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Human intestinal PEPT1 transporter expression and localization in preterm and term infants

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Human intestinal PEPT1 in infants

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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

MDR: multidrug-resistance protein

MRP: multidrug-resistance like protein

OATP: organic anion transporter polypeptide

PEPT1: oligopeptide transporter

PNA: postnatal age

RIN: RNA integrity number

RT-PCR: reverse transcription polymerase chain reaction

SLC: solute carrier

SLCO: solute carrier organic anion

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ABSTRACT

The intestinal influx oligopeptide transporter PEPT1 (*SLC15A1*) is best known for nutrient-derived di- and tripeptide transport. Its role in drug absorption is increasingly recognized. To better understand the disposition of PEPT1 substrate drugs in young infants, we studied intestinal PEPT1 mRNA expression and tissue localization across the pediatric age range. PEPT1 mRNA expression was determined using real time RT-PCR in small intestinal tissues collected from surgical procedures (neonates, infants) or biopsies (older children, adolescents). PEPT1 mRNA relative to villin mRNA expression were compared between neonates/infants and older children and adolescents. PEPT1 was visualized in infant tissue using immunohistochemical staining. Other transporters (MDR1, MRP2 and OATP2B1) were also stained to describe the localization in relation to PEPT1. 26 intestinal samples (n=20 neonates/infants, n=2 pediatric, n=4 adolescents) were analyzed. The young infant samples were collected at a median (range) gestational age at birth of 29.2 weeks (24.7 - 40) and postnatal age of 2.4 weeks (0 – 16.6). The PEPT1 mRNA expression of the neonates/infants was only marginally lower (0.8-fold) than the older children ($p<0.05$). Similar and clear apical PEPT1 and MRP2 staining, apical and lateral MDR1 staining, and intra-epithelial OATP2B1 staining at the basolateral membrane of the enterocyte was detected in 12 infant and 2 adolescent samples. Although small intestinal PEPT1 expression tended to be lower in neonates than in older children, this difference is small and tissue distribution is similar. This finding suggests similar oral absorption of PEPT1 substrates across the pediatric age range.

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INTRODUCTION

The influx oligopeptide transporter PEPT1 (*SLC15A1*) is a member of the solute carrier (SLC) superfamily and is situated on the apical membrane of the enterocyte. Its expression in the human adult jejunum is at least more than 2 to 10 times higher than that of other transporters such as from the ABC-transporter family (MDR1, MRP2, or BCRP) or from the solute carrier (SLC) family (OATP's) (Hilgendorf et al., 2007). In histologically normal intestinal biopsies from 10 adults, PEPT1 was expressed most abundantly in duodenum and ileum with a mean relative mRNA expression of 4 compared to <0.5 in colon (Meier et al., 2007). In 6 adult intestine organ donors the PEPT1 protein expression accounted for approximately 50% of the total expression of all transporter proteins in the small intestine. In colon, PEPT1 represents 5% of all transporter proteins (Drozdzik et al., 2014).

PEPT1 is best known for its function as a nutrient-derived di- and tripeptide transporter, but may also have a role in (pro)drug transport because it is the most abundant peptide transporter in the gut and drug properties mimicking di- and tripeptides may allow uptake by the peptide transporter (Brandsch, 2013). Its role as intestinal transporter has been demonstrated for several (pro)drugs. In PepT1 (orthologue of human PEPT1) knock-out mice, the small intestinal uptake of the pro-drug valacyclovir was attributed for 90% to the PepT1 transporter (Yang and Smith, 2013). Consequently these mice had a delayed T_{max} and decreased C_{max} of acyclovir (active metabolite of valacyclovir) relative to wild type mice (Yang et al., 2013).In human adults, however, PEPT1 mRNA expression was not correlated with valacyclovir pharmacokinetic parameters, even though, in vitro, valacyclovir was a PEPT1 substrate (Landowski et al., 2003). This suggests that valacyclovir may be not be a PEPT1 substrate in human adults, or that PEPT1 mRNA expression may not correlate to PEPT1 activity. The role of PEPT1 in valacyclovir pharmacokinetics in humans may differ from that in animals and remains to be elucidated. Several β-lactam antibiotics appeared to be PEPT1 substrates in Caco-2 cells

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with varying affinities: e.g. ceftibuten, cyclacillin, cefadroxil, cefaclor, benzylpenicillin, and cephalexin. Their structure resembles the tripeptide structure with additional groups (Brandsch et al., 2008). The ACE-inhibitor fosinopril is a PEPT1 substrate; other ACE-inhibitors might be substrates, although this needs to be confirmed (Brandsch et al., 2008).

Most drugs prescribed to children are administered orally. Some PEPT1 substrates are dosed to children even very early in life. Considering the wide age-related variation in the processes affecting oral drug absorption, including gastric pH, gastric motility and drug metabolizing enzyme activity, age-related changes in membrane transporters are also very likely (Mooij et al., 2012). Earlier we showed a transporter-dependent maturation in gene expression in young infants for MDR1, MRP2 and OATP2B1 (Mooij et al., 2014), but overall data on the intestinal expression of membrane transporters during growth and development are very scarce (Brouwer et al., 2015).

To the best of our knowledge, studies on the development of intestinal PEPT1 in humans are lacking. From a pharmacological point a view, it is important to elucidate the development of PEPT1 expression for known substrates. But this may also be for the development of new drugs in which PEPT1 could enhance oral absorption.

PEPT1 developmental changes have been studied in several animal species. A developmental pattern of PepT1 mRNA and protein expression has been shown in the duodenum, jejunum, ileum and colon of rats (Shen et al., 2001). In the small intestine of newborn rats the expression peaked 3-5 days after birth, after which it rapidly decreased and increased again by the time animals were weaning. The authors ascribed the increase postpartum to suckling. In another study in rats, PepT1 small intestinal mRNA expression was stable from postnatal day 4 till day 21, and then decreased from postnatal day 50 onwards (Rome et al., 2002). In neonatal miniature pigs, PepT1 mediated dipeptide (^{3}H-glycylsarcosine)

disappearance in the ileal segment was highest in the youngest age group (1 week) , but PepT1 expression in post-weaned pigs was higher than in sucklings (Nosworthy et al., 2013). These data suggest that in the first weeks of life, intestinal PEPT1 is important for nutritional intake and later for diet transition following weaning

Comparative mRNA expression of various peptide transporters in mice, rats and human adults shows a PEPT1 expression in all species, but expression levels varied in relation to that of other peptide transporters (HPT1, PEPT2) (Kim et al., 2007). This suggests that animal data cannot be directly extrapolated to humans and that human studies are needed.

To our knowledge expression or activity of PEPT1 in human fetal or pediatric population has not been described thus far, let alone a developmental expression pattern. To better understand the disposition of PEPT1 substrate drugs in neonates and young infants, we aimed to compare intestinal PEPT1 mRNA expression and tissue localization in these age groups with those in older children and adolescents. To describe PEPT1 protein staining in relation to other transporters with known mRNA expression data, we also aimed to detect MDR1, MRP2 and OATP2B1 protein in intestine.

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MATERIAL AND METHODS

1. Tissue samples

Intestinal tissue samples were obtained surgically at time of resection (neonates/infants/adolescents) or as biopsies during ileocolonoscopies (older children/adolescents: subjects 23-26 Table 1). For mRNA isolation, post-resection, tissue was snap frozen in liquid nitrogen and stored at -80˚C. For immunohistochemical analysis, tissue was immediately put in 4% formaldehyde in PBS and processed to paraffin cubes.

Collection of neonatal/infant intestinal tissue and the use of left-over material was approved by the "Central Committee of Research involving Human Subjects" (the Hague, Netherlands) (Puiman et al., 2011). The Erasmus MC research ethics board in two other protocols approved collection of intestinal residual tissue from adolescent patients and endoscopy biopsies of older children and adolescents.

Informed consent was obtained from all parents/care-givers and children older than 12 years of age for use of left-over tissue and clinical data.

2. Real-Time Reverse-Transcription Polymerase Chain Reaction (real time RT-PCR)

Isolation and cDNA synthesis has been previously described (Mooij et al., 2014). In brief, frozen tissue samples were mechanically homogenized on ice. RNA was extracted using the RNeasy Mini Kit (Qiagen). To digest genomic DNA remnants, RNA was treated with DNase. The RNA integrity numbers (RIN) of the samples were analyzed using the 2100 BioAnalyzer (Agilent, Santa Clara, CA), and a value <5 was considered poor quality and reason to discard the sample. The mRNA expression was measured by SYBR green quantitative real time RT-PCR with 7900 Sequence Detector (Applied Biosystems, ABI prism).

Primers were used for PEPT1, villin and GAPDH, and sequences were designed using

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Oligo 6.22 software. Primer sequences were: GAPDH forward 5'-GTCGGAGTCAACGGATT-3', GAPDH reverse 5'-AAGCTTCCCGTTCTCAG-3'; villin forward 5'-TGCCAACACCAAGAGACT-3', villin reverse 5'-TCCCAATCCAGAAGAAGAC-3'; PEPT1 forward 5'-TTGGCCCAATGTCTCA-3', PEPT1 reverse 5'-GGCCCTGCTTGAAGTC-3'. The melting curve was analyzed after every PCR to confirm product specificity. GAPDH and villin mRNA expression were used as the endogenous control. PEPT1 transcript levels were normalized to villin transcript levels (ratio PEPT1/villin), and relative expression was compared across the age groups (neonates/young infants vs children/adolescents)

3. Immunohistochemistry

Intestinal sections were dewaxed for immunohistochemistry (IHC), and endogenous peroxidases were quenched with 3% H_2O_2 in methanol for 20 min. Antigens were retrieved using Pepsin (0.1% in 0.01N HCL) pretreatment for 7 min at 37˚C stove. The sections were blocked for 1h in 10% normal human serum and 10% normal rabbit serum diluted in Teng-T (10 mMTris (pH 8), 5 mM EDTA (pH 8), 0.15 M SodiumCl, 0.25% gelatin and 0.05% Tween-20). Primary antibodies goat anti-PEPT1 C-20 (Santa Cruz Biotechnology Inc., Heidelberg, Germany) were incubated over night at 4˚C in 2% human serum. Immunoreactive sites were detected with biotinylated secondary rabbit anti-goat serum using the Vectastain ABC Elite Kit (Vector Laboratories, Burlingame, CA), and 3,3 diaminobenzidine tetrahydrochloride (DAB) solution (Sigma-Aldrich, Zwijndrecht, Netherlands). The nuclei were counterstained with hematoxylin (Vector Laboratories). A negative control staining lacking the primary antibody was performed for every slide. A matched goat-antibody negative control was performed to assess background staining. Images were acquired and analyzed with Leica microscope and LAS-AF image acquisition software. HC was also performed, on the same tissue samples for three other membrane transporters with known intestinal mRNA expression data, MDR1, MRP2 and OATP2B1, to compare tissue distribution of PEPT1 with these transporters. In case of MDR1

and OATP2B1 staining, microwave pretreatment in citrate buffer (10 mM, pH6.0) was used to retrieve antigens. In case of MRP2, pepsin pretreatment, similar as for PEPT1, was used. Primary antibodies mouse anti-MDR1 and mouse anti-MRP2 were obtained from EMD Millipore, and rabbit anti-OATP2B1 from Abnova. PEPT1 staining intensity was microscopically scored by two independent observers (low-1, moderate-2 or high-3).

4. Statistical analysis

Data are presented as median and range, unless indicated otherwise. Group comparison (neonates/young infants – older children/adolescents) was made using nonparametric Mann-Whitney U test. Within the neonates/young infants group, the association between postmenstrual age (gestational plus postnatal age) and PEPT1 mRNA expression was assessed using Spearman's rho correlation.

All statistical analyses were performed using GraphPad Prism version 5.00.2 and IBM SPSS Statistics software (SPSS Statistics for Windows, version 21.0; IBM, Armonk, NY). The level of significance was set at $P<0.05$.

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RESULTS

Descriptive results

Twenty-six samples (n=20 neonates/infants, n=2 children, n=4 adolescents) were collected (Table 1). The ages of the young infants ranged from gestational age at birth [median (range) GA] 29.2 weeks (24.7 - 40) and to postnatal age (PNA) 2.4 weeks (0 – 16.6). The main reasons for resection were stoma closure (in patients with history of necrotizing enterocolitis (NEC) (n=5), current NEC (n=6) and intestinal atresia (n=5). Other reasons were intestinal volvulus (=3), and Meckel's diverticulum (n=1). Samples of two children (9 and 10 years old) and two adolescents (16 and 17 years old) of whom frozen biopsy tissue was available for mRNA underwent endoscopy for suspicion of inflammatory bowel disease, and were previously classified as histologically normal. The two samples for immunohistochemistry were collected from two other children who underwent an ileocecal resection (age 15 years, history of Crohns disease, active disease at time of surgery) and ileoanal pouch surgery (age 17 years, history of ulcerative colitis, active disease at time of surgery), respectively.

mRNA was analyzed on samples of which snap frozen tissue was available; i.e. 17 young infant, 2 pediatric, and 2 adolescent samples. Immunohistochemistry was performed on samples of which paraffin-embedded tissue was available; i.e. 12 young infant and two adolescent samples (Table 1).

GAPDH mRNA strongly correlated with villin mRNA (n=21, ρ=0.6182, p<0.01). One sample was excluded due to low mRNA expression of villin and GAPDH suggesting loss of enterocytes. Twenty samples remained for mRNA expression analysis.

Nutritional intake up to three days before date of sampling (surgery or biopsy) was fully enteral in 6 patients, parenteral nutrition was given in 9 patients and 4 patients received both

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enteral and parenteral feeding. Data on nutritional intake was lacking in 7 subjects. No information on concomitant medications was available.

PEPT1 gene expression

PEPT1 mRNA was detected in all 20 samples. The relative intestinal PEPT1 mRNA expression (PEPT1/villin) in young infants slightly varied (0.15-fold) (Figure 1). In the neonatal/infant group the PEPT1 expression was 0.8 fold lower than in the older age group (p=0.01), with median relative mRNA expression of 0.80 (range 0.77 – 0.92) and 1.02 (1.01 – 1.04), respectively. In the neonatal/infant group postmenstrual age was not correlated to PEPT1 mRNA expression (ρ=0.453, p=0.078).

Presence of PEPT1 protein in enterocytes

PEPT1 staining was present at the apical membrane in the brush border of the enterocyte in all but one sample (Figure 2), this sample did not show clear villin and was also excluded from PEPT1 mRNA analysis for low villin and GAPDH mRNA expression levels..

PEPT1 apical localization was similar in neonatal and adolescent intestinal samples. No PEPT1 staining was detected in Goblet cells, most likely due to the artificial effect of enlargement of Goblet cells during the process of paraffin embedding. No staining was observed at the basolateral membrane or at the tight junctions. Microscopically, PEPT1 staining intensity was variable among samples. Median PEPT1 staining was high in neonatal and infantile samples (median 3, range 1-3) , and low in the two adolescent samples (both 1).

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Presence of MDR1, MRP2 and OATP2B1 protein in enterocytes

MDR1 staining was visible at the apical and lateral surfaces of the enterocyte (Figure 3). The lateral MDR1 staining is clearly visible in Figure 4. OATP2B1 staining was present intra-epithelial at the basolateral membrane (Figure 3). MRP2 was localized only in the brush border at the apical surface (Figure 3). Specific transporter staining was present in all neonatal, infantile and adolescent samples.

DISCUSSION

PEPT1 mRNA expression and protein expression were found in neonatal and young infant intestinal tissues immediately postnatally. The PEPT1 mRNA expression in young children was slightly lower than in older children, although the clinical relevance of this difference is probably negligible. This study is the first to demonstrate intestinal gene expression of the PEPT1 transporter across the pediatric age range. The gene expression of PEPT1 in neonatal intestine samples was confirmed by immunohistochemical staining showing protein PEPT1 expression in the brush border of the enterocyte. Localization of PEPT1 in the apical part along the brush border of villus epithelial cells was comparable with staining in human adolescents (this study) and adults, rat and mice (Groneberg et al., 2001; Hussain et al., 2002; Ziegler et al., 2002; Laforenza et al., 2010).

Based on the clear developmental expression patterns of intestinal drug metabolizing enzymes and hepatic transporters, with in general low expression at birth and increasing with postnatal age, we anticipated lower PEPT1 transporter expression in neonates (Brouwer et al., 2015). However, our results suggest slightly lower PEPT1 expression and similar localization as in adolescents. Hence the uptake of PEPT1 substrates in neonates and young infants is likely not to be affected by growth and maturation and dose-adjustments for PEPT1 activity therefore do not seem necessary. Nevertheless, adjustment of drug dosing of PEPT1 substrates may be needed in young children for other reasons, e.g. if the drug is metabolized or renally cleared, which may result in age-related changes in disposition.

Stable mRNA expression of small intestinal MDR1 from neonatal age onwards was also found in other studies (Fakhoury et al., 2005; Miki et al., 2005; Mizuno et al., 2013; Mooij et al., 2014). In a previous study from our group, MRP2 mRNA expression in the small intestines was also stable during infant age, but OATP2B1 expression in neonates was about three times

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higher than in adults (Mooij et al., 2014). This suggests that intestinal membrane transporters show stable or higher expression during childhood and that a developmental expression might occur before birth. These findings are supported by our immunohistochemistry data, which show clear localization of PEPT1 and the other transporters studied. Immunohistochemical staining of MDR1, MRP2 and OATP2B1 was done to compare the localization of PEPT1 in relation to the other transporters. MDR1 was stained at the apical border of the enterocyte, similar to MDR1 staining in 59 duodenal biopsies from infants up to 7 years of age and from fetuses from a gestational age of 16 and 20 weeks (van Kalken et al., 1992; Fakhoury et al., 2005). MRP2 apical staining matches to staining in human colorectal cancer tissue, as well as intestinal tissue from horse, rabbits and rats (Mottino et al., 2000; Van Aubel et al., 2000; Tyden et al., 2010). Intraepithelial OATP2B1 staining at the basolateral enterocyte border was similar to staining in human colonic biopsies from adults (Kleberg et al., 2012). The localization corresponds with the function of MDR1, MRP2 and PEPT1 to facilitate uptake of substrates in the enterocyte, whereas OATP2B1 facilitates excretion from the enterocyte to blood (Klaassen and Aleksunes, 2010).

Our data do not contradict previous data from juvenile animal studies. In rats, PepT1 expression was increased on days 3 to 5 after birth, after which it rapidly decreased and then increased at the time animals were weaning (Shen et al., 2001). If we would extrapolate this data to neonates, we would expect a PEPT1 elevation several days after birth, and time of weaning might be translated to infant age at the time of introducing food next to breastfeeding of formula. The slightly higher PEPT1 expression in older children and adolescents than in young infants might be compared to weaned rats from the animal study. Still, a clinical impact of slightly lower infantile PEPT1 expression is questionable.

PEPT1 has been studied in relation to feeding and nutrition (Spanier, 2014). Interestingly, the PEPT1 transporter expression seems sensitive to nutritional status. In adult

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short-bowel syndrome patients, small intestinal and colonic mRNA expression of PEPT1 was upregulated compared to healthy controls (Ziegler et al., 2002). Studies in several animal species have further explored the impact of nutrition on intestinal PEPT1 expression. After maternal overnutrition during pregnancy (but not after maternal undernutrition), PepT1 mRNA expression was significantly increased in jejunum of newborn or weaned piglets (Cao et al., 2014). In contrast, maternal protein restriction in rats also led to higher duodenal PepT1 mRNA expression in 3- and 16-week-old offspring. Irrespective of feeding, PepT1 mRNA expression in 16-week-old rats was higher than in 3-week-old rats (Pinheiro et al., 2013). In another study in adult rats on a protein-rich diet, PepT1 mRNA and protein expressions, as well as transporter activity were increased (Shiraga et al., 1999). In low-birth-weight piglets colonic PepT1 activity (measured by flux of cephalexin) was increased after high- instead of normal-protein formula feeding (Boudry et al., 2014). The effects of feeding and nutrition may in part explain the observed interindividual variability in PEPT1 gene expression in young infants in the present study. Unfortunately the available nutritional data were too diverse and partially lacking to test this hypothesis. Moreover, immunohistochemistry is not the preferred method to quantify the observed changes. Other approaches such as Western Blots are more informative, but tissue was lacking to perform this analysis..

Several hormones (insulin, leptin, growth hormone) appear to induce PEPT1 activity in Caco-2 cells (Thamotharan et al., 1999; Buyse et al., 2001; Alteheld et al., 2005). Also, dexamethasone was shown to enhance PepT1 activity in Xenopus oocytes and mice, but human data are, to our knowledge, lacking (Rexhepaj et al., 2009). In our cohort, a large proportion of premature neonates have very likely been exposed prenatally to betamethasone as a routine for the prevention of respiratory distress syndrome in imminent premature birth. Unfortunately, informed consent was lacking to retrieve prenatal corticosteroid use by the mother from the medical charts. Hence, our data should be interpreted with caution, taking into

account a potential effect of prenatal corticosteroid exposure on the observed intestinal PEPT1 expression.

Several limitations of this study should be addressed. One, most samples were obtained during the first few weeks of life, and pediatric samples after the age of 4 months up to 9 years are lacking. Therefore the expression in this age range is still unknown. Second, variability in our samples might be due to co-medication, nutritional differences, disease or extent of inflammation. Still, most samples were obtained during surgery of stoma closure and therefore the intestinal tissue can be expected to be relatively normal. Third, variability might be due by genetic polymorphisms in *SLC15A1* gene causing a change in PEPT1 expression. Thus far, no polymorphism has been proven to be clinically relevant, however, and therefore we did not genotype our samples (Anderle et al., 2006).

In conclusion, although small intestinal PEPT1 expression was slightly lower in neonates than in older children, this difference is small and tissue distribution is similar. Therefore, this finding suggests similar oral absorption of PEPT1 substrates across the pediatric age range.

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AUTHORSHIP CONTRIBUTIONS

- Participated in research design: Mooij, de Koning, Samsom, Tibboel, de Wildt,
- Conducted experiments: Mooij, de Koning, Lindenbergh-Kortleve, Simons-Oosterhuis, van Groen, de Wildt
- Contributed new reagents or analytic tools: Samsom
- Performed data analysis: Mooij, de Wildt
- Wrote or contributed to the writing of the manuscript: Mooij, Samsom, van Groen, Tibboel, de Wildt.

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FIGURE LEGENDS

Figure 1 Relationship of age with intestinal PEPT1 gene expression. Relative mRNA expression of PEPT1 in relation to age normalized to villin mRNA expression.

Figure 2 Age and PEPT1 protein localization in enterocyte. Immunohistochemical detection of PEPT1 in paraffin-embedded intestinal sample: pediatric intestinal sample (A), negative control (B); intestinal sample from adolescent (C); negative control (D). Apical staining of PEPT1 on enterocyte. Black arrows indicate PEPT1 detection, grey arrows indicate artifacts.

Figure 3 Age and MDR1, OATP2B1 and MRP2 protein localization in enterocyte. Immunohistochemical detection of MDR1 (A-D), OATP2B1 (E-H) and MRP2 (I-L) in paraffin-embedded intestinal samples: pediatric intestinal sample (A, E, I), negative control (B, F, J); intestinal sample from adolescent (C, G, K); negative control (D, H, L). Apical and lateral MDR1 staining, intra-epithelial OATP2B1 staining at the basolateral membrane, and apical MRP2 staining in the enterocyte. Black arrows indicate transporter detection, grey arrows indicate artifacts.

Figure 4 MDR1 protein localization in enterocyte. Immunohistochemical detection of MDR1 in paraffin-embedded intestinal samples: 40x magnification of pediatric intestinal sample (A), negative control (B). Clear apical and lateral MDR1 staining (black arrows). Grey arrows indicate non-specific signal.

TABLE

Table 1 Patient characteristics

	IHC	mRNA	Gender	Ethnicity	Diagnosis	Gestational age at birth (weeks)	Postnatal age (weeks)	Nutrition up to 3 days before date of sampling Enteral Feeding (EF), Parenteral Nutrition (PN)	Pathology report	Resection area
1	*		Male	Caucasian	NEC And stoma closure	25.3	0.2 and 6.9	EF and PN	Necrotizing enterocolitis and stoma closure	Jejunum
2	*		Male	Caucasian	Stoma closure (history of NEC)	30.3	5.7	EF	-	Ileum
3		*	Male	Caucasian	volvulus jejunum, malrotation	39.3	3.9	PN	-	Jejunum
4	*		Male	Caucasian	Stoma closure	25.6	16.6	EF	-	Ileum

					(history of NEC)					
5	*	*	Male	Unknown	NEC	30.9	8.9	EF and PN	Resection stenosis with necrotizing enterocolitis	Cecum
6	*	*	Male	Unknown	jejunum atresia	35.7	0	PN	stenosis	Jejunum
7		*	Male	Unknown	NEC in patient with complex cor vitium Death - Enterobacter-sepsis	33.0	0.9	EF	NEC	Ileum
8	*	*	Female	Caucasian	NEC	26.9	2.7	EF and PN	NEC	Ileum
9	*	*	Male	Unknown	CHD complicated by volvulus and intestinal necrosis after repair hernia	36.9	2.0	EF and PN	ischemia	Jejunum
10	*	*	Female	Caucasian	NEC	26.4	2.0	PN	NEC	Ileum
11		*	Male	Unknown	NEC	25.3	3.1	?	Ischemic enteritis	Jejunum

12		*	Female	Caucasian	Stoma closure (history of NEC)	24.7	9.57	PN	-	Ileum
13		*	Male	Caucasian	midgut volvulus	28.1	3.86	PN	Infarction and peritonitis	Ileum
14	*	*	Male	Unknown	Meckel's diverticulum	40.0	3.1	EF	Meckel's diverticulum with ulcerations and normal small intestine	Ileum
15	*	*	Male	Caucasian	jejunum atresia	38.3	0.1	PN	stenosis	Jejunum
16	*	*	Male	Caucasian	jejunum atresia	38.0	0.1	PN	Small reactive changes	Jejunum
17		*	Male	Caucasian	jejunum atresia	38.3	0	PN	Atresia with extensive reactive changes	Jejunum

18	*	*	Male	Unknown	Ileum atresia	38.9	0.1	EF	Ileumatresia, no ischemia	Ileum
19		*	Female	Caucasian	NEC	24.9	1.1	PN	NEC	Ileum
20		*	Female	Unknown	Stoma closure (history of NEC)	25.6	13.9	EF		Ileum
21	*		Male	Caucasian	M.Crohn: active	*Adolescent*	15 years	?	*Ileocaecal resection*	Ileum
22	*		Female	Caucasian	Ulcerative colitis: active	*Adolescent*	17years	?	*Ileoanal pouch surgery*	Ileum
23		*		Caucasian	Biopsy in case of abdominal complaints; non-IBD	*Adolescent*	9 years	?	*Normal*	Ileum
24		*		Caucasian	Biopsy in case of abdominal complaints; non-IBD	*Adolescent*	10 years	?	*Normal*	Ileum

25	*	Caucasian	Biopsy in case of abdominal complaints; non-IBD	*Adolescent*	16 years	?	*Normal*	Ileum
26	*	Caucasian	Biopsy in case of abdominal complaints; non-IBD	*Adolescent*	17 years	?	*Normal*	Ileum

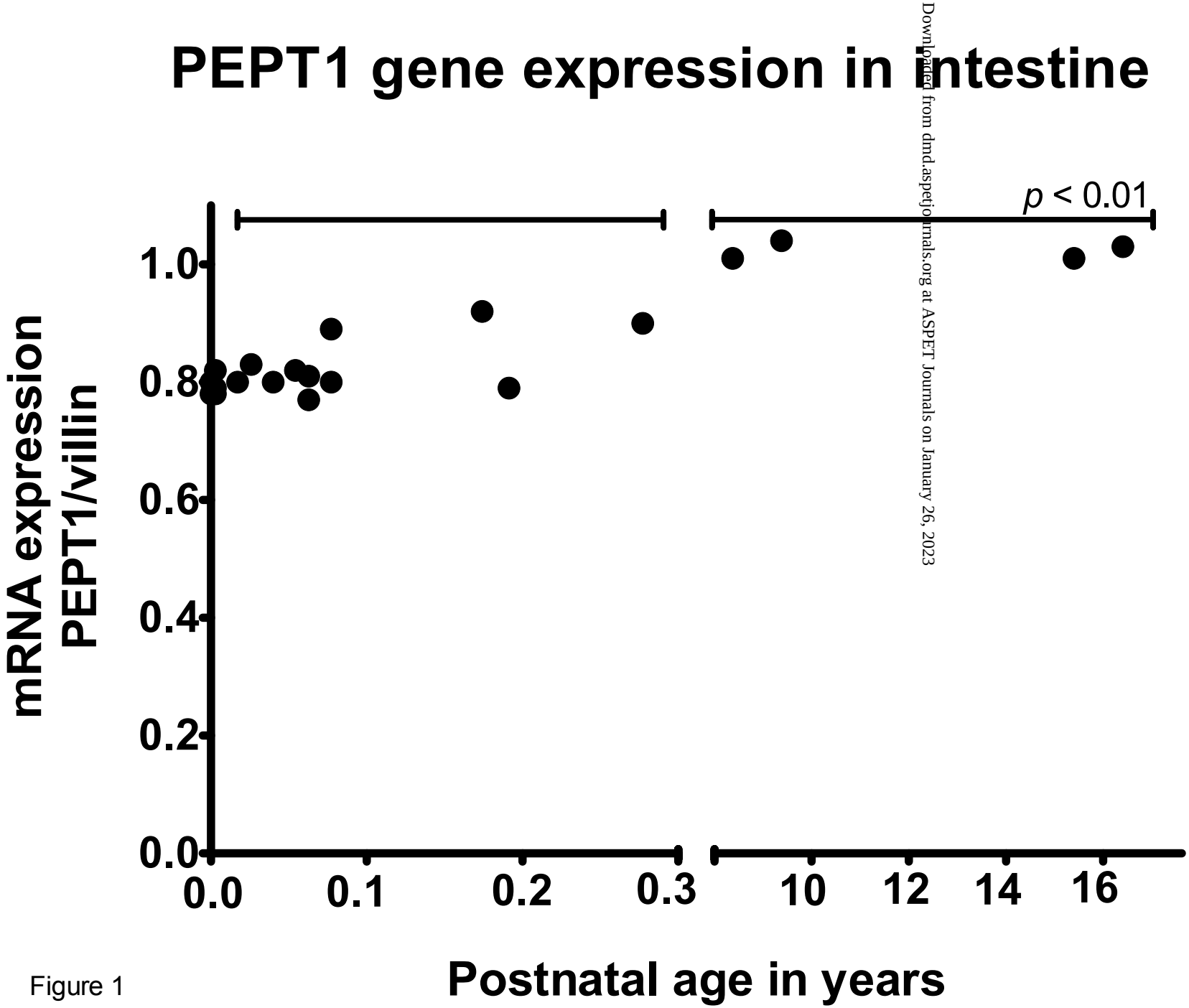

Figure 1

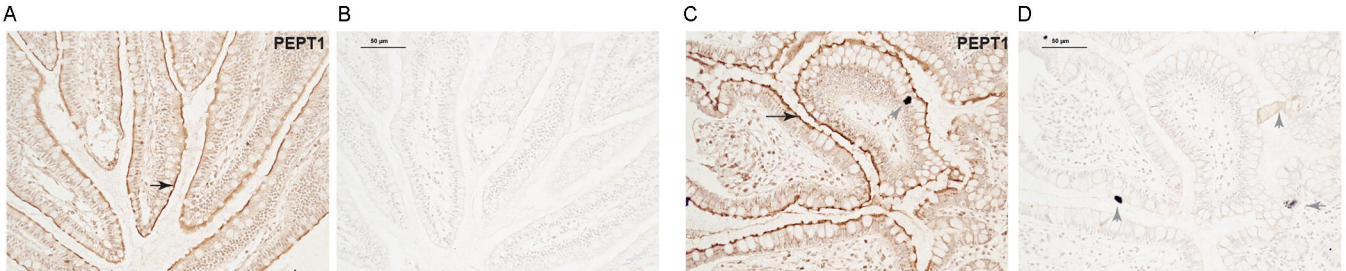

Figure 2

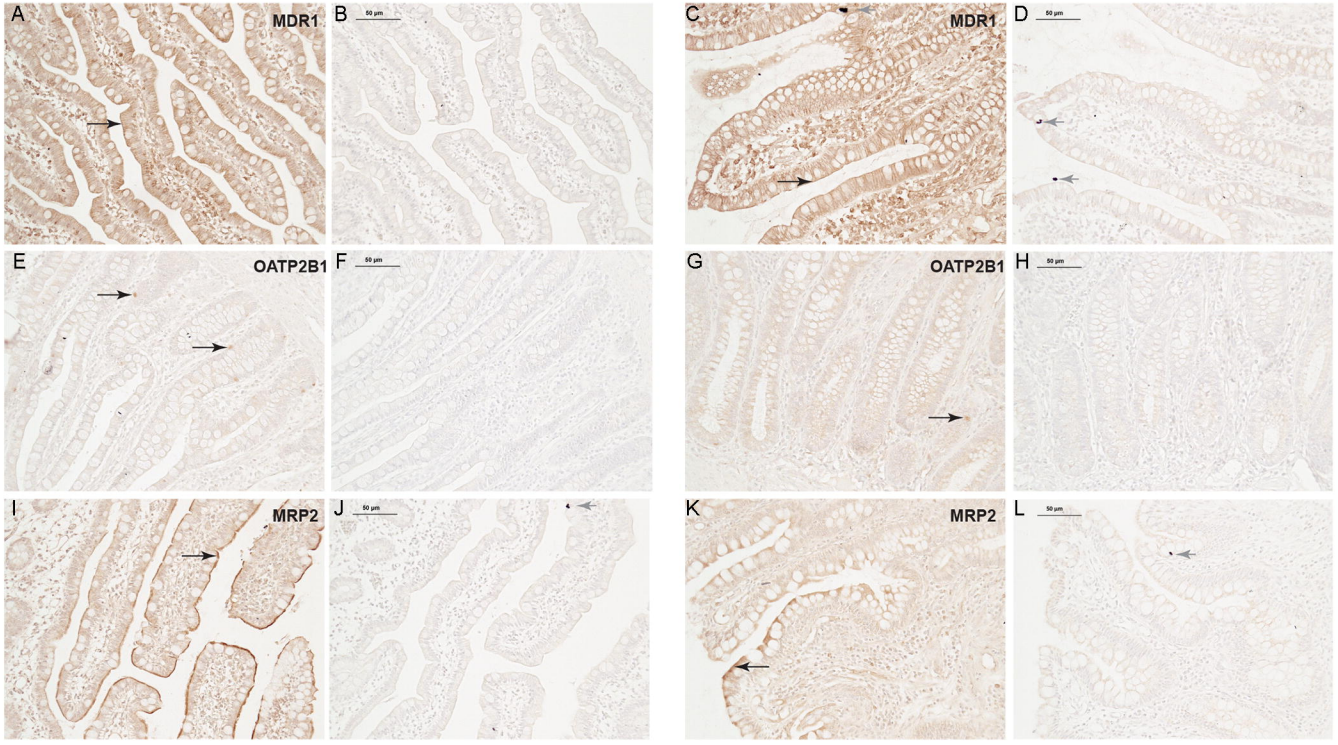
A
MDR1
B
50 µm
C
MDR1
D
50 µm
E
OATP2B1
F
50 µm
G
OATP2B1
H
50 µm
I
MRP2
J
50 µm
K
MRP2
L
50 µm

Figure 3

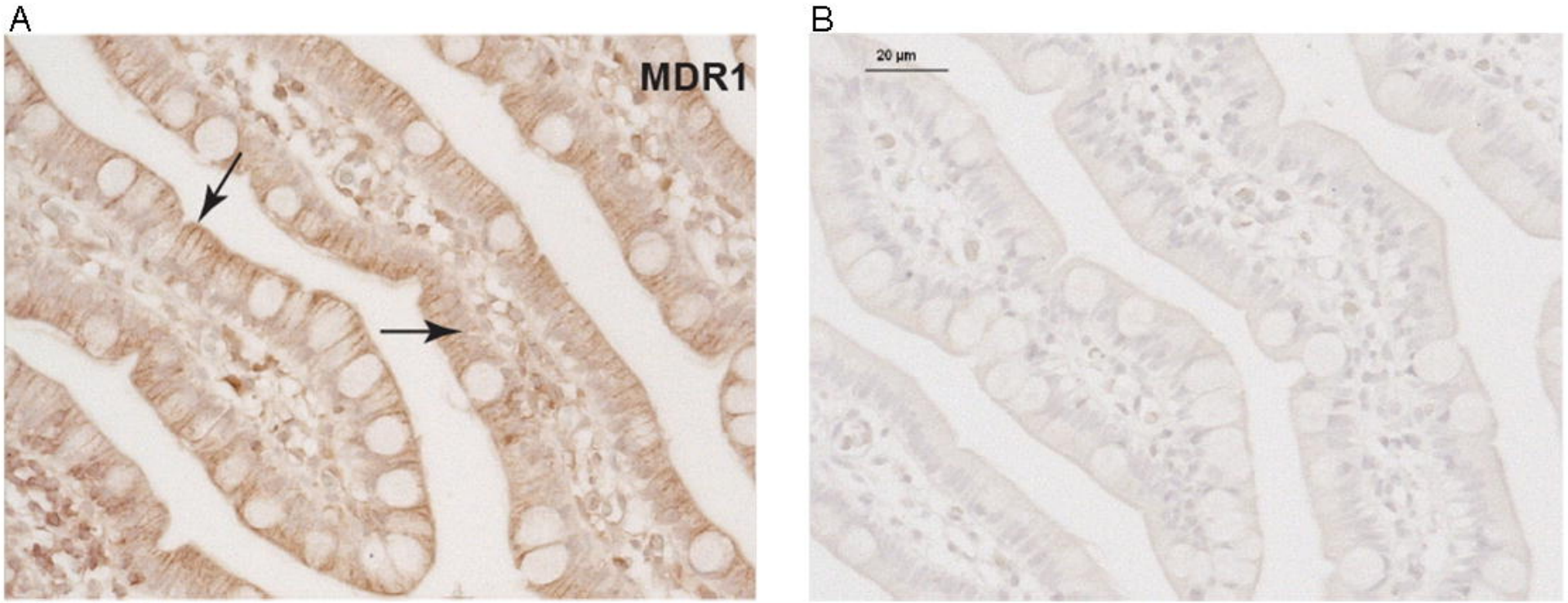

Figure 4