DMD Fast Forward. Published on December 28, 2015 as DOI: 10.1124/dmd.115.067801
This article has not been copyedited and formatted. The final version may differ from this version.

DMD # 67801

Mini Review

Ontogeny of Hepatic Drug Transporters and Relevance to Drugs Used in Paediatrics

Yasmine Elmorsi, Jill Barber and Amin Rostami-Hodjegan

Manchester Pharmacy School, University of Manchester, United Kingdom (Y.E., J.B., A.R-H.); Simcyp Limited, a Certara Company, Sheffield, United Kingdom (A.R-H.).

Running Title: Age-Dependent Hepatic Drug Transport in Paediatrics

Corresponding author: Professor A. Rostami-Hodjegan,
Manchester Pharmacy School,
University of Manchester, Stopford Building
Oxford Road, Manchester, M13 9PT, UK
Tel: (+) 44 161 306 0634
Fax: (+) 44 161 275 8349
Email: amin.rostami@manchester.ac.uk

Number of Text Pages: 25 (inc references)

Number of Tables: 1

Number of Figures: 3

Number of References: 43

Number of Words in Abstract: 184 words

Abbreviations: IVIVE, in vitro to in vivo extrapolation; PBPK, physiologically based pharmacokinetic; PK/PD, Pharmacokinetic/Pharmacodynamic; ABC: ATP-binding cassette; BCRP: breast cancer resistance protein; BSEP: bile salt export pump; NTCP: Na+-taurocholate co-transporting polypeptide; OAT: organic anion transporter; OATP: organic anion transporting polypeptide; OCT: organic cation transporter; MATE: multidrug and toxin extrusion protein; P-gp: P-glycoprotein; MRP: multidrug resistance- associated protein; OSTα/β: organic solute transporter; SLC: solute carrier; QconCAT, quantification concatemer; AQUA, Absolute quantification.

ABSTRACT

Most of the pharmacokinetic studies conducted to calculate paediatric drug doses are based on scaling from adult data using various allometric parameters related to body size. However, these uniform scaling methods cannot account for all physiological changes occurring during maturation which influence various drugs in different ways. The ontogeny of physiological and biological functions accompanying the progression from infancy to childhood to adulthood does not proceed in a simple monotonic rate with body size for various elimination pathways. The transporters and their interplay with enzymes have a substantial role in drug metabolism and disposition. Although much is known about enzymes and their ontogeny, there is a scarcity of information on the ontogenic profile of drug transporters, particularly during the early years of human life. These ontogeny data are required for the enhancement of PBPK models, and consequently for the prediction of pharmacokinetic profiles of new therapeutic compounds in paediatric populations. This review points to the relative ontogeny rate for enzymes and transporters and how these may confound our understanding of the role that transporters may or may not play in childhood compared with adulthood.

Introduction

Commonly Encountered Issues in Paediatric Drug Dosing

One of the most prevalent problems in paediatrics is the high incidence of adverse drug reactions associated with the use of off-label or unlicensed drugs. In 2000, it was reported that about 70% of the drugs prescribed to paediatric in five European countries are off-label or unlicensed (Conroy et al., 2000).

It is not surprising that at the time of approval of new drugs, they have seldom been tested in children, even when there might be paediatric applications. Of the formulae that have been used to scale adult doses to children, rules such as Clark's body weight (BW) and body surface area (BSA) which are purely based on allometric scaling (Anderson and Meakin, 2002) are typically used. However, such simple extrapolation methods usually fail to predict pharmacokinetic behaviour in younger paediatrics (Johnson, 2008). This is mainly because of developmental changes in organ function (including the ontogeny of drug disposition pathways) and variations in body composition across the different age ranges of children (Kearns et al., 2003).

Physiologically Based Pharmacokinetic (PBPK) Models in Paediatric Populations

Development of PBPK models in paediatrics has facilitated the evaluation of paediatric population exposure to drugs and xenobiotics. These models take into consideration drug related data such as biochemical, demographic and physiological data - specifically the ontogeny of drug disposition and elimination pathways. The models can be used to assist in the extrapolation of in vitro data to predict the in vivo behaviour of drugs in any age group, including children (Barrett et al., 2012).

Robust PBPK models require good data, in particular quantitative data on paediatric drug metabolising enzymes (Cytochrome P450, CYPs, or UDP-glucuronosyltransferases, UGTs and transporters. Further, it is not sufficient to take a single snapshot of the childhood drug metabolising capability, because it changes with age and needs to be described as an age-dependent ontogeny function. Hence, measurements of the levels of these proteins over the whole childhood period are necessary for in vitro to in vivo extrapolation (IVIVE) (Prasad and Unadkat, 2014).

Drug Transporters

According to the direction in which transporters flux their drug substrates through membranes, they can be grouped as efflux or uptake transporters. Efflux transporters drive their substrates out of cells, whereas uptake transporters transfer them into cells. Alternatively, transporters may belong to the ABC (ATP-binding cassette), the SLC (solute carrier) transporter, or the OST (organic solute transporter) families.

Liver Transporters

Liver transporting proteins are crucial factors for the uptake and efflux of various drugs and endogenous substances (Klaassen and Lu, 2008; Klaassen and Aleksunes, 2010). They are therefore major determinants of drug efficacy and toxicity; they affect drug concentrations in plasma through their roles in metabolic or biliary clearance (Borst and Elferink, 2002). Figure 1 shows the most important hepatic uptake and efflux transport proteins and their locations in the hepatocytes.

Ontogeny of Liver Transporters

Although there is some metabolizing enzymes known to be overexpressed in the early days of infant life compared with adulthood (CYP3A7 for example), drug transporters expression is expected to be fairly low at birth, because of the observation that infants disposition machineries are unable to handle toxic xenobiotics. The liver matures rapidly after birth from simply an organ for formation of blood cells to an organ holding the major metabolism and elimination machinery for drugs and xenobiotics. Therefore, liver transporters maturation is important for the proper flux of xenobiotics across the cells (Cui et al., 2012). Despite improved knowledge of drug transporters in human, the developmental patterns of individual drug transporters remains incompletely known, particularly in the perspective of transporter developmental and ontogenic expression in paediatric population. This is largely because of the scarcity of paediatric clinical studies in this area (Wei et al., 2014).

For the determination of the ontogenic profile of liver transporters a literature search was conducted for identification of studies dealing with major drug transporters in the liver. Relevant publications on the abundance data of the efflux transporters, the uptake transporters, and the bidirectional transporter were searched through PubMed using the following keyword combinations: “hepatic/liver” plus “MATE1, BSEP, BCRP, MRP2, MRP3, MRP4, MRP6, NTCP, P-gp, OATP1B1, OATP1B3, OATP2B1, OAT2, OCT1, OAT7, OSTα/β” plus “uptake OR influx OR efflux OR flux OR transport” plus “abundance, ontogeny, correlation of expression, quantification”. Searches were limited to Humans, Child: birth-18 years. Titles and abstracts were reviewed to keep the search centred on the levels of transporters expression in paediatric subjects. The literature was reviewed to assess the development of drug

transporter expression with age, and the following data were obtained regarding each of the relevant liver transporter ontogeny.

Hepatic Uptake Transport Proteins

Sodium Taurocholate Cotransporting Polypeptide (NTCP)

Sodium taurocholate cotransporting polypeptide is a basolateral transporter entirely expressed in the liver and constituting the main hepatic pathway for conjugated bile acids uptake. NTCP was detectable in the liver samples of foetuses of 14-20 weeks of gestation (Chen et al., 2005). NTCP protein expression was not significantly different between adults and neonates (Yanni et al., 2011).

Organic Anion Transporting Polypeptides (OATPs)

Out of the 11 identified Organic Anion Transporting Polypeptides in humans, there are only 4 transporters with an extensive role in substrate uptake at the hepatic sinusoidal membrane; these are OATP1A2, OATP1B1, OATP1B3, and OATP2B1 (Kalliokoski and Niemi, 2009).

Foetal livers showed mRNA expression for OATP1B1, OATP1B3 and OATP2B1. Some studies suggested that there were no significant differences between neonatal and adult liver in the expression of OATP1B1 and OATP1B3 (Yanni et al., 2011). One study on 45 liver samples, however, suggested that the mRNA expression for both transporters was age-dependent until the seventh year of life, at which point the levels stabilised (Mooij et al., 2014). The only available study on protein expression for OATPs showed no correlation between the OATP1B1, OATP1B3 and OATP2B1 expression and age (Prasad et al., 2014).

Organic Anion Transporters (OATs)

Although most of the available data about Organic Anion Transporters are related to kidney drug transport, they are highly expressed in sinusoidal hepatocyte membrane. OAT2 was detected in foetal liver and showed an increased expression with age from neonatal to older children and adults' livers (Klaassen and Aleksunes, 2010).

Organic Cation Transporters (OCTS)

There are two expressed isoforms of human organic cation transporters in the human liver, OCT1 and OCT3. OCT1 is the major transporter in human in terms of its expression and is believed to be confined to the liver sinusoidal membrane (Zhang et al.,1997, Hilgendorf et al., 2007). It has 13 times the expression level of OCT3, which has broader tissue distribution (Nies et al., 2009). While there were no studies about OCT3 ontogeny, the very limited number of studies available assessing the age related maturation of OCT1 did not report any significant difference in mRNA expression between adults and paediatrics (Kim et al., 2012).

Hepatic Efflux Transport Proteins

These may be classified into either Canalicular or Basolateral Efflux Transport Proteins.

Canalicular Transport Proteins

The role of canalicular transport proteins is to excrete drugs and their metabolites through the hepatic apical membrane to the bile (canalicular membrane).

P-glycoprotein (P-gp)

Having different functions in various tissues, P-glycoprotein is the transport protein with the highest number of studies. The mRNA levels of P-gp in a group of 61 post-mortem liver samples from foetuses and neonates were about 20-30 fold lower when compared to the adult group. P-gp mRNA expression of first year infants was found to be higher than the neonatal levels, and was estimated at about 5 fold lower than adults, whereas there were similar expression levels in older children, adolescents, and adults (Mooij et al., 2014). This suggests that the P-gp mRNA increases throughout the first year of human life and these findings were consistent with previous results (van Kalken et al., 1992, Miki et al., 2005, Fakhoury et al., 2009). P-gp protein was expressed in neonates including premature neonates, but its expression was independent of age in a group of paediatrics from 4 months to 12 years of age, where P-gp expression levels were not statistically significantly different in 65 different liver samples (Tang et al., 2007). This result was somewhat supported by a study for P-gp quantification through the age interval from 7 to 70 years which revealed that age and P-gp protein expression were not correlated (Prasad et al., 2014).

MDR3

Available literature data proves a similar MDR3 substrate specificity to P-gp. However, there is not enough data available about its ontogenic profile.

Bile Salt Export Pump (BSEP)

The mRNA expression of this transporter was found to be very low in foetuses with a 10 to 20 fold increase in expression in neonates; it continues to rise in adulthood (Chen et al., 2005).

Multidrug Resistance-Associated Protein 2 (MRP2)

Multidrug resistance-associated protein 2 was found to be expressed in the second trimester of gestation. The expression level was higher in 19 weeks old foetuses than those of 14 weeks of age (Čížková et al., 2005). The expression continues to increase from the foetal period to neonatal and infantile periods (Mooij et al., 2014, Klaassen and Aleksunes, 2010). The MRP expression was stable over the age range from 7 to 70 years (Deo et al., 2012).

Breast Cancer Resistance Protein (BCRP)

Although breast cancer resistance protein was found in samples from foetuses aged only 6 weeks (Konieczna et al., 2011), BCRP protein expression did not differ significantly between neonates and adults (Yanni et al., 2011). Analysis by (LC-MS/MS) revealed no association between BCRP levels and age in 65 liver samples from 7 to 70 years of age (Prasad et al., 2013).

Multidrug and Toxin Extrusion 1 (MATE1)

Analysis of the early foetuses' livers detected the expression of mRNA of MATE1 with an increase in expression with age till adulthood (Klaassen and Aleksunes, 2010).

Basolateral Efflux Transport Proteins

These transporters are a major class of export proteins that mediates xenobiotic excretion from the liver into the sinusoidal blood across the basolateral membrane.

Multidrug Resistance-Associated Protein 3 (MRP3)

There was a similarity in the ontogeny of MRP3 to that of MATE1 and OAT2 in terms of its expression in foetal liver and its increase with development from early neonatal life to adulthood (Klaassen and Aleksunes, 2010).

Multidrug Resistance-Associated Protein 4 & 5 (MRP4 and MRP5)

While there was a scarcity of ontogeny data related to MRP5, the mRNA expression of MRP4 was found to be unrelated to age (Sharma et al., 2013).

Multidrug Resistance-Associated Protein 6 (MRP6)

In line with the ontogeny data of MRP3, MATE1 and OAT2 transporters, MRP6 was also detected in foetal liver and its expression increased with age from neonatal to older children and adult livers (Klaassen and Aleksunes, 2010).

Organic Solute Transporter Alpha/ Beta (Ostα-Ostβ)

The Ostα-Ostβ transporters are basolateral hepatic bidirectional liver transporters mediating bile acids flux. The mRNA for both transporters was detected at low levels in paediatric livers (Chen et al., 2008), but there is little reported data on their ontogeny.

Drugs with Known Paediatric Application which Are Substrates for Transporters

To appreciate the importance (or lack of relevance) of transporter ontogeny for paediatric drug treatment, it seems natural to assess the overlap between the sets of drugs which are used in paediatric drug treatment and the drugs acting as substrates or modulators of drug transporting proteins. However, as described later, this approach might be misleading.

Management of preterm infants and children of all ages often involves using a variety of drugs which are frequently transported by one or more transporter proteins.

For identifying drugs with important uses in paediatric populations that are substrates for liver transporters, a review of literature was carried out in PUBMED using the keywords combination (hepatic OR liver) plus (NTCP OR OATP OR OAT OR OCT OR P-gp OR P-glycoprotein OR MDR OR BSEP OR MRP OR BCRP OR MATE1 OR OST) plus (substrate); only human studies were taken into account and titles/ abstracts were looked into for relevant information. References in each report were scrutinised for further sources of published data on drug transporter substrates. Data collected are shown in Table 1. Drugs known to be substrates of liver transporters were then compared to drugs used in paediatrics from BNFc 2014 / 2015, and the matching drugs were indicated in bold in Table 1.

According to Table 1, there are about 175 drug substrates for liver transporters, 104 of which are of paediatric application. This suggests that about 60% of the drugs prescribed to children may be affected by the function of one or more liver transporters (see Figure 2). The accuracy of this calculated proportion may, of course, be compromised by the fact that there are many drugs used off-label or unlicensed. It seems likely, however, that the proportion of off-label drugs which are substrates for transporters is similar to the value obtained for the BNFc drugs, and that the calculated value might be taken as a rough estimate for the scale of the relevance of transporters in paediatric drug treatment. Obviously, the involvement of transporters does not necessarily translate to a crucial impact for them in the drug disposition; assessing the significance of transporter involvement is an area which has only started to mature (see later sections).

Based on the increasing number of drugs used in paediatrics that are substrates of or modulators for liver transporters, it is apparent therefore that studies about developmental changes of these transport proteins should be improved. Most of the available studies on liver transporters have concentrated only on snapshots of gene or protein expression and few have focused on the determination of age-dependent transporter activities (Fattah et al., 2015).

In the next section, the theoretical aspects related to the relative importance of transporters, as opposed to enzymes, with age are discussed. It cannot be assumed that the transporter effect in a certain drug's disposition (as prominent determinant, regardless of absolute value) does not vary with age.

Problems Associated with Estimating Drug Transporter Relevance to Paediatric Drugs

There is a general deficiency of data on the developmental changes in transporters in humans. However, from the available studies, it is clear that there is a large variability in the developmental scenarios between individual transporters. This is also known for enzymes (Salem et al., 2013). Although several efflux and uptake transporters were found to be expressed in the foetal liver, some transporters show some developmental maturation in expression from foetal to neonatal and adulthood periods such as BSEP, MRP3, MRP6, MATE1 and OAT2 while the expression of other transporters like MRP2, MDR1, OAT1B1 and OAT1B3 increases from the neonatal period to some point in childhood and then stabilizes at adult levels. On the other hand, the maturation of some transporters is not related to age; these include BCRP, OCT1 and NTCP. These results are broadly in line with a recently published

review that assessed the ontogeny of human transporters in intestine, liver and kidney (Brouwer et al., 2015).

Of the available transporter ontogeny data, some are in the form of mRNA, and others are in the form of protein data. Further, most of these data still need to be correlated with activity and the relative significance of the transporters in the distribution of drugs and the ways in which this significance changes with age requires further investigation.

For a transporter to be of relevance to the disposition of a certain drug, there are a range of factors to be considered.

- Firstly, the availability of this transporter and its abundant expression in the tissue of interest,
- Secondly, the relative contribution from this transporter in drug distribution compared to contribution from other transporters or from passive diffusion (f_t for each transporter),
- Thirdly, the degree of the drug affinity to the transporter and the proportion of the drug transported by this specific transporter,
- Finally, modulation of this transporter by induction or inhibition through endogenous or exogenous substances.

Drugs may be affected by transporters to varying degrees according to the fraction of drug dose being absorbed, distributed between body tissues, or cleared out of the body. Therefore, the concept of fraction transported is of great importance in the determination of the transporter effect on the concentration of drugs in any organ by estimating their role in the ADME profile of drugs. When this fraction of drug

transported is high, then the transporters influencing this drug and their modulation by DDI is very important to the drug ADME.

Prasad and Unadkat (2015), summarized the CNS affecting drugs and the antiretroviral agents that are at the same time substrates for P-gp, BCRP, or MRPs in the blood-brain barrier (BBB), together with their f_T values. Drugs with high f_T values (0.67-0.98) are underlined in Table 1. However, this picture becomes more complex when the dimension of age is added to it where the fraction of drug transported may not be constant across various age groups.

To indicate the varying relative contribution of different transporters with age, two hypothetical transporters T1 and T2 were assumed to have relative importance, f_{T1} and f_{T2} of 0.1 and 0.9 respectively in adults. In the first case (A), both transporters were assumed to be expressed at birth with relative values of 0.6 and 0.5 for T1 and T2 respectively compared to the adults. In the second case (B), T2 was assumed not to be expressed at birth while T1 relative value was the same as in the first case. The relative abundance values for each transporter were assumed to follow a different trajectory (See Figure 3 Part (B)), unlike the first scenario when the ontogeny was similar for both transporters (Figure 3 Part (A)).

Under these circumstances it can be shown that the relative importance of transporters (f_T) can be age-dependent for the scenario described for Case B (Figure 3 part (D)) whilst the relative importance may remain the same as adult despite the ontogeny of transporters in Case A (Figure 3 Part (C)).

This concept is a general case and can be considered not just between two transporters but also in terms of the relative importance of a non-transporter route vs

a transporter related route. The parallels between the above case with the results of another recent investigation (Salem et al. submitted) on selection of covariates and their age-dependence, are obvious. This study by Salem et al, discussed the validity of the commonly known fact that hepatic extraction ratio, E_H is a characteristic property of the drug. They concluded that caution should be taken before assuming that the extraction ratio in paediatrics is the same as in adults because classification of a drug as a high or low extraction ratio does not take into account the variation of the E_H calculation parameters with age. In conclusion, it is clear that the relative ontogeny of transporters and enzymes may not follow the same trajectory and they can differ in their relative importance to a specific drug and its disposition.

Progress with Experimental Methods for the Study of Transporter Expression

The majority of data about the abundance of drug transporters originates from in vitro methods based on, for example, primary hepatocytes or cell-lines with transfected human transporting protein (Hirano et al., 2004; Kitamura et al., 2008). Nevertheless, these in vitro models are not available for the paediatric population. Preclinical animal studies are of value in assessing the ontogenic profile of transporters and much of that information seems to be in agreement with clinical findings in adults. Nonetheless, most of these data are in the form of transcriptional information limited to the gene expression level and most of the abundance data are obtained through Western blot analysis and immunohistochemistry which suffer from poor reproducibility (Al Feteisi et al., 2015).

Transcript levels (as measured by conventional mRNA methods) have been shown to have only a weak association with the expression levels of proteins. LC-MS/MS experiments, however, can be designed to give sensitive characterization and

quantification of proteins. Unlike relative quantification methods that rely on comparing the protein concentrations in two samples relative to each other, absolute quantification, is highly recommended to aid in the measurement of the absolute values of proteins in samples and consequently facilitate inter-laboratory comparisons.

Mass Spectrometry-Based Absolute Quantification

Because of the high sensitivity and selectivity of mass spectrometry, it is of crucial importance in quantitative analysis of paediatric samples, especially because of the minute size and the limited availability of these samples.

Mass spectrometry-based absolute quantification is mainly based on isotope dilution, where a pre-determined amount of a heavy and isotope labelled internal standard is mixed with the analyte protein as a reference. Comparison of the signal intensity of labelled and unlabelled peptides leads to the concentration of the protein of interest.

Internal standards may be isolated peptides labelled with stable isotopes (sometimes known as AQUA peptides) (Brun et al., 2007), this technique has been successfully used in the hepatic CYP 2D6 quantification in human (Langenfeld et al., 2009). Alternatively, marker quantotypic peptides of various proteins of interest may be expressed (using an artificial gene) concatenated in an artificial protein and released on proteolytic digestion. This approach (known as the QconCAT method) allows the quantification of up to 50 proteins using a single standard (Beynon et al., 2005, Pratt et al., 2006). The robustness of the QconCAT approach for simultaneous quantification of a few 10s of proteins has been demonstrated by Al-Majdoub et al (2014), and the approach has been used in the quantification of enzymes and transporters (Russell et al., 2013).

Label-free quantification of transporters and enzymes by LC-MS/MS is also possible and is especially useful for establishing an initial overview (Kito and Ito, 2008). Labelled standards allow for more direct measurements, dependent upon fewer assumptions about the relationship of peptide signal to protein abundance, and are normally to be preferred for repeated precise measurements.

CONCLUSION

The quest to understand and manage the paediatric drug dose requires the knowledge of changes that occur to various body functions with age. The biology of enzymes metabolising the drugs and it ontogeny has been ahead of the efforts on transporters. Some of the concepts regarding the importance (or lack of importance) of transporters are challenged when the adults data are used without considerations for age-dependent impact of these transporting proteins in the disposition of any particular drug. The invention of methodologies which enable quantitative measurement of transporter proteins using small biological samples will help to gain insight into ontogeny trajectories of various transporters. These in turn, will assist with building more robust PBPK models making use of the in vitro data of drugs and their affinities to various transporters and enzymes to aid in the prediction of drug behaviour in paediatrics.

ACKNOWLEDGEMENTS

We would like to thank The Egyptian Government for funding the work undertaken by Yasmine Elmorsi and Miss Eleanor Savill for assistance with preparation of the manuscript.

AUTHORSHIP CONTRIBUTIONS

Participated in research design = Elmorsi, Barber, Rostami-Hodjegan

Performed data analysis = Elmorsi, Rostami-Hodjegan

Wrote or contributed to the writing of the manuscript = Elmorsi, Barber, Rostami-Hodjegan

REFERENCES

Al Feteisi H, Achour B, Rostami-Hodjegan A, and Barber J (2015) Translational value of liquid chromatography coupled with tandem mass spectrometry-based quantitative proteomics for in vitro – in vivo extrapolation of drug metabolism and transport and considerations in selecting appropriate techniques. *Expert Opin Drug Metab Toxicol* **11:**1357–1369.

Anderson BJ, and Meakin GH (2002) Scaling for size: Some implications for paediatric anaesthesia dosing. *Paediatr Anaesth* **12**:205–219.

Barrett JS, Della Casa Alberighi O, Läer S, and Meibohm B (2012) Physiologically Based Pharmacokinetic (PBPK) Modeling in Children. *Clin Pharmacol Ther* **92**:40–49.

Beynon RJ, Doherty MK, Pratt JM, and Gaskell SJ (2005) Multiplexed absolute quantification in proteomics using artificial QCAT proteins of concatenated signature peptides. *Nat Methods* **2**:587–589.

Borst P, and Elferink RO (2002) Mammalian ABC transporters in health and disease. *Annu Rev Biochem* **71**:537–592.

Brouwer KLR, Aleksunes LM, Brandys B, Giacoia GP, Knipp G, Lukacova V, Meibohm B, Nigam SK, Rieder M, and de Wildt SN (2015) Human ontogeny of drug transporters: Review and recommendations of the pediatric transporter working group. *Clin Pharmacol Ther* **98**:266-287.

Brun V, Dupuis A, Adrait A, Marcellin M, Thomas D, Court M, Vandenesch F, and Garin J (2007) Isotope-labeled protein standards: toward absolute quantitative proteomics. *Mol Cell Proteomics* **6**:2139–2149.

Chen H-L, Chen H-L, Liu Y-J, Feng C-H, Wu C-Y, Shyu M-K, Yuan R-H, and Chang M-H (2005) Developmental expression of canalicular transporter genes in human liver. *J Hepatol* **43**:472–477.

Chen HL, Liu YJ, Chen HL, Wu SH, Ni YH, Ho MC, Lai HS, Hsu WM, Hsu HY, Tseng HC, Jeng YM, and Chang MH (2008) Expression of hepatocyte transporters and nuclear receptors in children with early and late-stage biliary atresia. *Pediatr Res* **63**:667–673.

Čížková D, Mokrý J, Mičuda S, Österreicher J, and Martínková J (2005) Expression of MRP2 and MDR1 transporters and other hepatic markers in rat and human liver and in WRL 68 cell line. *Physiol Res* **54**:419–428.

Conroy S, Choonara I, Impicciatore P, Mohn A, Arnell H, Rane A, Knoeppel C, Seyberth H, Pandolfini C, Raffaelli MP, Rocchi F, Bonati M, Jong G, de Hoog M, and van den Anker J (2000) Survey of unlicensed and off label drug use in paediatric wards in European countries. European Network for Drug Investigation in Children. *BMJ* **320**:79–82.

Cui JY, Gunewardena SS, Yoo B, Liu J, Renaud HJ, Lu H, Zhong XB, and Klaassen CD (2012) RNA-seq reveals different mRNA abundance of transporters and their alternative transcript isoforms during liver development. *Toxicol Sci* **127**:592–608.

Deo AK, Prasad B, Balogh L, Lai Y, and Unadkat JD (2012) Interindividual variability in hepatic expression of the multidrug resistance-associated protein 2 (MRP2/ABCC2): Quantification by liquid chromatography/tandem mass spectrometry. *Drug Metab Dispos* **40**:852–855.

Fakhoury M, de Beaumais T, Guimiot F, Azougagh S, Elie V, Medard Y, Delezoide A-L, and Jacqz-Aigrain E (2009) mRNA expression of MDR1 and major metabolising enzymes in human fetal tissues. *Drug Metab Pharmacokinet* **24**:529–536.

Fattah S, Augustijns P, and Annaert P (2015) Age-Dependent Activity of the Uptake Transporters Ntcp and Oatp1b2 in Male Rat Hepatocytes□: From Birth Till Adulthood. *Drug Metab Dispos* **43**:1-8.

Hilgendorf C, Ahlin G, Seithel A, Artursson P, Ungell A, and Karlsson J (2007) Expression of Thirty-Six Drug Transporter Genes in Human Intestine, Liver, Kidney, and Organotypic Cell Lines. *Drug Metab Dispos* **35**:1333–1340.

Hirano M, Maeda K, Shitara Y, and Sugiyama Y (2004) Contribution of OATP2 (OATP1B1) and OATP8 (OATP1B3) to the hepatic uptake of pitavastatin in humans. *J Pharmacol Exp Ther* **311**:139–146.

Johnson TN (2008) The problems in scaling adult drug doses to children. *Arch Dis Child* **93**:207–211.

Kalliokoski A, and Niemi M (2009) Impact of OATP transporters on pharmacokinetics. *Br J Pharmacol* **158**:693–705.

Kearns GL, Abdel-Rahman SM, Alander SW, Blowey DL, Leeder JS, and Kauffman RE (2003) Developmental pharmacology--drug disposition, action, and therapy in infants and children. *N Engl J Med* **349**:1157–1167.

Kim M-H, Shin HJ, Lim SJ, Park J-S, Lee S-S, Song I-S, and Shin J-G (2012) Inter-individual Variability in OCT1 Expression and Its Relationship with OCT1 Genotype in Liver Samples from a Korean Population. *Drug Metab Pharmacokinet* **27**:530–535.

Kitamura S, Maeda K, and Sugiyama Y (2008) Recent progresses in the experimental methods and evaluation strategies of transporter functions for the prediction of the pharmacokinetics in humans. *Naunyn Schmiedebergs Arch Pharmacol* **377**:617–628.

Kito K, and Ito T (2008) Mass spectrometry-based approaches toward absolute quantitative proteomics. *Curr Genomics* **9**:263–74.

Klaassen CD, and Aleksunes LM (2010) Xenobiotic, bile acid, and cholesterol transporters: function and regulation. *Pharmacol Rev* **62**:1–96.

Klaassen CD, and Lu H (2008) Xenobiotic transporters: Ascribing function from gene knockout and mutation studies. *Toxicol Sci* **101**:186–196.

Konieczna A., Erdösová B, Lichnovská R, Jandl M, Čížková K, and Ehrmann J (2011) Differential expression of ABC transporters (MDR1, MRP1, BCRP) in developing human embryos. *J Mol Histol* **42**:567–574.

Langenfeld E, Zanger UM, Jung K, Meyer HE, and Marcus K (2009) Mass spectrometry-based absolute quantification of microsomal cytochrome P450 2D6 in human liver. *Proteomics* **9**:2313–2323.

Miki Y, Suzuki T, Tazawa C, Blumberg B, and Sasano H (2005) Steroid and xenobiotic receptor (SXR), cytochrome P450 3A4 and multidrug resistance gene 1 in human adult and fetal tissues. *Mol Cell Endocrinol* **231**:75–85.

Mooij MG, Schwarz UI, De Koning BAE, Leeder JS, Gaedigk R, Samsom JN, Spaans E, Van Goudoever JB, Tibboel D, Kim RB, and De Wildt SN (2014) Ontogeny of human hepatic and intestinal transporter gene expression during childhood: Age matters. *Drug Metab Dispos* **42**:1268–1274.

Nies AT, Koepsell H, Winter S, Burk O, Klein K, Kerb R, Zanger UM, Keppler D, Schwab M, and Schaeffeler E (2009) Expression of organic cation transporters OCT1 (SLC22A1) and OCT3 (SLC22A3) is affected by genetic factors and cholestasis in human liver. *Hepatology* **50**:1227–1240.

Prasad B, Evers R, Gupta A, Hop CE A Salphati L, Shukla S, Ambudkar S V., and Unadkat JD (2014) Interindividual variability in hepatic organic anion-transporting polypeptides and P-glycoprotein (ABCB1) protein expression: Quantification by liquid chromatography tandem mass spectroscopy and influence of genotype, age, and sex. *Drug Metab Dispos* **42**:78–88.

Prasad B, Lai Y, Lin Y, and Unadkat, J.D. 2013. Interindividual variability in the hepatic expression of the human breast cancer resistance protein (BCRP/ABCG2): effect of age, sex, and genotype. J Pharm Sci **102**:787-793.

Prasad B, and Unadkat J (2015) The concept of fraction of drug transported (ft) with special emphasis on BBB efflux of CNS and antiretroviral drugs. *Clin Pharmacol Ther* **97**:320–323.

Prasad B, and Unadkat JD (2014) Optimized Approaches for Quantification of Drug Transporters in Tissues and Cells by MRM Proteomics. *AAPS J* **16**:634–648.

Pratt JM, Simpson DM, Doherty MK, Rivers J, Gaskell SJ, and Beynon RJ (2006) Multiplexed absolute quantification for proteomics using concatenated signature peptides encoded by QconCAT genes. *Nat Protoc* **1**:1029–1043.

Russell MR, Achour B, McKenzie EA, Lopez R, Harwood MD, Rostami-Hodjegan A, and Barber J (2013) Alternative fusion protein strategies to express recalcitrant

QconCAT proteins for quantitative proteomics of human drug metabolizing enzymes and transporters. *J Proteome Res* **12**:5934–5942.

Salem F, Johnson TN, Barter ZE, Leeder JS, and Rostami-Hodjegan A (2013) Age related changes in fractional elimination pathways for drugs: Assessing the impact of variable ontogeny on metabolic drug-drug interactions. *J Clin Pharmacol* **53**:857–865.

Sharma S, Ellis EC, Gramignoli R, Dorko K, Tahan V, Hansel M, Mattison DR, Caritis SN, Hines RN, Venkataramanan R, and Strom SC (2013) Hepatobiliary disposition of 17-OHPC and taurocholate in fetal human hepatocytes: A comparison with adult human hepatocytes. *Drug Metab Dispos* **41**:296–304.

Tang L, Hines RN, Schuetz EG, and Meibohm B (2007) Age-associated protein expression of P-glycoprotein (MDR1/P-gp) and MRP2 in human pediatric liver. *Clin Pharmacol Ther* **81**:S101.

Van Kalken CK, Giaccone G, van der Valk P, Kuiper CM, Hadisaputro MM, Bosma S a, Scheper RJ, Meijer CJ, and Pinedo HM (1992) Multidrug resistance gene (P-glycoprotein) expression in the human fetus. *Am J Pathol* **141**:1063–1072.

Wei J, Ding C, Zhang J, Mi W, Zhao Y, Liu M, Fu T, Zhang Y, Ying W, Cai Y, Qin J, and Qian X (2014) High-throughput absolute quantification of proteins using an improved two-dimensional reversed-phase separation and quantification concatemer (QconCAT) approach. *Anal Bioanal Chem* **406**:4183–4193.

Yanni SB, Smith PB, Benjamin DK, Augustijns PF, Thakker DR, and Annaert PP (2011) Higher clearance of micafungin in neonates compared with adults: Role of age-dependent micafungin serum binding. *Biopharm Drug Dispos* **32**:222–232.

Zhang L, Dresser MJ, Gray AT, Yost SC, Terashita S, and Giacomini KM (1997) Cloning and functional expression of a human liver organic cation transporter. *Mol Pharmacol* **51**:913–921.

FIGURE LEGENDS

Figure 1: The most important hepatic uptake and efflux transporters and their locations in the hepatocytes.

BCRP: breast cancer resistance protein; BSEP: bile salt export pump; MATE1: multidrug and toxin extrusion protein1; P- GP: P-glycoprotein; MRP: multidrug resistance- associated protein; NTCP: Na+-taurocholate co-transporting polypeptide; OAT: organic anion transporter; OATP: organic anion transporting polypeptide; OCT: organic cation transporter; OSTα/β: organic solute transporter

Figure 2: The number of drugs of paediatric application that are substrates for liver transporters.

Figure 3: The relative abundance values (A) and (B), and the age related changes in the relative importance of two transporters T1 and T2 (C) and (D) in different age groups. (A) The relative values of T1 and T2 are 0.6 and 0.5 respectively at birth compared to the adult value. (B) T1 relative value at birth is 0.6 compared to the adult value while T2 is not expressed at birth. (C) Both T1 and T2 have a constant relative importance all over the different age groups. (D) T1 has a higher relative importance than T2 in neonates compared to adults.

Table 1. Drugs known to be Substrates for Liver Transporters

Transporter	Substrates
NTCP	Atorvastatin, Fluvastatin, Pitavastatin, **Rosuvastatin**, Taurocholate covalently bound drugs.
OATP1A2	**Acebutolol, Atenolol**, Atrasentan, **Celiprolol**, **D-penicillamine** , Deltorphin-II, Erythromycin, **Fexofenadine**, Imatinib, **Levofloxacin**, **Lopinavir**, **Methotrexate**, Microcystin-LR, Ouabain, Pitavastatin, **Rosuvastatin**, **Rocuronium**, **N-Methyl Quinine**, **Saquinavir**, **Sotalol**, **Thyroxine**, Talinolol, Tebipenem pivoxil, Unaprostone
OATP1B1	Atrasentan, **Atorvastatin**, **Benzylpenicillin**, **Bosentan** ,**Caspofungin**, Cerivastatin, D-penicillamine , Enalapril, **Ezetimibe** glucouronide , **Fexofenadine**, **Fluvastatin**, **Methotrexate**, Microcystin-LR, **Olmesartan**, Ouabain, Pitavastatin, Phalloidin, **Pravastatin**, **Repaglinide**, **Rifampicin**, **Rosuvastatin** , **Simvastatin**, SN-38, Troglitazone-Sulfate , Temocapril, **Valsartan**
OATP1B3	Atrasentan, Amanitin, **Bosentan**, **Cyclosporin**, **Digoxin**, **Docetaxel**, Deltorphin-II, D-Penicillamine , **Enalapril**, Erythromycin, **Fexofenadine**, **Fluvastatin**, Imatinib, **Methotrexate**, Microcystin,**Olmesartan**, Ouabain, **Paclitaxel**, Pitavastatin, Phalloidin, Pravastatin, **Rifampicin**, **Rosuvastatin**, SN-38,**Telmisartan**, **Valsartan**
OATP2B1	**Atorvastatin**, **Benzylpenicillin**, **Bosentan**, **Fluvastatin**, **Fexofenadine**, Glibenclamide, **Pravastatin**, Pitavastatin, **Rosuvastatin**, Unaprostone
OAT2	**Allopurinol**, L-Ascorbic Acid, **Bumetanide**, **Erythromycin**, **5-Fluorouracil**, **Methotrexate**, Paclitaxel, **Ranitidine**, **Salicylate**,

DMD Fast Forward. Published on December 28, 2015 as DOI: 10.1124/dmd.115.067801
This article has not been copyedited and formatted. The final version may differ from this version.

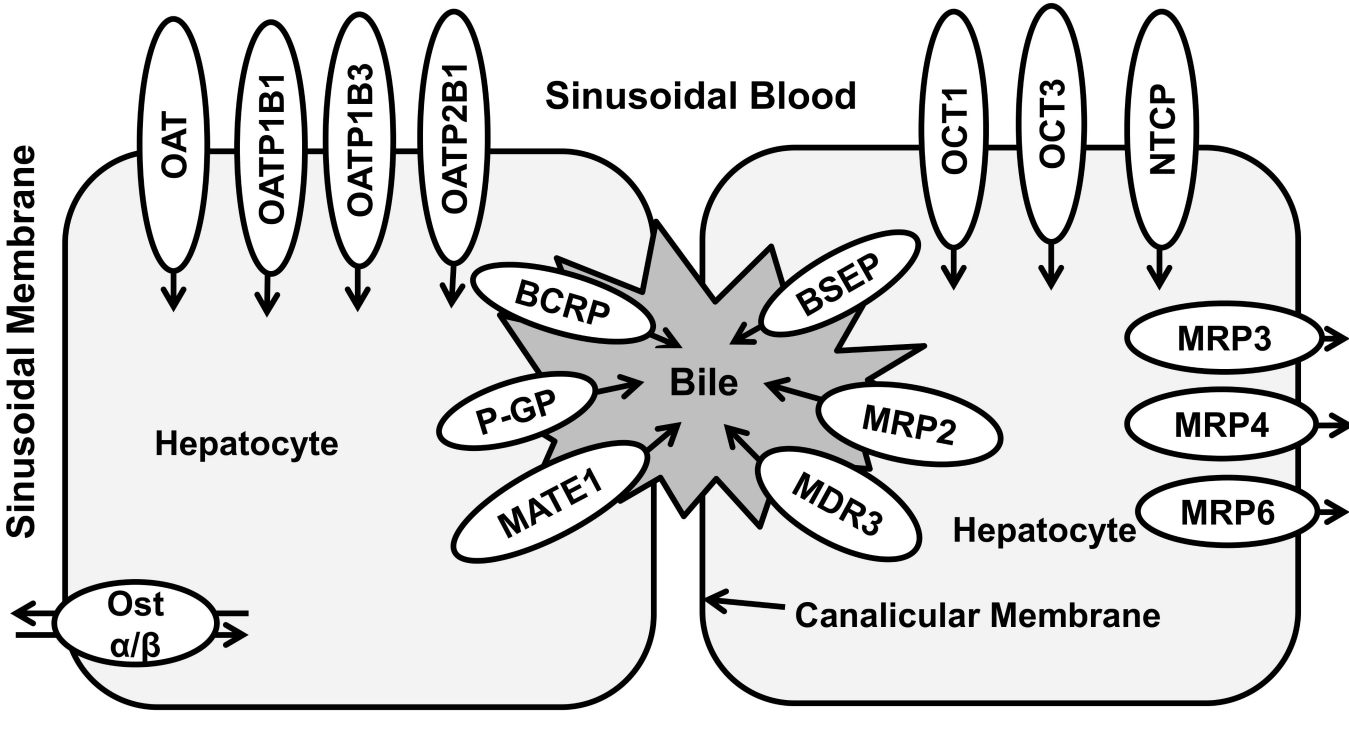
	Tetracycline, Taxol, **Theophylline** , **Zidovudine**
OCT1	**Acyclovir**, Azidoprocainamide, Berberine, **Citalopram**, **Cimetidine**, **Cisplatin**, **Famotidine**, Furamidine, **Ganciclovir**, **Imatinib**, Irinotecan, Lamivudine, **Metformin**, Methoiodide, **Morphine**, **Oxaliplatin**, **Ondansetron**, **Procainamide**, **Pentamidine**, Picoplatin, Paclitaxel, Quinidine, **Ranitidine**, Tropisetron, **Tramadol**, **Verapamil**
OCT3	**Adefovir**, **Atropine**, **Amantadine**, **d-Amphetamine**, **Cimetidine**, Clonidine, Citalopram, Desipramine, Diphenhydramine, Dizoclipine, Etilefrine, **Granisetron**, **Imipramine**, **Ketamine**, **Lidocaine**, Lamivudine, Metformin, Mitoxantrone, **Memantine**, o-Methylisoprenaline, **Nicotine**, **Phenoxybenzamine**, **Phencyclidine**, **Prazosin**, **Procainamide**, **Quinidine**, **Ranitidine,** Tropisetron, Verapamil
P-gp	Amprenavir, **Atorvastatin**, **Aldosterone**, Berberine, Corticosterone; **Cimetidine**, **Cyclosporin** A, **Dexamethasone**, **Digoxin**, **Daunorubicin**, **Doxorubicin**, Debrisoquine, **Diltiazem**, **Erythromycin**, **Etoposide**, **Fexofenadine**, Grapafloxacin, **Hydrocortisone**, **Indinavir**, **Imatinib**, Irinotecan, Lovastatin, **Losartan**, **Levofloxacin**, **Loperamide**, **Mitoxantrone** , **Morphine**, Norverapamil, Nelfinavir, **Paclitaxel**, Pitavastatin, **Phenytoin**, Quinidine, **Rosuvastatin**, **Ritonavir**, Rhodamine 123, **Saquinavir**, **Simvastatin**, **Tacrolimus**, **Taxanes**, Talinolol, **Terfenadine**, **Verapamil**, **Vinblastine**, **Vincristine**
BSEP	**Fexofenadine**, **Pravastatin**, Vinblastine
MRP2	**Acetaminophen** Glucuronide, Carboxydichlorofluorescein, Camptothecin, Cerivastatin, **Cisplatin**, **Doxorubicin**, **Etoposide** , **Fexofenadine**, **Glibenclamide**, **Indomethacin**, **MTX** , Mitoxantrone, Olmesartan,

DMD Fast Forward. Published on December 28, 2015 as DOI: 10.1124/dmd.115.067801
This article has not been copyedited and formatted. The final version may differ from this version.

	Pitavastatin, **Pravastatin**, **Rifampin** Conjugates, **Rosuvastatin**, **Spiramycin**, SN-38 glucouronide, **Vincristine**, Valsartan
BCRP	**Albendazole** Sulfoxide, Cerivastatin, **Ciprofloxacin**, **Daunorubicin**, **Doxorubicin**, Dirithromycin, **Erythromycin**, **Epirubicin**, Genistein , **Irinotecan**, **Imatinib**, **Lamivudine**, **Methotrexate**, MX, **Norfloxacin** , **Nitrofurantoin**, Oxfendazole, **Ofloxacin**, Pitavastatin, **Prazosin**, Pantoprazole, **Rifampicin**, Rhodamine 123, **Rosuvastatin**, **Sulfasalazine**, SN-38, **Testosterone**, **Tamoxifen**, Topotecan, **Zidovudine**
MATE1	Acyclovir, Cephalexin, **Fexofenadine,** Gancyclovir, Metformin, Oxaliplatin
MRP1	Adfovir, Apicidin, Berberine, Camptothecins, Ciprofloxacin, **Citalopram**, **Daunorubicin**, **Doxorubicin**, Difloxacin, **Etoposide**, Edatrexate, Epirubicin, **Flutamide**, Fifloxacin, Grepafloxacin, **Irinotecan**, Indinavir, **Idarubicin**, **Methotrexate**, Pirarubicin, Paclitaxel, **Ritonavir**, Romidepsin, **Raltitrexed**, SN-38, **Saquinavir**, Tomudex, **Vincristine**, Vinblastine,
MRP3	**Acetaminophen** glucuronide, **Etoposide**, **Fexofenadine**, **MTX**, Teniposide, Vincristine
MRP4	**Azidothymidine** , **Lamivudine**, **Methotrexate**, PMEA, **Stavudine** **Zidovudine**
MRP5	**Adefovir**, Atorvastatin, 5-Flurouracil, **Methotrexate**, PMEA, Rosuvastatin
MRP6	Endothelin Receptor Antagonist BQ-123
Ost α/Ost β	Digoxin

* Drugs in bold have paediatric application * Drugs underlined have high (f_T) valu

Figure 1



Re-drawn from UCSF-FDA TransPortal. Retrieved from http://dbts.ucsf.edu/fdatransportal/organs/liver/

Figure 2

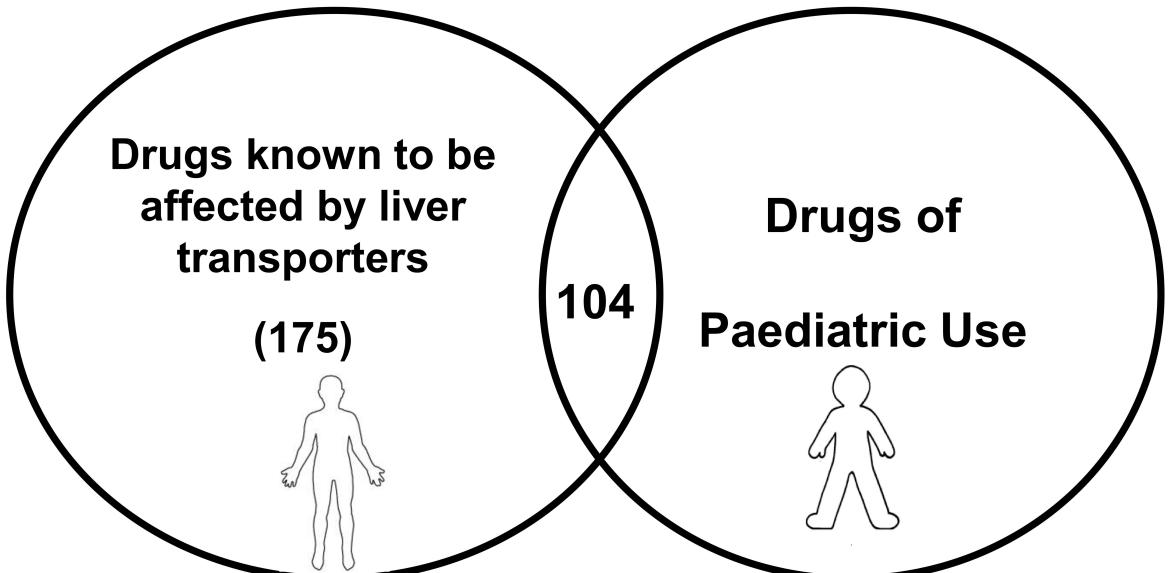
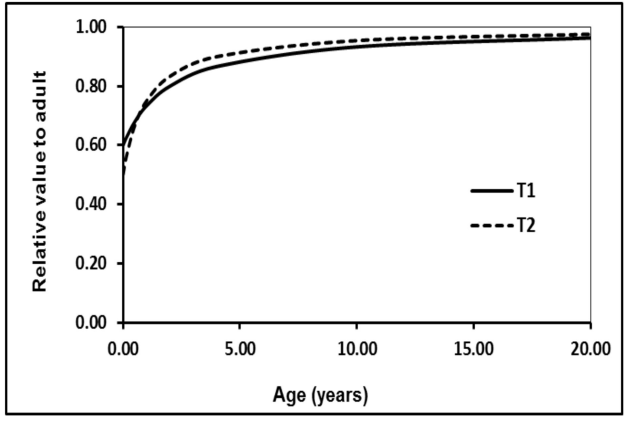
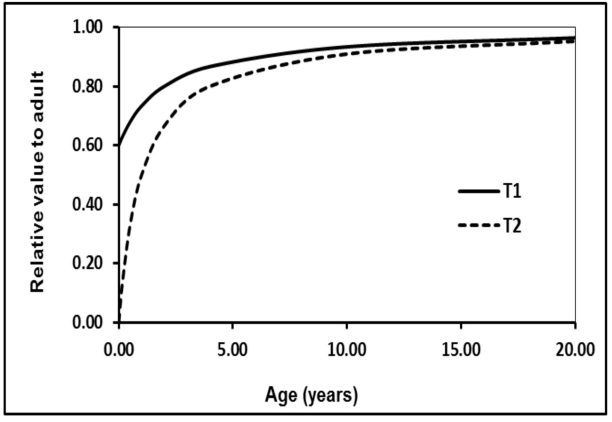

Drugs known to be affected by liver transporters
(175)
104
Drugs of
Paediatric Use

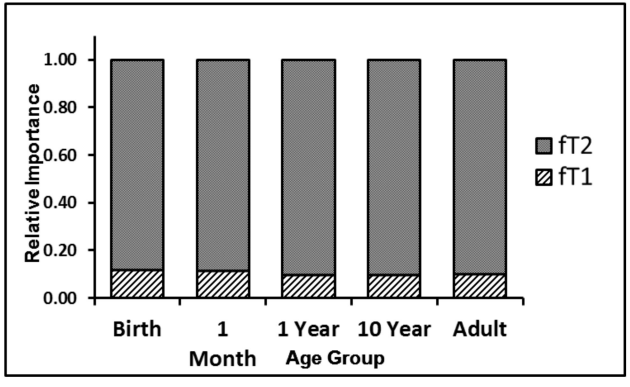
Figure 3



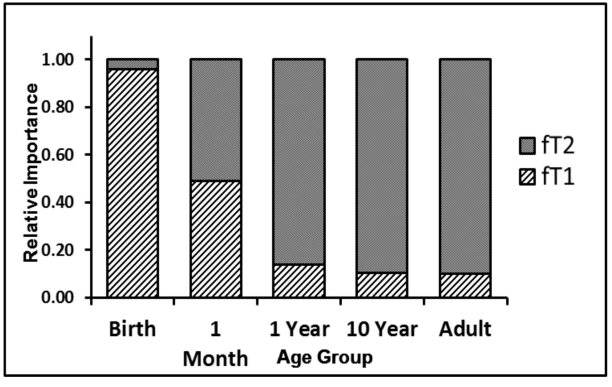
(A)



(B)



(C)



(D)