRNA (RT-PCR)	The expression of Oat1, which was initially observed at 15.5 days of gestation, progressively increased to adult levels.	[97]* Parreira
Oat1 Oat3	Mouse	Cultured embryonic kidney, renal slices from knockout tissue	Protein function (functional uptake assay)	Embryonic kidney in organ culture transports antiviral drugs by Oat1 and Oat3. Uptake of fluorescent tracers in coronal slices of adult kidney from Oat1 and Oat3 knockout mice revealed functional localization of Oat1 and Oat3. Oat1 function appeared to be consistent with localization in the proximal tubule, while Oat3 function seemed to be found in both the proximal and distal tubule.	[98]* Nagle
Oat1 Oat3	Mouse	N=13 Fetal: 13–18 days of gestation Postnatal: days 1, 4, 7, 14 and 21 days after birth Adult: 8–10 weeks of age	Protein (IHC)	Oat1 was first detected on day 15 of gestation and localized to the proximal tubule of the inner cortex. Oat1 expression continued to increase, appearing in the outer cortex at 7 days after birth and by 3 weeks Oat1 distribution was comparable to that seen in adult kidneys. Oat3 was first detectable on day 14 of gestation in the distal tubule and was later localized to the S2 segment of the proximal tubule. Around the time of birth, Oat3 was localized to the S1 and S3 segments of the proximal tubule, and Oat3 expression continued to increase through postpartum day 21.	[99]* Hwang

Protein	Human/ Animal	Age range and number of samples	Methods used	Major results	Reference First author
Oat1 Oat3	Mouse	cultured embryonic kidney knockout tissue	Protein (functional uptake assay)	Cultured whole embryonic kidneys from Oat1 and Oat3 knockout mice were able to transport many antivirals ex vivo. Certain antivirals were found to have selective dependency on Oat1 or Oat3.	[100]* Truong
Oat1 Oat2 Oat3	Mouse	N=90 Postnatal: 0, 5, 10, 15, 20, 25, 30, 35, and 40 days after birth	mRNA (RT-PCR; bDNA)	Postnatal Oat1 and Oat2 levels of expression increased after 25 days of age, while Oat3 expression levels gradually increased from birth to 45 days of age. Overall Oat2 levels increased 30-fold, while Oat1 and Oat3 levels increased about 2-fold.	[101]* Buist
Oat1 Oat3	Sheep	N=28 Fetal: 80, 100, 120, 130, and 145 days of gestation Postnatal: 1 and 7 days after birth	mRNA (RT-PCR) Protein (IHC)	Oat1 was expressed at 80 days of gestation and was most highly expressed at 145 days of gestation; the postnatal kidney showed comparable levels of expression. Oat3 displayed a similar pattern of expression, although significant increases were evident in the postnatal kidney. Immunohistochemical analysis revealed localization of the transporters to tubular epithelia consistent with the proximal tubule.	[102]* Wood
Oat1 Oat3 Oct1 Oct2	Rat	Fetal: 13–18 days of gestation; cultured embryonic kidney; induced metanephric mesenchyme	mRNA (RT-PCR) Protein function (functional uptake assay)	Oat1, Oat3 and Oct2 are expressed early in kidney development, while Oct1 is expressed late in development. The ontogeny of the transporters in ex vivo cultures of embryonic kidney or its component tissues is comparable to that seen *in vivo*. Cultured embryonic kidneys and induced metanephric mesenchyme are functionally capable of organic anion transport.	[50] Sweet
Oat1 Oat2 Oat3 Oct1	Mouse	Fetal: 14–19 days of gestation Postnatal: 0 days of age Adult: 8–10 weeks	mRNA (Northern blot, *in situ* hybridization)	Oat1, Oat2 and Oat3 expression was seen as early as day 15 of gestation. The expression levels of the organic anion transporters continued to increase to adult levels.	[48] Pavlova

*References 1–85 are located in the published manuscript and references 86–103 are located in Appendix 3.

IHC, Immunohistochemistry

Table 4

RECOMMENDATIONS

- Build multidisciplinary, international collaborative networks to facilitate collection and sharing of data on pediatric transporters, including expertise in preclinical studies (e.g., knockout and *in vitro* models), pediatrics, clinical pharmacology, pharmacogenomics, pharmacometrics, and pharmacovigilance
- Establish central (perhaps regional) tissue repositories where surgical and postmortem samples can be stored with clear guidelines for tissue collection and handling to preserve sample integrity
- Continue to support the training of scientists in pediatric clinical pharmacology with expertise in transporters, pharmacogenomics, pharmacometrics, and pharmacovigilance
- Increase the awareness of clinicians regarding the importance of transporters in pediatric drug disposition
- Identify examples relevant to pediatric pharmacotherapy where developmental differences in transporter expression or activity could translate into clinically relevant effects
- Work with professional groups to develop guidelines on how drug therapy may be altered due to variations in transporter expression or activity
- Identify selective and specific biomarkers for transporter activity in pediatric patients
- Investigate basic developmental mechanisms regulating transporter expression and activity in the different organs in pediatric health and disease
- Develop pediatric-relevant *in vitro/in silico* and systems biology models to predict transporter function in the context of overall drug disposition