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Ontogeny of human hepatic and intestinal transporter gene expression during childhood: age matters

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Ontogeny of hepatic and intestinal transporter expression

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List of nonstandard abbreviations:

ABC: ATP-binding cassette transporters

BRCP: Breast cancer resistance protein

CYP3A: Cytochrome P450 family 3A

GA: gestational age

HCC: hepatocellular carcinoma

MDR1: multidrug resistance 1

MRP2: multidrug resistance protein 2

OATP: organic anion transporting polypeptide

OCT: organic cation transporter

PXR: pregnane X receptor

RIN: RNA integrity number

RT-PCR: reverse transcription polymerase chain reaction

SLCO: solute carrier organic anion

ABSTRACT

Many drugs prescribed to children are drug transporter substrates. Drug transporters are membrane-bound proteins that mediate the cellular uptake or efflux of drugs, and which are important to drug absorption and elimination. Very limited data are available on the effect of age on transporter expression. The aim of this study was to assess age-related gene expression of hepatic and intestinal drug transporters. MRP2, OATP1B1 and OATP1B3 expression was determined in postmortem liver samples (fetal n=6, neonatal n=19; infant n=7; child n=2; adult n=11) and MDR1 expression in 61 pediatric liver samples. Intestinal expression of MDR1, MRP2 and OATP2B1 was determined in surgical small bowel samples (neonates n=15, infants n=3, adults n=14). Using real time RT-PCR, fetal and pediatric gene expression was determined relative to 18S rRNA (liver) and villin (intestines), and compared to adults using the $2^{-\Delta\Delta Ct}$ method. Hepatic expression of MRP2, OATP1B1 and OATP1B3 in all pediatric age groups was significantly lower than in adults. Hepatic MDR1 mRNA expression in fetuses, neonates and infants was significantly lower than in adults. Neonatal intestinal expressions of MDR1 and MRP2 were comparable to those in adults. Intestinal OATP2B1 expression in neonates was significantly higher than in adults. We provide new data that show organ- and transporter-dependent differences in hepatic and intestinal drug transporter expression in an age-dependent fashion. This suggests that substrate drug absorption mediated by these transporters may be subject to age-related variation in a transporter dependent pattern.

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INTRODUCTION

Drug transporters are membrane-bound proteins whose primary function is to facilitate the trafficking of drugs and their metabolites across a biological membrane. Expressed in structural cells that compose the organs of importance to drug disposition, such as enterocytes and hepatocytes, they are involved in the active uptake and elimination of orally administered drugs. In adults, drug transporters are recognized as key determinants of variation in the pharmacokinetics of many drugs, as shown in studies using primary cell and ex vivo organ cultures as well as clinical studies (DeGorter et al., 2012). In contrast, such data in children are scarce and clinical studies are absent (Chen et al., 2005; Fakhoury et al., 2005; Miki et al., 2005; Fukudo et al., 2006; Chen et al., 2008; Mizuno et al., 2013).

Pharmacokinetics in children is known to be affected by developmental changes with age (Kearns et al., 2003). Drug metabolizing enzyme activity, for example, changes significantly from fetal to adolescent age associated with alterations in metabolic clearance of many drugs (de Wildt, 2011). Notably, hepatic and intestinal expression of cytochrome P450 (CYP) 3A4 shows a clear developmental pattern, and systemic clearance of midazolam, a known substrate of CYP3AA changes with age (Johnson et al., 2001; de Wildt et al., 2002; Leeder et al., 2005). Similarly, compared to adults, differences in body composition and renal function in children affect drug tissue distribution and renal drug excretion, respectively.

As a consequence, it has become apparent that simply extrapolating drug doses from adults to children based on body weight is likely inaccurate due to lack of our understanding of age-dependent differences in the maturation of the drug absorption and elimination processes involved in drug disposition. As the expression of drug metabolizing enzymes appears to be age-related, it is also likely that this is the case for drug transporter expression. Data from animal studies report occurrence of transporter-specific maturation (Klaassen and Aleksunes, 2010). In humans, several efflux transporters that belong to the ATP binding cassette family (ABC) including the multidrug resistance protein 1 (MDR1/ABCB1), multidrug resistance-associated protein 2 (MRP2/ABCC2), and breast cancer resistance protein

(BCRP/ABCG2) were found to be expressed in fetal liver or intestine, but otherwise little is known on developmental patterns of individual drug transporters (Chen et al., 2008; Fakhoury et al., 2009; Sharma et al., 2013). Systematic characterization of age-related differences in transporter expression will aid the selection of the most appropriate dose for substrate drugs in children.

In a first step towards elucidating the developmental changes, this study determined the gene expression of clinically relevant hepatic and intestinal drug transporters across the pediatric age range. We focused on transporters with well-defined roles in drug pharmacokinetics in adults (DeGorter et al., 2012) (Fig. 1). These included the efflux carriers MDR1 and MRP2 in liver and intestine, and uptake carriers of the organic anion transporting polypeptide (OATP/SLCO) family including the liver-specific transporters OATP1B1 and OATP1B3, and OATP2B1 in the intestine.

MATERIALS AND METHODS

1. Tissue samples

Autopsy liver tissue samples from fetuses and from children up to 18 years of age were obtained from the Erasmus MC Tissue Bank. An opt-out clause was in place for use of tissue from the Tissue Bank. The Erasmus MC Research Ethics Board (REB) provided a waiver for Ethics approval according to the Dutch Law on Research in humans. Fresh intestinal tissue was collected during surgery at the time of resection. Intestinal samples from children were derived from other research projects, and informed consent was previously obtained for the use of this tissue in the context of these Erasmus MC REB approved studies. After resection, all liver and intestinal tissues were immediately snap frozen in liquid nitrogen, stored at -80ºC and processed on ice for mRNA isolation.

Healthy human adult liver samples were obtained from The Liver Tissue Cell Distribution System, University of Minnesota under NIH Contract #N01-DK-7-0004/HHSN267200700004C. Adult intestinal samples were obtained from the University of Western Ontario, and the use of these samples had been REB approved.

Additional pediatric tissue specimens for MDR1 analyses were obtained from the NIH-supported tissue programs: the Liver Tissue Cell Distribution System (LTCDS) from the Minnesota and Pittsburgh collection centers; the University of Maryland Brain and Tissue bank for Developmental Disorders (Baltimore, MD); and the Laboratory of Developmental Biology at the University of Washington (Seattle, WA). Additional samples were from Xenotech, LLC (Lenexa, KS). The use of these tissues was declared non-human subjects research by the Children's Mercy Hospitals and Clinics Pediatric Institutional Review Board. The source of the obtained samples is listed in Table 1.

2. mRNA Isolation and cDNA synthesis

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Frozen human tissue samples were mechanically homogenized on ice. Homogenate was applied to a QiaShredder column (Qiagen) and RNA was extracted using the RNeasy Mini Kit (Qiagen) according to the instructions provided by the manufacturer. RNA was treated with DNase to digest genomic DNA remnants. Quantity and integrity of RNA were determined by microfluidics-based analyses using the 2100 BioAnalyzer (Agilent). Samples with an RNA integrity number (RIN) < 5 were excluded from this study. Complementary DNA (cDNA) was synthesized using the High-capacity cDNA Archive Kit (Applied Biosystems) and random priming.

3. Real Time Reverse Transcription Polymerase Chain Reaction (RT-PCR)

mRNA expression was measured by SYBR green quantitative real-time polymerase chain reaction (PCR) with the ABI 7500 sequence detection system (Applied Biosystems). Previously optimized primers were used (supplemental file) (Glaeser et al., 2007). Modified primers were used for villin and OATP2B1 (reverse primer only), and primer sequences were obtained via qPrimer Depot (http://primerdepot.nci.nih.gov/). After each PCR, a melting curve analysis was performed to confirm product specificity. Eukaryotic 18S rRNA was assessed as endogenous control with the use of a TaqMan VIC/MGB probe (4319413E, Applied Biosystems). Transporter transcript levels were normalized to18s rRNA, and relative expression was determined using the $2^{-\Delta\Delta CT}$ method comparing pediatric samples to adult liver or intestine (calibrator samples) (Schmittgen and Livak, 2008). The adult target gene value was determined by using the median of the adult Ct values.

For comparison, all 96 well plates analyzing liver samples included a specific liver control sample to normalize expression for the gene of interest and 18S rRNA, if appropriate. Similarly, a specific intestinal sample was added to plates to normalize for the genes of interest, 18S rRNA and villin if appropriate.

4. Statistics

All data were analyzed by Kruskal-Wallis analysis, followed by group comparisons using a nonparametric Mann-Whitney test and post-hoc Bonferroni multiple comparison test. All statistical analyses were performed using GraphPad Prism version 5.00.2 and IBM SPSS Statistics software (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0, Armonk, NY). Level of significance was $p<0.05$. Data are presented as median and range, unless indicated otherwise.

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RESULTS

Sample characteristics

Characteristics of all samples are provided in Table 1. Forty-one liver samples from anonymous donors were collected from the Erasmus MC Tissue Bank. Seven samples were excluded because of poor mRNA quality and 34 samples remained for analysis. The causes of death were the following: congenital disorders (n=9), [hydrops fetalis (n=2), ruptured giant omphalocele, hemangioendothelioma, extended congenital abnormalities, trisomy 7 or 8, cardiac, osteogensis imperfecta], cardiac failure (n=6), respiratory disorders (n=6), sepsis (n=4), gastrointestinal disorders (n=4), neurological disorders (n=3), and unknown causes (n=2). Adult liver samples (n=11) were derived from histologically normal parts of transplanted livers. Death was related to anoxia (n=5), trauma (n=3), neurologic disorders (n=2) or unknown cause (n=1). Age was unknown. RIN median of fetal, pediatric and adult liver samples was 7 (range 5 - 10). MDR1 mRNA expression was also measured in additional pediatric liver samples; 3 fetuses, 1 neonate, 4 children age 1 month to 12 months, and 17 children aged 12 months to 17 years. Thus the total set of liver samples in which MDR1 mRNA expression was measured consisted of 9 fetal samples and 52 neonatal/pediatric samples. Cause of death of the additional pediatric donors was unknown. Twenty-eight intestinal resection samples were obtained, of which 10 were excluded due to poor mRNA quality. The main reasons for resection were necrotizing enterocolitis and intestinal atresia (n=11); other reasons (n=7) were volvulus, persistent ductus omphaloentericus, Meckels diverticulum, meconium peritonitis and ileostomy closure. Resection areas were aimed at the most proximal part of the intestine. Adult intestinal samples (n=14) were endoscopic biopsy samples and negative for any pathology. RIN median of pediatric intestinal samples was 7 (range 5 - 9). RIN values of samples from adult intestinal donors were not measured. In pediatric and adult intestinal samples, 18S was significantly correlated with villin mRNA (n=32, r=0.6110, $p<0.05$). We also determined

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CYP3A4 gene expression. The mRNA expression of CYP3A4 is well reported in the literature. Patterns of intestinal and hepatic CYP3A4 mRNA expression were the same as those reported in the literature (data not shown) (Miki et al., 2005).

Information about medication use by the donors was not available.

Transporter-specific ontogenetic profile of MDR1, MRP2, OATP1B1 and OATP1B3 mRNA expression in liver

Hepatic transporter expression was significantly associated with age: MDR1 $H(4) = 35.3$, $p<0.05$; MRP2 $H(4) = 18.0$, $p<0.05$; OATP1B1 $H(4) = 27.4$, $p<0.05$; OATP1B3 $H(4) = 28.1$, $p<0.05$. Fetal, neonatal and infant (up to 12 months of age) gene expression of all hepatic transporters was lower than in adults. In the age group 1 to 7 years only two samples were assessed for the hepatic transporters MRP2, OATP1B1 and OATP1B3, as a result of which median mRNA expression could not be determined and comparison with adult expression was not possible.

MDR1 mRNA expression in fetal (0.061 (0.027 - 0.792)) and neonatal (0.036 (0.011 - 0.364)) age groups was 20 to 30-fold lower than in adults (Fig. 2). MDR1 mRNA expression in infants was slightly lower (0.248 (0.010 - 1.46)) than in adults (Fig. 2). Median MDR1 gene expression in children aged 1 to 7 years was not different from adults (0.777 (0.012 - 8.928); $p=0.663$). Inter-individual variability in all age groups was large.

MRP2 mRNA expression was about 30-fold lower in fetal liver samples (0.074 (0.004 - 0.162)), 200-fold lower in neonates (0.012 (0.001 - 0.238)), and 100-fold lower in infants (0.022 (0.008 - 0.357)) than in adult liver samples (Fig. 2). Compared to adult liver, OATP1B1 mRNA expression was 20-fold lower in fetal liver samples (0.042 (0.0004 - 0.683)), 500-fold lower in neonates (0.002 (0.0003 - 0.018)), and 90-fold lower in infants (0.010 (0.001 - 0.086)) (Fig. 2). Expression profiles of OATP1B3 in the liver samples were similar to those of OATP1B1. Expression of hepatic OATP1B3 was 30-fold lower in fetuses

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(0.046 (0.001 - 1.910)), 600-fold lower in neonates (0.002 (0.0004 - 0.0187)), and 100-fold lower in infants (0.010 (0.001 - 0.021)) than in adults (Fig. 2).

Similar MDR1 and MRP2 mRNA expression in neonatal and adult intestines, and higher OATP2B1 in neonatal intestines

Intestinal transporter expression was significantly associated with age for MRP2 $H(2) = 9.1$, $p<0.05$ and OATP2B1 $H(2) = 15.8$, $p<0.05$, but not for MDR1, $H(2) = 1.3$, $p=0.5$. MDR1 expression in intestinal samples of neonates was 0.8382 (0.370 - 97.0; $p=0.395$) and of infants was 1.454 (0.409 - 3.174; $p=0.413$) - and hence similar to adults (Fig. 3). Expression of MRP2 mRNA in intestinal samples of neonates (1.262 (0.338 - 9.266); $p=0.248$) was similar to adult expression (Fig. 3). In infant intestinal samples, however, MRP2 mRNA expression was significantly lower (0.038 (0.023 - 0.074); $p<0.05$) than in adult samples. Interestingly, the uptake transporter OATP2B1 showed a higher mRNA expression in neonates (2.86 (1.212 - 34.45); $p<0.0001$). In infants up to 12 months of age, OATP2B1 was expressed at the same level as in adult intestinal samples (1.21 (0.328 – 2.288); $p=0.147$) (Fig. 3).

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DISCUSSION

Little is known about transporter gene expression in children. In this study utilizing samples from fetal to adult age, gene expression of hepatic transporters MDR1, MRP2, OATP1B1, and OATP1B3 was found to be age-related, as their expression was lowest in fetal and pediatric liver samples. In contrast, intestinal OATP2B1 expression in neonates was higher than in adults. Intestinal MDR1 and MRP2 expression was similar between neonates and adults.

Ontogeny in hepatic transporters

Hepatic MDR1 expression is already detectable in early human fetal life. The maturation pattern in the present study is consistent with previous data on hepatic MDR1 mRNA expression (van Kalken et al., 1992; Miki et al., 2005; Fakhoury et al., 2009). Our findings are also supported by immunohistochemical staining showing that MDR1 expression followed the architectural changes during fetal development (van Kalken et al., 1992). We show that MDR1 expression changes after birth with low mRNA expression until 12 months of age, thereupon increasing to adult levels. In a recent study, using a novel LC-MS-MS method to estimate protein expression, from 7 years onwards, MDR1 was stable up to 70 years of age in a cohort of more than 50 patients (Prasad et al., 2014).

Low fetal MRP2 expression in humans has been reported earlier in two small studies (n=10, 14-20 weeks GA; and n=3, 20-21 weeks GA) (Chen et al., 2005; Sharma et al., 2013). In the latter, however, 2 of 5 control adult liver samples were from normal tissue in patients with hepatocellular carcinoma (HCC). Since organic cation transporter (OCT)1 expression has been shown to be lower in non-tumor HCC liver than healthy liver, a similar phenomenon with other transporters cannot be excluded (Schaeffeler et al., 2011). Using immunohistochemical staining, distinct canalicular expression of MRP2 in adult liver was observed, but a fuzzier canalicular occurrence in fetal liver which may suggest immaturity of the localization pattern (Chen et al., 2005). In addition, fetal human liver tissues obtained at

19 weeks GA showed higher MRP2 signal intensity than tissues obtained at 14 weeks (Cizkova et al., 2005) In our study, neonatal MRP2 gene expression was lower than in adults. From 7 years onwards, MRP2 protein expression was stable. (Prasad et al., 2013). The observed lower fetal OATP1B1 and OATP1B3 mRNA expression levels in the present study are also in line with human data of 3 fetal (21-23 weeks GA) and 3 adult livers (Sharma et al., 2013). In our study, OATP1B1 and OATB1B3 expression was also low in neonates. In contrast, reported neonatal OATP1B1 and OATP1B3 protein expression (n=5), was similar in adults (Yanni et al., 2011). In 80 human pediatric liver samples ranging from age 2 months to 12 years, OATP1B1 was low until 6 years of age, while OATP1B3 protein levels were high at birth, then rapidly decreased in the first year to then increase again from 8 years of age onwards to adult levels (Thomson et al., 2013). From 7 years onwards, OATP1B1 and OATP1B3 protein expression was stable, with only genotype related to OATP1B1 abundance (Prasad et al., 2014).

Ontogeny in intestinal transporters

We observed stable intestinal MDR1 expression from neonatal to adult age, which finding fills a gap between currently available fetal and pediatric data. Previously, fetal intestinal tissue obtained after induced abortion, showed no or minimal MDR1 mRNA expression in samples after 11, 13 and 14 weeks, but clear expression at 16 and 20 weeks gestational age (van Kalken et al., 1992). Another study reported similar small intestinal MDR1 mRNA expression in fetuses (14 to 20 weeks gestational age), neonates and adults (Miki et al., 2005). However, a limitation is the small sample size [n=5, n=12 (fetus plus neonates)] (van Kalken et al., 1992; Miki et al., 2005). Stable MDR1 mRNA expression in children from one month to adulthood was shown in non-diseased duodenal biopsy and jejunal tissue from liver donor recipients (Fakhoury et al., 2005; Mizuno et al., 2013). We also found stable intestinal MRP2 expression, but higher expression levels of OATP2B1 in neonates compared to adults.

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Transporter maturation observed in the present study likely reflects physiological roles of these transporters in organ development. Epigenetic mechanisms such as DNA methylation are thought to be involved in maturation of drug metabolizing enzymes. For example, DNA methylation seems to partly explain a developmental switch from expression of CYP3A7 to CYP3A4 in human fetal and adult liver (Kacevska et al., 2012). Epigenetic mechanisms may be also involved in the developmental regulation of drug transporter expression, however studies are currently lacking.

Due to mediation by transcription factors (which in their turn can be induced by chemicals or pathological conditions) some transporters show relatively high whereas others show low expression (Klaassen and Aleksunes, 2010). For example, expression of the steroid- and xenobiotic-sensing pregnane X receptor (PXR) was shown to correlate with intestinal BCRP, MRP2 and MDR1 mRNA in many different human tissues (e.g. intestine and liver) (Synold et al., 2001; Albermann et al., 2005; Miki et al., 2005). We may further speculate that the ontogeny of transporters is a combined function of developmental changes, genetic heterogeneity, and exposure to substrates (drug or environmental) that induce or inhibit expression and/or activity. For example, CYP1A2 and CYP3A4 activity, assessed by phenotyping caffeine and dextromethorphan, respectively, was shown to be affected by the type of infant feeding (breast milk or formula) (Blake et al., 2006). Dietary supplements in infant formula decreased MDR1 expression in colon cell cultures (Bebawy et al., 2009). Moreover, the effect of feeding is more variable when a child's diet is expanded, as specific nutrients can influence MDR1 expression (Schwarz et al., 2005; Schwarz et al., 2007).

This study is one of the first exploratory studies characterizing developmental changes in hepatic and intestinal transporters. Some limitations of the data should be addressed. First, most fetal liver samples were obtained in the second trimester of gestation, thus results might not be representative for the entire range of gestational age. Second, the variation in fetal transporter mRNA expression may be partly due to the small number of samples

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representing ages more distant from the median age. Given the small number per age group, the range of expression might not be representative for the entire population.
Third, all liver samples were collected within 24 hours after death, but the exact time is unknown. Still, RNA stability was minimized by RNA integrity determination.

Also, our samples may display disease-dependent variability in transporter expression. Inflammation has been shown to affect drug metabolism and transport. Decreased midazolam clearance in critically ill children is likely a result of reduced CYP3A activity (Vet et al., 2012). Inconsistency has been reported from studies assessing the effect of pro-inflammatory cytokines on intestinal MDR1 expression *in vitro* and in animal models, but suggest a trend towards decreased expression (Fernandez et al., 2004). In children with Crohn disease, MDR1 expression was higher in both inflamed and non-inflamed duodenal biopsies compared to healthy age-matched controls (Fakhoury et al., 2006). Inflammation was also shown to affect the expression of other transporters in primary human hepatocytes (i.e. OATP1B1, OATP1B3, MDR1, MRP2) resulting in reduced mRNA expression, protein, and activity (Le Vee et al., 2011). Thus, evidence exists to consider potential effects of underlying disease when interpreting age-related changes in transporter expression.

Finally, transporter genetic polymorphisms likely contribute to the observed variability in gene expression (DeGorter et al., 2012). Recently, Nies et al and Prasad et al showed that hepatic expression of OATP1B1, but not OATP1B3, is significantly affected by genetic variants (Nies et al., 2013; Prasad et al., 2014).
The observed age-dependent maturation and large inter-individual variability in drug transporter expression may have implications for oral drug absorption and hepatic drug excretion. The question whether posttranscriptional modifications occur and if expressed mRNA level can be extrapolated to protein expression and ultimately in vivo activity remains to be answered (Johnson et al., 2001; Fakhoury et al., 2005). Human hepatic OATP1B1 and OATP1B3 protein expression showed a trend with high inter-individual variability in the first year of life followed by lower variability until the age of 8 years, when variability increases again (Thomson et al., 2013). This typical age-related change in variability at younger age is

also reflected by our findings on OATP1B1 and OATP1B3 mRNA expression. In contrast, others could not correlate protein levels with OATP1B1 or MDR1 mRNA expression (Ulvestad et al., 2013). Further protein expression and transporter activity studies are needed to confirm these mRNA expression results.

In conclusion, hepatic MDR1, MRP2, OATP1B1, OATP1B3 and intestinal MDR1, MRP2, OATP2B1 drug transporter expression show organ- and transporter-specific maturation patterns during childhood. Therefore, the disposition of drugs substrates for these transporters may be subject to age-related changes other than that in body size alone.

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AUTHORSHIP CONTRIBUTIONS

- Participated in research design: Mooij, de Koning, Schwarz, Spaans, de Wildt
- Conducted experiments: Mooij, de Koning, de Wildt
- Contributed new reagents or analytic tools: Leeder, Gaedigk, Kim, Samsom
- Performed data analysis: Mooij, Gaedigk, de Koning, de Wildt
- Wrote or contributed to the writing of the manuscript: Mooij, de Wildt, Kim, Tibboel, Van Goudoever

REFERENCES

Albermann N, Schmitz-Winnenthal FH, Z'Graggen K, Volk C, Hoffmann MM, Haefeli WE, and Weiss J (2005) Expression of the drug transporters MDR1/ABCB1, MRP1/ABCC1, MRP2/ABCC2, BCRP/ABCG2, and PXR in peripheral blood mononuclear cells and their relationship with the expression in intestine and liver. *Biochem Pharmacol* **70:**949-958.

Bebawy M, Rasmussen C, Sambasivam S, and Bao S (2009) Dietary nucleotide supplements in infant formula modify the expression of P-glycoprotein in the intestinal epithelium in vitro. *Int J Vitam Nutr Res* **79:**381-387.

Blake MJ, Abdel-Rahman SM, Pearce RE, Leeder JS, and Kearns GL (2006) Effect of diet on the development of drug metabolism by cytochrome P-450 enzymes in healthy infants. *Pediatr Res* **60:**717-723.

Chen HL, Chen HL, Liu YJ, Feng CH, Wu CY, Shyu MK, Yuan RH, and Chang MH (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.

Cizkova D, Morky J, Micuda S, Osterreicher J, and Martinkova 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.

de Wildt SN (2011) Profound changes in drug metabolism enzymes and possible effects on drug therapy in neonates and children. *Expert Opin Drug Metab Toxicol* **7:**935-948.

de Wildt SN, Kearns GL, Hop WC, Murry DJ, Abdel-Rahman SM, and van den Anker JN (2002) Pharmacokinetics and metabolism of oral midazolam in preterm infants. *Br J Clin Pharmacol* **53:**390-392.

DeGorter MK, Xia CQ, Yang JJ, and Kim RB (2012) Drug transporters in drug efficacy and toxicity. *Annu Rev Pharmacol Toxicol* **52:**249-273.

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

Fakhoury M, Lecordier J, Medard Y, Peuchmaur M, and Jacqz-Agrain E (2006) Impact of inflammation on the duodenal mRNA expression of CYP3A and P-glycoprotein in children with Crohn's disease. *Inflamm Bowel Dis* **12:**745-749.

Fakhoury M, Litalien C, Medard Y, Cave H, Ezzahir N, Peuchmaur M, and Jacqz-Aigrain E (2005) Localization and mRNA expression of CYP3A and P-glycoprotein in human duodenum as a function of age. *Drug Metab Dispos* **33:**1603-1607.

Fernandez C, Buyse M, German-Fattal M, and Gimenez F (2004) Influence of the pro-inflammatory cytokines on P-glycoprotein expression and functionality. *J Pharm Pharm Sci* **7:**359-371.

Fukudo M, Yano I, Masuda S, Goto M, Uesugi M, Katsura T, Ogura Y, Oike F, Takada Y, Egawa H, Uemoto S, and Inui K (2006) Population pharmacokinetic and pharmacogenomic analysis of tacrolimus in pediatric living-donor liver transplant recipients. *Clin Pharmacol Ther* **80:**331-345.

Glaeser H, Bailey DG, Dresser GK, Gregor JC, Schwarz UI, McGrath JS, Jolicoeur E, Lee W, Leake BF, Tirona RG, and Kim RB (2007) Intestinal drug transporter expression and the impact of grapefruit juice in humans. *Clin Pharmacol Ther* **81:**362-370.

Johnson TN, Tanner MS, Taylor CJ, and Tucker GT (2001) Enterocytic CYP3A4 in a paediatric population: developmental changes and the effect of coeliac disease and cystic fibrosis. *Br J Clin Pharmacol* **51:**451-460.

Kacevska M, Ivanov M, Wyss A, Kasela S, Milani L, Rane A, and Ingelman-Sundberg M (2012) DNA methylation dynamics in the hepatic CYP3A4 gene promoter. *Biochimie* **94:**2338-2344.

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.

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

Le Vee M, Jouan E, Moreau A, and Fardel O (2011) Regulation of drug transporter mRNA expression by interferon-gamma in primary human hepatocytes. *Fundam Clin Pharmacol* **25:**99-103.

Leeder JS, Gaedigk R, Marcucci KA, Gaedigk A, Vyhlidal CA, Schindel BP, and Pearce RE (2005) Variability of CYP3A7 expression in human fetal liver. *J Pharmacol Exp Ther* **314:**626-635.

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.

Mizuno T, Fukuda T, Masuda S, Uemoto S, Matsubara K, Inui KI, and Vinks AA (2013) Developmental trajectory of intestinal MDR1/ABCB1 expression in children. *Br J Clin Pharmacol*.

Nies AT, Niemi M, Burk O, Winter S, Zanger UM, Stieger B, Schwab M, and Schaeffeler E (2013) Genetics is a major determinant of expression of the human hepatic uptake transporter OATP1B1, but not of OATP1B3 and OATP2B1. *Genome Med* **5:**1.

Prasad B, Evers R, Gupta A, Hop CE, Salphati L, Shukla S, Ambudkar SV, 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 JD (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.

Schaeffeler E, Hellerbrand C, Nies AT, Winter S, Kruck S, Hofmann U, van der Kuip H, Zanger UM, Koepsell H, and Schwab M (2011) DNA methylation is associated with downregulation of the organic cation transporter OCT1 (SLC22A1) in human hepatocellular carcinoma. *Genome Med* **3:**82.

Schmittgen TD and Livak KJ (2008) Analyzing real-time PCR data by the comparative C(T) method. *Nat Protoc* **3:**1101-1108.

Schwarz UI, Hanso H, Oertel R, Miehlke S, Kuhlisch E, Glaeser H, Hitzl M, Dresser GK, Kim RB, and Kirch W (2007) Induction of intestinal P-glycoprotein by St John's wort reduces the oral bioavailability of talinolol. *Clin Pharmacol Ther* **81:**669-678.

Schwarz UI, Seemann D, Oertel R, Miehlke S, Kuhlisch E, Fromm MF, Kim RB, Bailey DG, and Kirch W (2005) Grapefruit juice ingestion significantly reduces talinolol bioavailability. *Clin Pharmacol Ther* **77:**291-301.

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.

Synold TW, Dussault I, and Forman BM (2001) The orphan nuclear receptor SXR coordinately regulates drug metabolism and efflux. *Nat Med* **7:**584-590.

Thomson MMS, Krauel S, Hines RN, Scheutz EG, and Meibohm B (2013) Lack of effect of genetic variants on the age-associated prtoein expression of OATP1B1 and OATP1B3 in human pediatric liver, in: *[Abstract for the 114th American Society for Clinical Pharmacology and Therapeutics Annual Meeting]*.

Ulvestad M, Skottheim IB, Jakobsen GS, Bremer S, Molden E, Asberg A, Hjelmesaeth J, Andersson TB, Sandbu R, and Christensen H (2013) Impact of OATP1B1, MDR1, and CYP3A4 expression in liver and intestine on interpatient pharmacokinetic variability of atorvastatin in obese subjects. *Clin Pharmacol Ther* **93:**275-282.

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

Vet NJ, de Hoog M, Tibboel D, and de Wildt SN (2012) The effect of critical illness and inflammation on midazolam therapy in children. *Pediatr Crit Care Med* **13:**e48-50.

Yanni SB, Smith PB, Benjamin DK, Jr., 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.

FOOTNOTES

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FIGURE LEGENDS

Table 1 Tissue sources and age distribution

Figure 1 Transporters in enterocyte and hepatocyte. Only transporters in enterocyte (A) and hepatocyte (B) selected in this paper.

Figure 2 Transporter gene expression in liver. Relative mRNA expression of MDR1 (A) MRP2 (B) OATP1B1 (C) OATP1B3 (D) from fetal, pediatric and adult liver samples, normalized to 18S mRNA expression and adult expression using the $2^{-\Delta\Delta C}$ method. Lines represent median and interquartile range.

Figure 3 Transporter gene expression in intestine. Relative mRNA expression of MDR1 (A) MRP2 (B) OATP2B1 (C) from pediatric and adult intestine samples, normalized to villin mRNA expression and adult expression using the $2^{-\Delta\Delta C}$ method. Lines represent median and interquartile range.

TABLE

Tissue	**Collection**	**Transporter examined**	**Number of samples**	**Sex**	**Gestational age at birth** median(range) in weeks	**Postnatal age** median(range)	**Source**
Fetal and pediatric liver	Postmortem Autopsy	MRP2, OATP1B1, OATP1B3	Fetal 6 Pediatric 28 *Total 34*	Male 19 Female 15	Fetuses: 22 (22-23) Pediatric: 36 (25 - 41)	Pediatric 1 week (0 – 7 years)	Erasmus MC Tissue Biobank
Fetal and pediatric liver	Postmortem Autopsy	MDR1	Fetal 9 Pediatric 52 *Total 61*	Male 38 Female 22 Unknown 1	Fetuses: 22 (8-23)	Pediatric 11 weeks (0-17 years)	1) Erasmus MC Tissue Biobank 2) The Liver Tissue Cell Distribution System; from the Minnesota and Pittsburgh collection centers; the University of Maryland Brain and Tissue bank for Developmental Disorders (Baltimore, MD); and the

							Laboratory of Developmental Biology at the University of Washington (Seattle, WA). Additional samples were from Xenotech, LLC (Lenexa, KS).
Adult liver	Biopsy	MDR1, MRP2, OATP1B1, OATP1B3	11	Male 6 Female 2 Unknown 1	-	Unknown	The Liver Tissue Cell Distribution System; University of Minnesota under NIH Contract #N01-DK-7-0004/HHSN267200700004C.
Pediatric intestine	Surgical Jejunum 7 Ileum 9 Caecum 2	MDR1, MRP2, OATP2B1	18	Male 12 Female 6	Neonates: 32 (25-40)	1 (0 – 14) weeks	Erasmus MC
Adult intestine	Biopsy Ileum 14	MDR1, MRP2, OATP2B1	14	Male 3 Female 11	-	Unknown	University of Western Ontario

Figure 1

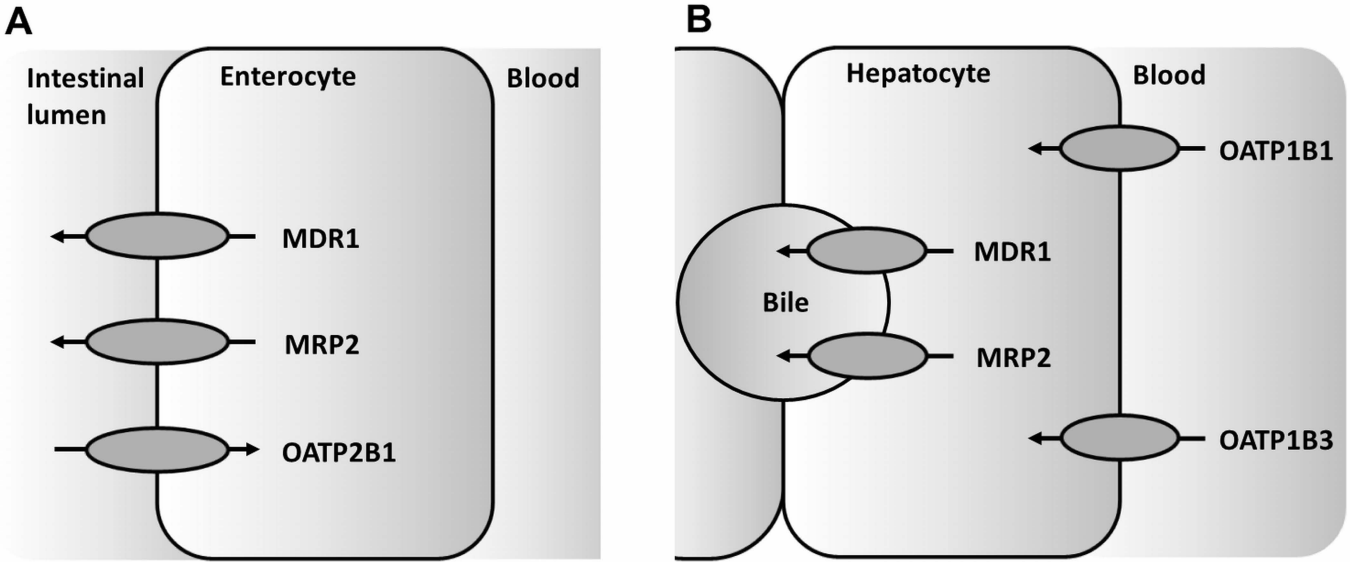

A
Intestinal lumen
Enterocyte
Blood
MDR1
MRP2
OATP2B1
B
Hepatocyte
Blood
OATP1B1
MDR1
Bile
MRP2
OATP1B3

Figure 2

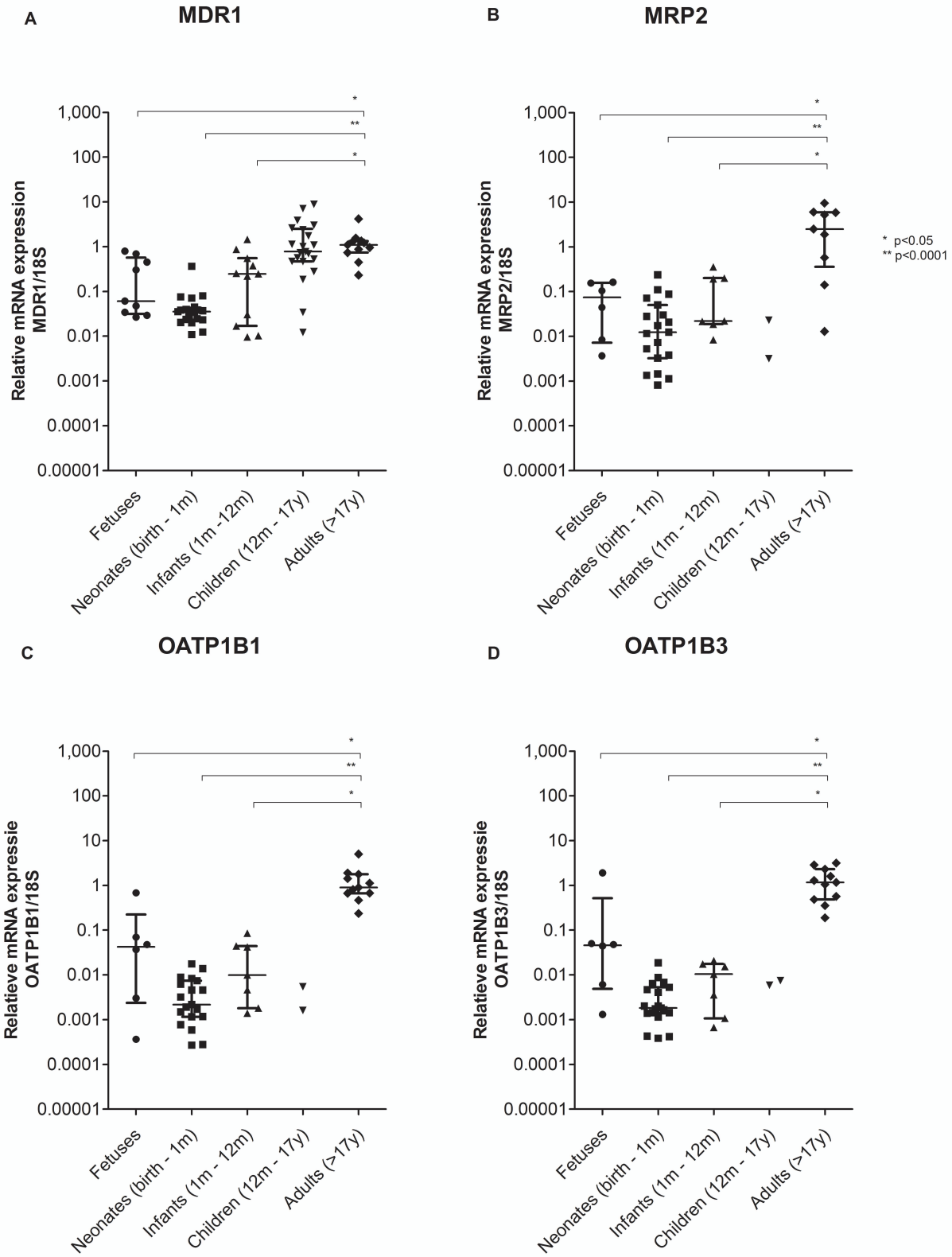
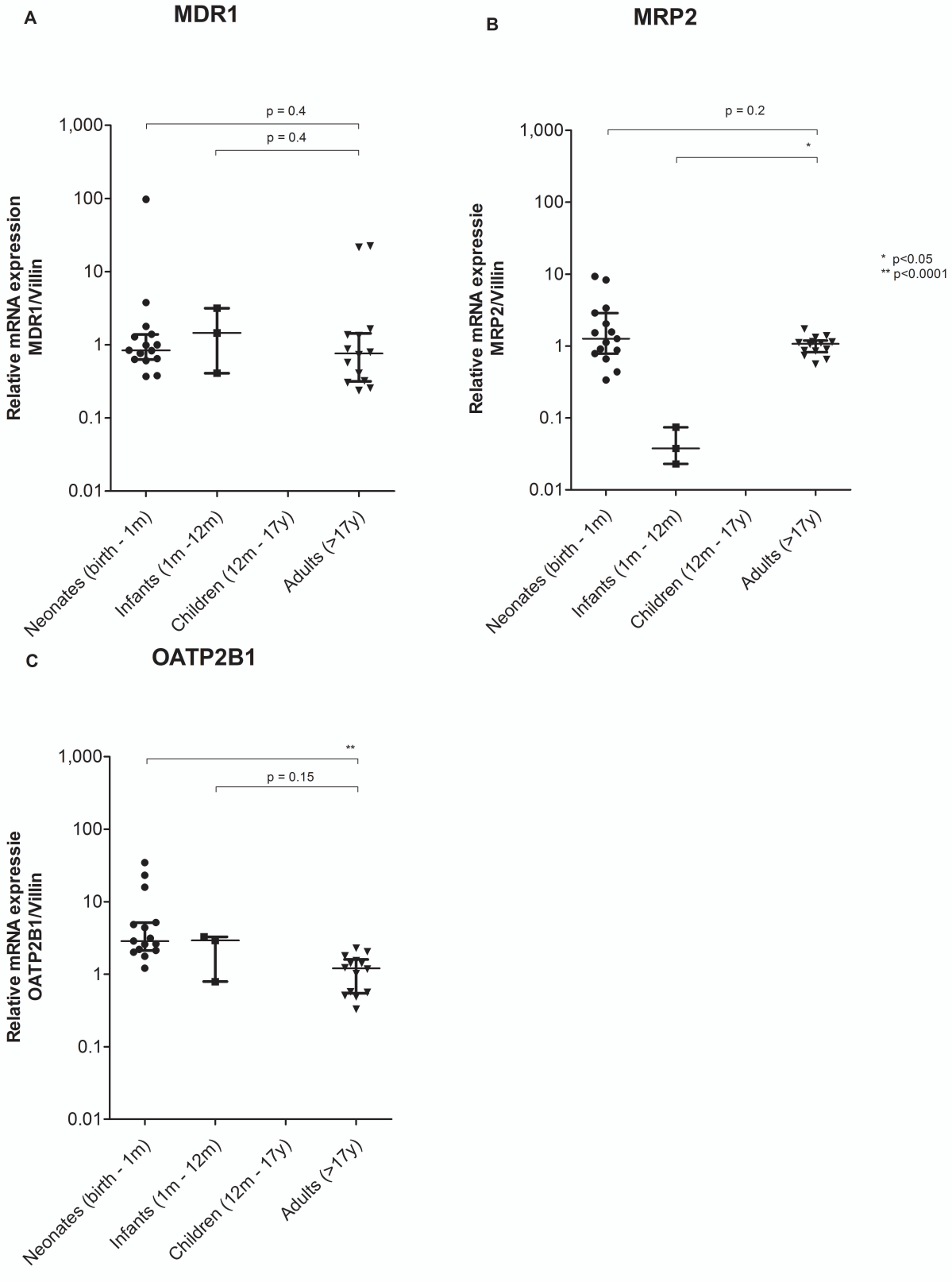

A
MDR1
Relative mRNA expression MDR1/18S
1,000
100
10
1
0.1
0.01
0.001
0.0001
0.00001
*
**
*
Fetuses
Neonates (birth - 1m)
Infants (1m -12m)
Children (12m - 17y)
Adults (>17y)
B
MRP2
Relative mRNA expression MRP2/18S
* p<0.05
** p<0.0001
C
OATP1B1
Relatieve mRNA expressie OATP1B1/18S
D
OATP1B3
Relatieve mRNA expressie OATP1B3/18S

Figure 3


A
MDR1
p = 0.4
p = 0.4
1,000
100
10
1
0.1
0.01
Relative mRNA expression
MDR1/Villin
Neonates (birth - 1m)
Infants (1m -12m)
Children (12m - 17y)
Adults (>17y)
B
MRP2
p = 0.2
*
1,000
100
10
1
0.1
0.01
Relative mRNA expressie
MRP2/Villin
Neonates (birth - 1m)
Infants (1m - 12m)
Children (12m - 17y)
Adults (>17y)
* p<0.05
** p<0.0001
C
OATP2B1
**
p = 0.15
1,000
100
10
1
0.1
0.01
Relative mRNA expressie
OATP2B1/Villin
Neonates (birth - 1m)
Infants (1m - 12m)
Children (12m - 17y)
Adults (>17y)