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Application of proteomics to understand maturation of drug metabolizing enzymes and transporters for the optimization of pediatric drug therapy

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Drug disposition in children is different compared to adults. Growth and developmental change the processes involved in drug disposition and efficacy, including membrane transporters and drug metabolizing enzymes, but for many of these proteins, the exact changes have not been fully elucidated to date. Quantitative proteomics offers a solution to analyze many DME and DT proteins at once and can be performed with very small tissue samples, overcoming many of the challenges previously limiting research in this pediatric field. Liquid chromatography tandem mass spectrometry (LC–MS/MS) based methods for quantification of (membrane) proteins has evolved as a golden standard for proteomic analysis. The last years, big steps have been made in maturation studies of hepatic and renal drug transporters and drug metabolizing enzymes using this method. Protein and organ specific maturation patterns have been identified for the human liver and kidney, which aids pharmacological modelling and predicting drug dosing in the pediatric population. Further research should focus on other organs, like intestine and brain, as well as on innovative methods in which proteomics can be used to further overcome the limited access to pediatric tissues, including liquid biopsies and organoids. In this review there is aimed to provide an overview of available human pediatric proteomics data, discuss its challenges and provide guidance for future research.

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Introduction

Drug disposition in children is different compared to adults. Still, doses are often taken from adult PK data with linear adjustment for body weight. We now know that the processes involved in the absorption, distribution, metabolism and excretion (ADME), which determine pharmacokinetics are all impacted by growth and development [3]. Hence, dose extrapolation which does not take these changes into account may result in over- or underdosing.

Membrane drug transporters (DTs) and drug metabolizing enzymes (DMEs) importantly impact all ADME processes and thereby pharmacokinetics (PK) (Box 1). Consequently, maturational variation in the expression of DTs and DMEs may result in differences in drug efficacy and safety in pediatric patients compared to adults. To be able to predict and set a safe and effective dose for this vulnerable population, a thorough understanding of the variation in the underlying ADME processes is required.

This is especially true in the first two years of life, when most changes in DMEs and DTs appear to occur [4]. The most well-known and oldest example is the grey baby syndrome resulting from accumulation of chloramphenicol due to immature glucuronidation by UGT2B7 [5,6]. While major advances in our knowledge have been made towards understanding maturation of individual DMEs and DTs, still important information gaps remain.

Several approaches have been used to study ontogeny of human DTs and DMEs, such as immunohistochemistry, protein expression (Western blot), gene expression (RT-qPCR, RNAseq) and DME activity. There are quite some limitations with these approaches, including the need for larger tissue volumes (Western blot and DME activity), the semi-quantifying character (immunohistochemistry, Western blot), and posttranslational variation (gene expression). With the introduction of liquid chromatography tandem mass spectrometry (LC–MS/MS) methods for protein quantification, many of these limitations can be overcome [7]. Over the last years, LC–MS/MS has evolved to become a golden standard for the quantification of absolute protein abundance of DTs and DMEs. Quantification of protein expression enables in vitro-in vivo extrapolation (IVIVE), and the prediction of PK processes [8]. Next to that, proteomic approaches have also been used to elucidate age-related variation in drug transport and metabolism. This review aims to provide an overview of available human pediatric proteomics data, discuss challenges and provide guidance for future research.

Box 1. Drug metabolizing enzymes and membrane transporters.

Membrane drug transporters (DTs) and drug metabolizing enzymes (DMEs) play a crucial role in drug disposition and efficacy, as they determine drug concentrations in plasma and tissues by translocating drugs across membranes and metabolizing drugs to active or inactive metabolites. Important transport families include ATP-binding cassette (ABC) and solute carrier (SLC) transporters [1]. Drug metabolism knows two phases (phase I and phase II metabolism) and takes place inside the cell. The cytochrome P450 (CYP450) superfamily is involved in the majority of phase I reactions, mainly resulting in oxidation, reduction or hydrolysis of a compound. In contrast, phase II metabolism conjugates metabolites. Major phase II DME families are UDP-glucuronosyltransferase (UGT), carboxylesterases (CES), sulfotransferases (SULTs) [2]. Variation in DT and DME activities impact variation in drug disposition and thereby efficacy and safety of drugs, therefore a thorough understanding of variation due to growth and development is important to optimize pediatric pharmacotherapy.

Proteomic methods

The term proteomics refers to a large variety of protein abundance analyses, which can be performed in tissues, blood and cells. Proteomics has developed from a qualitative methodology to a precise quantitative way of determining protein abundance [9]. Immunohistochemistry (IHC) is used for the subcellular membrane localization of specific DTs and DMEs, but the method is rather qualitative. Traditionally, Western Blot analysis has been used for semi-quantitative analysis of protein expression levels in tissues or cells [10,11], but comparable to IHC, the method is hampered by the lack of specific antibodies and difficulties to quantitate expression levels [12].

In contrast, for protein quantification, LC–MS/MS is by far superior. For this technology, the (membrane bound) proteins need to be extracted and digested, before they are separated on the chromatography column. Subsequently, they are injected into a mass spectrometer were the fragmented ions are quantified as surrogates for protein abundance [13,14]. With LC–MS/MS methodology, proteins can be quantitated using a targeted or global approach. Targeted protein quantification allows the identification of proteins of interest (the targets) with the use of a pre-made standard, containing the prototypic peptides of these proteins of interest. By comparing the mass peaks of the sample with the standard, proteins can be identified and abundance is calculated. Global or non-targeted protein quantification is aimed at measuring up to 1000 proteins per sample. Afterwards, signal abundances are compared with a sequence database to identify and calculate a protein's concentration. The global method is more efficient, however, less sensitive, as it mainly works for the most abundant proteins in a sample and it has a lower reproducibility [7,8,15].

Impact of age on DME and DT expression using proteomics

To better understand the absolute change in DTs and DMEs protein expression, proteomics has been applied over the last couple of years to determine protein expression profiles across the fetal and pediatric age range. In Tables 1 and 2,

Table 1. Overview of age-related drug transporter protein expression studies in human organs performed with mass spectrometry methods. Fetus: 0 years, neonates: 0–1 months, infants 1 month–2 years, early child: 2–5 years, middle child: 6–11 years, children: 11 years, adolescents 12–17 years, adults: 18 year. The study of Mooij et al., 2016 was excluded due to sample overlap with van Groen et al., 2018.

Organ	DT	Method	Tissue details	Relation to age	Population	Study
Liver	BCRP	Targeted protein quantification (LC–MS/MS), pmol/gram protein	Post mortem, crude membrane fractions	Stable expression	Fetus n = 36 Preterm neonates n = 12 Term neonates n = 10 Children n = 4 Adults n = 8	van Groen et al., 2018 [17]
		Targeted protein quantification (LC–MS/MS), fmol/ug membrane protein	Post mortem, crude membrane fractions	Stable expression	Neonates n = 4 Infants n = 19 Children n = 32 Adolescents n = 14 Adults n = 41	Prasad t al., 2016 [19]
		Targeted protein quantification (LC–MS/MS), fmol/ug membrane protein	Post mortem, crude membrane fractions	Stable expression	Children n = 8 Adults n = 57	Prasad et al., 2014 [18]
	BSEP	Targeted protein quantification (LC–MS/MS), pmol/gram protein	Post mortem, crude membrane fractions	Lower expression in fetus vs. term neonates/adults	Fetus n = 36 Preterm neonates n = 12 Term neonates n = 10 Children n = 4 Adults n = 8	van Groen et al., 2018 [17]
		Targeted protein quantification (LC–MS/MS), fmol/ug membrane protein	Post mortem, crude membrane fractions	Stable expression	Neonates n = 4 Infants n = 19 Children n = 32 Adolescents n = 14 Adults n = 41	Prasad t al., 2016 [19]
	GLUT1	Targeted protein quantification (LC–MS/MS), pmol/gram protein	Post mortem, crude membrane fractions	Higher expression in fetus vs term neonates/children/adults	Fetus n = 36 Preterm neonates n = 12 Term neonates n = 10 Children n = 4 Adults n = 8	van Groen et al., 2018 [17]
	MATE1	Targeted protein quantification (LC–MS/MS), fmol/ug membrane protein	Post mortem, crude membrane fractions	Stable expression	Neonates n = 4 Infants n = 19 Children n = 32 Adolescents n = 14 Adults n = 41	Prasad t al., 2016 [19]
	MCT1	Targeted protein quantification (LC–MS/MS), pmol/gram protein	Post mortem, crude membrane fractions	Stable expression	Fetus n = 36 Preterm neonates n = 12 Term neonates n = 10 Children n = 4 Adults n = 8	van Groen et al., 2018 [17]
	MDR1/P-gp	Targeted protein quantification (LC–MS/MS), pmol/gram protein	Post mortem, crude membrane fractions	Lower expression in fetus vs. adults	Fetus n = 36 Preterm neonates n = 12 Term neonates n = 10 Children n = 4 Adults n = 8	van Groen et al., 2018 [17]
		Targeted protein quantification (LC–MS/MS), fmol/ug membrane protein	Post mortem, crude membrane fractions	Lower expression in neonates/infants vs. children/adolescents/adults	Neonates n = 4 Infants n = 19 Children n = 32 Adolescents n = 14 Adults n = 41	Prasad t al., 2016 [19]
		Targeted protein quantification (LC–MS/MS) fmol/ug membrane protein	Post mortem, crude membrane fractions	Stable expression	Children n = 8 Adults n = 57	Prasad et al., 2014 [18]

Table 1 (*Continued*)

Organ	DT	Method	Tissue details	Relation to age	Population	Study
	MRP1	Targeted protein quantification (LC–MS/MS), pmol/gram protein	Post mortem, crude membrane fractions	Lower expression in fetus/term neonates vs. adults	Fetus n = 36 Preterm neonates n = 12 Term neonates n = 10 Children n = 4 Adults n = 8	van Groen et al., 2018 [17]
		Targeted protein quantification (LC–MS/MS), fmol/ug membrane protein	Post mortem, crude membrane fractions	Stable expression	Neonates n = 4 Infants n = 19 Children n = 32 Adolescents n = 14 Adults n = 41	Prasad t al., 2016 [19]
	MRP2	Targeted protein quantification (LC–MS/MS), pmol/gram protein	Post mortem, crude membrane fractions	Lower expression in fetus/term neonates vs. adults	Fetus n = 36 Preterm neonates n = 12 Term neonates n = 10 Children n = 4 Adults n = 8	van Groen et al., 2018 [17]
	MRP3	Targeted protein quantification (LC–MS/MS), pmol/gram protein	Post mortem, crude membrane fractions	Lower expression in fetus/term neonates vs. adults	Fetus n = 36 Preterm neonates n = 12 Term neonates n = 10 Children n = 4 Adults n = 8	van Groen et al., 2018 [17]
		Targeted protein quantification (LC–MS/MS), fmol/ug membrane protein	Post mortem, crude membrane fractions	Lower expression in infants/adolescents vs. adults	Neonates n = 4 Infants n = 19 Children n = 32 Adolescents n = 14 Adults n = 41	Prasad t al., 2016 [19]
	NTCP	Targeted protein quantification (LC–MS/MS), pmol/gram protein	Post mortem, crude membrane fractions	Lower expression in fetus vs. term neonates/children/adults, preterm vs. adults	Fetus n = 36 Preterm neonates n = 12 Term neonates n = 10 Children n = 4 Adults n = 8	van Groen et al., 2018 [17]
		Targeted protein quantification (LC–MS/MS), fmol/ug membrane protein	Post mortem, crude membrane fractions	Stable expression	Neonates n = 4 Infants n = 19 Children n = 32 Adolescents n = 14 Adults n = 41	Prasad t al., 2016 [19]
	OATP1B1	Targeted protein quantification (LC–MS/MS), pmol/gram protein	Post mortem, crude membrane fractions	Higher expression in fetus vs. term neonates	Fetus n = 36 Preterm neonates n = 12 Term neonates n = 10 Children n = 4 Adults n = 8	van Groen et al., 2018 [17]
		Targeted protein quantification (LC–MS/MS), fmol/ug membrane protein	Post mortem, crude membrane fractions	Stable expression	Neonates n = 4 Infants n = 19 Children n = 32 Adolescents n = 14 Adults n = 41	Prasad t al., 2016 [19]
		Targeted protein quantification (LC–MS/MS), fmol/ug membrane protein	Post mortem, crude membrane fractions	Stable expression	Children n = 8 Adults n = 57	Prasad et al., 2014 [18]

Table 1 (*Continued*)

Organ	DT	Method	Tissue details	Relation to age	Population	Study
	OATP1B3	Targeted protein quantification (LC–MS/MS), pmol/gram protein	Post mortem, crude membrane fractions	Stable expression	Fetus n = 36 Preterm neonates n = 12 Term neonates n = 10 Children n = 4 Adults n = 8	van Groen et al., 2018 [17]
		Targeted protein quantification (LC–MS/MS), fmol/ug membrane protein	Post mortem, crude membrane fractions	Lower expression in neonates vs. adolescents/adults, infants vs. children/adolescents/adults	Neonates n = 4 Infants n = 19 Children n = 32 Adolescents n = 14 Adults n = 41	Prasad t al., 2016 [19]
		Targeted protein quantification (LC–MS/MS), fmol/ug membrane protein	Post mortem, crude membrane fractions	Stable expression	Children n = 8 Adults n = 57	Prasad et al., 2014 [18]
	OATP2B1	Targeted protein quantification (LC–MS/MS), pmol/gram protein	Post mortem, crude membrane fractions	Stable expression	Fetus n = 36 Preterm neonates n = 12 Term neonates n = 10 Children n = 4 Adults n = 8	van Groen et al., 2018 [17]
		Targeted protein quantification (LC–MS/MS), fmol/ug membrane protein	Post mortem, crude membrane fractions	Stable expression	Neonates n = 4 Infants n = 19 Children n = 32 Adolescents n = 14 Adults n = 41	Prasad t al., 2016 [19]
		Targeted protein quantification (LC–MS/MS) fmol/ug membrane protein	Post mortem, crude membrane fractions	Stable expression	Children n = 8 Adults n = 57	Prasad et al., 2014 [18]
	OCT1	Targeted protein quantification (LC–MS/MS), pmol/gram protein	Post mortem, crude membrane fractions	Lower expression in fetus/term neonates vs. adults	Fetus n = 36 Preterm neonates n = 12 Term neonates n = 10 Children n = 4 Adults n = 8	van Groen et al., 2018 [17]
		Targeted protein quantification (LC–MS/MS), fmol/ug membrane protein	Post mortem, crude membrane fractions	Lower expression in fetus/infants vs. adolescents/adults	Neonates n = 4 Infants n = 19 Children n = 32 Adolescents n = 14 Adults n = 41	Prasad t al., 2016 [19]
Kidney	BCRP	Targeted protein quantification (LC–MS/MS), pmol/ng total membrane protein *Gene expression[a] (RT-qPCR)*	Post mortem autopsy, renal cortex tissue	Stable expression *Higher expression in term neonates vs. infants/children/adolescents/adults*	Term neonates n = 11 Infants n = 60 Children n = 31 Adolescents n = 10 Adults n = 10	Cheung et al., 2019 [20]
	GLUT2	Targeted protein quantification (LC–MS/MS), pmol/ng total membrane protein *Gene expression (RT-qPCR)*	Post mortem autopsy, renal cortex tissue	Stable expression *Stable expression*	Term neonates n = 11 Infants n = 60 Children n = 31 Adolescents n = 10 Adults n = 10	Cheung et al., 2019 [20]
	MATE1	Targeted protein quantification (LC–MS/MS), pmol/ng total membrane protein *Gene expression[a] (RT-qPCR)*	Post mortem autopsy, renal cortex tissue	Stable expression *Stable expression*	Term neonates n = 11 Infants n = 60 Children n = 31 Adolescents n = 10 Adults n = 10	Cheung et al., 2019 [20]
		Targeted protein quantification (LC–MS/MS), pmol/gram of tissue normalized to Aquaporin 1	Post mortem, renal cortex tissue	Stable expression	Children n = 12 Adolescents n = 13 Adults n = 17	Li et al., 2019 [21]

Table 1 (*Continued*)

Organ	DT	Method	Tissue details	Relation to age	Population	Study
	MATE2-K	Targeted protein quantification (LC–MS/MS), pmol/ng total membrane protein *Gene expression[a] (RT-qPCR)*	Post mortem autopsy, renal cortex tissue	Stable expression *Lower expression in term neonates vs. adults*	Term neonates n = 11 Infants n = 60 Children n = 31 Adolescents n = 10 Adults n = 10	Cheung et al., 2019 [20]
	MDR1/P-gp	Targeted protein quantification (LC–MS/MS), pmol/ng total membrane protein *Gene expression[a] (RT-qPCR)*	Post mortem autopsy, renal cortex tissue	Lower expression in term neonates & infants vs. children/adults *Lower expression in reaching adults levels in children*	Term neonates n = 11 Infants n = 60 Children n = 31 Adolescents n = 10 Adults n = 10	Cheung et al., 2019 [20]
		Targeted protein quantification (LC–MS/MS), pmol/gram of tissue normalized to Aquaporin 1	Post mortem, renal cortex tissue	Stable expression	Children n = 12 Adolescents n = 13 Adults n = 17	Li et al., 2019 [21]
	MRP2	Targeted protein quantification (LC–MS/MS), pmol/gram of tissue normalized to Aquaporin 1	Post mortem, renal cortex tissue	Stable expression	Children n = 12 Adolescents n = 13 Adults n = 17	Li et al., 2019 [21]
	OAT1	Targeted protein quantification (LC–MS/MS), pmol/ng total membrane protein *Gene expression[a] (RT-qPCR)*	Post mortem autopsy, renal cortex tissue	Lower expression in term neonates & infants vs. children/adults *Lower expression in preterm neonates vs. infants/children*	Term neonates n = 11 Infants n = 60 Children n = 31 Adolescents n = 10 Adults n = 10	Cheung et al., 2019 [20]
		Targeted protein quantification (LC–MS/MS), pmol/gram of tissue normalized to Aquaporin 1	Post mortem, renal cortex tissue	Stable expression	Children n = 12 Adolescents n = 13 Adults n = 17	Li et al., 2019 [21]
	OAT2	Targeted protein quantification (LC–MS/MS), pmol/gram of tissue normalized to Aquaporin 1	Post mortem, renal cortex tissue	Stable expression	Children n = 12 Adolescents n = 13 Adults n = 17	Li et al., 2019 [21]
	OAT3	Targeted protein quantification (LC–MS/MS), pmol/ng total membrane protein *Gene expression[a] (RT-qPCR)*	Post mortem autopsy, renal cortex tissue	Lower expression in term neonates & infants vs. children/adolescents/adults *Lower expression in preterm neonates vs. infants/children/adults, term neonates vs. children*	Term neonates n = 11 Infants n = 60 Children n = 31 Adolescents n = 10 Adults n = 10	Cheung et al., 2019 [20]
		Targeted protein quantification (LC–MS/MS), pmol/gram of tissue normalized to Aquaporin 1	Post mortem, renal cortex tissue	Stable expression	Children n = 12 Adolescents n = 13 Adults n = 17	Li et al., 2019 [21]
	OAT4	Targeted protein quantification (LC–MS/MS), pmol/gram of tissue normalized to Aquaporin 1	Post mortem, renal cortex tissue	Stable expression	Children n = 12 Adolescents n = 13 Adults n = 17	Li et al., 2019 [21]

Table 1 (*Continued*)

Organ	DT	Method	Tissue details	Relation to age	Population	Study
	OATP2C1	Targeted protein quantification (LC–MS/MS), pmol/gram of tissue normalized to Aquaporin 1	Post mortem, renal cortex tissue	Stable expression	Children n = 12 Adolescents n = 13 Adults n = 17	Li et al., 2019 [21]
	OCT2	Targeted protein quantification (LC–MS/MS), pmol/ng total membrane protein *Gene expression[a] (RT-qPCR)*	Post mortem autopsy, renal cortex tissue	Lower expression in term neonates & infants vs. children *Lower expression in neonates vs. infants/children/adults, term neonates vs. children*	Term neonates n = 11 Infants n = 60 Children n = 31 Adolescents n = 10 Adults n = 10	Cheung et al., 2019 [20]
		Targeted protein quantification (LC–MS/MS), pmol/gram of tissue normalized to Aquaporin 1	Post mortem, renal cortex tissue	Higher expression in adolescents vs. adults	Children n = 12 Adolescents n = 13 Adults n = 17	Li et al., 2019 [21]
	OCTN1	Targeted protein quantification (LC–MS/MS), pmol/gram of tissue normalized to Aquaporin 1	Post mortem, renal cortex tissue	Stable expression	Children n = 12 Adolescents n = 13 Adults n = 17	Li et al., 2019 [21]
	OCTN2	Targeted protein quantification (LC–MS/MS), pmol/gram of tissue normalized to Aquaporin 1	Post mortem, renal cortex tissue	Stable expression	Children n = 12 Adolescents n = 13 Adults n = 17	Li et al., 2019 [21]
	URAT1	Targeted protein quantification (LC–MS/MS), pmol/ng total membrane protein *Gene expression[a] (RT-qPCR)*	Post mortem autopsy, renal cortex tissue	Lower expression in term neonates & infants vs. children *Lower expression in term neonates vs. infants/children*	Term neonates n = 11 Infants n = 60 Children n = 31 Adolescents n = 10 Adults n = 10	Cheung et al., 2019 [20]

[a] Gene expression performed by Cheung et al., 2019 in a larger study population, which did include the samples also used for proteomics: preterm neonates: n = 9, term neonates n = 19, infants n = 81, children n = 38, adolescents n = 10, adults n = 27.

Table 2. Overview of age-related drug metabolizing enzyme protein expression studies in human organs performed with mass spectrometry methods. Fetus: 0 years, neonates: 0–1 months, infants 1 month–2 years, children: 3 months–8 years, early child: 2–5 years, middle child: 6–11 years, children: 2–11 years, adolescents 12–17 years, adults: 18 year.

Organ	Enzyme	Method	Tissue details	Relation with age	Population	Study
Liver	ADH1A	Protein quantification (LC–MS/MS), pmol/mg of cystolic protein	Post mortem, liver cytosol	Non-linear expression, lower expression in neonates vs. early child/middle child/ adolescents, higher expression in early child/ middle child/adolescents vs. adults	Neonates n = 4 Infants n = 17 Early child n = 30 Middle child n = 38 Adolescents n = 48 Adults n = 57	Bhatt et al., 2017 [25]
	ADH1B	Protein quantification (LC–MS/MS), pmol/mg of cystolic protein	Post mortem, liver cytosol	Lower expression in neonates/infants vs. early child/ middle child/ adolescents/adults	Neonates n = 4 Infants n = 17 Early child n = 30 Middle child n = 38 Adolescents n = 48 Adults n = 57	Bhatt et al., 2017 [25]

Table 2 (*Continued*)

Organ	Enzyme	Method	Tissue details	Relation with age	Population	Study
	ADH1C	Protein quantification (LC–MS/MS), pmol/mg of cystolic protein	Post mortem, liver cytosol	Lower expression in neonates/infants vs. early child/ middle child/ adolescents/adults	Neonates n = 4 Infants n = 17 Early child n = 30 Middle child n = 38 Adolescents n = 48 Adults n = 57	Bhatt et al., 2017 [25]
	ALDH1A1	Protein quantification (LC–MS/MS), pmol/mg of cystolic protein	Post mortem, liver cytosol	Lower expression in neonates/infants vs. early child/ middle child/ adolescents, neonates vs. adults	Neonates n = 4 Infants n = 17 Early child n = 30 Middle child n = 38 Adolescents n = 48 Adults n = 57	Bhatt et al., 2017 [25]
	CYP1A1	Global protein quantification (LC–MS/MS), quantified using AMT database	Post mortem, hepatic microsomes	Stable expression	Fetus n = 30 Neonates n = 2 Infants n = 2 Children n = 10 Adolescents n = 1 Adults n = 9	Sadler et al., 2016 [22]
	CYP1A2	Global protein quantification (LC–MS/MS), quantified using AMT database	Post mortem, hepatic microsomes	Lower expression in fetus vs. postnatal samples	Fetus n = 30 Neonates n = 2 Infants n = 2 Children n = 10 Adolescents n = 1 Adults n = 9	Sadler et al., 2016 [22]
	CYP2A6	Global protein quantification (LC–MS/MS), quantified using AMT database	Post mortem, hepatic microsomes	Lower expression in fetus vs. postnatal samples	Fetus n = 30 Neonates n = 2 Infants n = 2 Children n = 10 Adolescents n = 1 Adults n = 9	Sadler et al., 2016 [22]
	CYP2B6	Global protein quantification (LC–MS/MS), quantified using AMT database	Post mortem, hepatic microsomes	Lower expression in fetus vs. postnatal samples	Fetus n = 30 Neonates n = 2 Infants n = 2 Children n = 10 Adolescents n = 1 Adults n = 9	Sadler et al., 2016 [22]
	CYP2C8	Global protein quantification (LC–MS/MS), quantified using AMT database	Post mortem, hepatic microsomes	Lower expression in fetus vs. postnatal samples	Fetus n = 30 Neonates n = 2 Infants n = 2 Children n = 10 Adolescents n = 1 Adults n = 9	Sadler et al., 2016 [22]
	CYP2C9	Targeted protein quantification (LC–MS/MS), nmol/ng protein	Post mortem, hepatic microsome	Lower expression in fetus vs. children/adults	Fetus n = 7 Children n = 16 Adults n = 10	Zane et al., 2018 [23]
		Global protein quantification (LC–MS/MS), quantified using AMT database	Post mortem, hepatic microsomes	Lower expression in fetus vs. postnatal samples	Fetus n = 30 Neonates n = 2 Infants n = 2 Children n = 10 Adolescents n = 1 Adults n = 9	Sadler et al., 2016 [22]

Table 2 (*Continued*)

Organ	Enzyme	Method	Tissue details	Relation with age	Population	Study
	CYP2C19	Targeted protein quantification (LC–MS/MS), nmol/ng protein	Post mortem, hepatic microsome	Non-linear expression, lower expression in fetus vs. children, higher expression in children vs. adults	Fetus n = 7 Children n = 16 Adults n = 10	Zane et al., 2018 [23]
		Global protein quantification (LC–MS/MS), quantified using AMT database	Post mortem, hepatic microsomes	Lower expression in fetus vs. postnatal samples	Fetus n = 30 Neonates n = 2 Infants n = 2 Children n = 10 Adolescents n = 1 Adults n = 9	Sadler et al., 2016 [22]
	CYP2D6	Global protein quantification (LC–MS/MS), quantified using AMT database	Post mortem, hepatic microsomes	Lower expression in fetus vs. postnatal samples	Fetus n = 30 Neonates n = 2 Infants n = 2 Children n = 10 Adolescents n = 1 Adults n = 9	Sadler et al., 2016 [22]
	CYP2E1	Global protein quantification (LC–MS/MS), quantified using AMT database	Post mortem, hepatic microsomes	Lower expression in fetus vs. postnatal samples	Fetus n = 30 Neonates n = 2 Infants n = 2 Children n = 10 Adolescents n = 1 Adults n = 9	Sadler et al., 2016 [22]
	CYP2J2	Global protein quantification (LC–MS/MS), quantified using AMT database	Post mortem, hepatic microsomes	Lower expression in fetus vs. postnatal samples	Fetus n = 30 Neonates n = 2 Infants n = 2 Children n = 10 Adolescents n = 1 Adults n = 9	Sadler et al., 2016 [22]
	CYP2W1	Global protein quantification (LC–MS/MS), quantified using AMT database	Post mortem, hepatic microsomes	Stable expression	Fetus n = 30 Neonates n = 2 Infants n = 2 Children n = 10 Adolescents n = 1 Adults n = 9	Sadler et al., 2016 [22]
	CYP3A4	Targeted protein quantification (LC–MS/MS), nmol/ng protein	Post mortem, hepatic microsome	Lower expression in fetus vs. children/adults	Fetus n = 7 Children n = 16 Adults n = 10	Zane et al., 2018 [23]
		Global protein quantification (LC–MS/MS), quantified using AMT database	Post mortem, hepatic microsomes	Lower expression in fetus vs. postnatal samples	Fetus n = 30 Neonates n = 2 Infants n = 2 Children n = 10 Adolescents n = 1 Adults n = 9	Sadler et al., 2016 [22]
	CYP3A5	Targeted protein quantification (LC–MS/MS), nmol/ng protein	Post mortem, hepatic microsome	Stable expression	Fetus n = 7 Children n = 16 Adults n = 10	Zane et al., 2018 [23]
		Global protein quantification (LC–MS/MS), quantified using AMT database	Post mortem, hepatic microsomes	Lower expression in fetus vs. postnatal samples	Fetus n = 30 Neonates n = 2 Infants n = 2 Children n = 10 Adolescents n = 1 Adults n = 9	Sadler et al., 2016 [22]

Table 2 (*Continued*)

Organ	Enzyme	Method	Tissue details	Relation with age	Population	Study
	CYP3A7	Targeted protein quantification (LC–MS/MS), nmol/ng protein	Post mortem, hepatic microsome	Higher expression in fetus vs. children/adults	Fetus n = 7 Children n = 16 Adults n = 10	Zane et al., 2018 [23]
		Global protein quantification (LC–MS/MS), quantified using AMT database	Post mortem, hepatic microsomes	Higher expression in fetus vs. postnatal samples	Fetus n = 30 Neonates n = 2 Infants n = 2 Children n = 10 Adolescents n = 1 Adults n = 9	Sadler et al., 2016 [22]
	CYP4F2	Global protein quantification (LC–MS/MS), quantified using AMT database	Post mortem, hepatic microsomes	Lower expression in fetus vs. postnatal samples	Fetus n = 30 Neonates n = 2 Infants n = 2 Children n = 10 Adolescents n = 1 Adults n = 9	Sadler et al., 2016 [22]
	CYP4F3	Global protein quantification (LC–MS/MS), quantified using AMT database	Post mortem, hepatic microsomes	Lower expression in fetus vs. postnatal samples	Fetus n = 30 Neonates n = 2 Infants n = 2 Children n = 10 Adolescents n = 1 Adults n = 9	Sadler et al., 2016 [22]
	CYP4F11	Global protein quantification (LC–MS/MS), quantified using AMT database	Post mortem, hepatic microsomes	Lower expression in fetus vs. postnatal samples	Fetus n = 30 Neonates n = 2 Infants n = 2 Children n = 10 Adolescents n = 1 Adults n = 9	Sadler et al., 2016 [22]
	CYP4F12	Global protein quantification (LC–MS/MS), quantified using AMT database	Post mortem, hepatic microsomes	Lower expression in fetus vs. postnatal samples	Fetus n = 30 Neonates n = 2 Infants n = 2 Children n = 10 Adolescents n = 1 Adults n = 9	Sadler et al., 2016 [22]
	CYP4V2	Global protein quantification (LC–MS/MS), quantified using AMT database	Post mortem, hepatic microsomes	Lower expression in fetus vs. postnatal samples	Fetus n = 30 Neonates n = 2 Infants n = 2 Children n = 10 Adolescents n = 1 Adults n = 9	Sadler et al., 2016 [22]
	CYP7B1	Global protein quantification (LC–MS/MS), quantified using AMT database	Post mortem, hepatic microsomes	Higher expression in fetus vs. postnatal samples	Fetus n = 30 Neonates n = 2 Infants n = 2 Children n = 10 Adolescents n = 1 Adults n = 9	Sadler et al., 2016 [22]
	CYP8B1	Global protein quantification (LC–MS/MS), quantified using AMT database	Post mortem, hepatic microsomes	Lower expression in fetus vs. postnatal samples	Fetus n = 30 Neonates n = 2 Infants n = 2 Children n = 10 Adolescents n = 1 Adults n = 9	Sadler et al., 2016 [22]
	CYP17A1	Global protein quantification (LC–MS/MS), quantified using AMT database	Post mortem, hepatic microsomes	Stable expression	Fetus n = 30 Neonates n = 2 Infants n = 2 Children n = 10 Adolescents n = 1 Adults n = 9	Sadler et al., 2016 [22]

Table 2 (*Continued*)

Organ	Enzyme	Method	Tissue details	Relation with age	Population	Study
	CYP19A1	Global protein quantification (LC–MS/MS), quantified using AMT database	Post mortem, hepatic microsomes	Higher expression in fetus vs. postnatal samples	Fetus n = 30 Neonates n = 2 Infants n = 2 Children n = 10 Adolescents n = 1 Adults n = 9	Sadler et al., 2016 [22]
	CYP20A1	Global protein quantification (LC–MS/MS), quantified using AMT database	Post mortem, hepatic microsomes	Lower expression in fetus vs. postnatal samples	Fetus n = 30 Neonates n = 2 Infants n = 2 Children n = 10 Adolescents n = 1 Adults n = 9	Sadler et al., 2016 [22]
	CYP27A1	Global protein quantification (LC–MS/MS), quantified using AMT database	Post mortem, hepatic microsomes	Lower expression in fetus vs. postnatal samples	Fetus n = 30 Neonates n = 2 Infants n = 2 Children n = 10 Adolescents n = 1 Adults n = 9	Sadler et al., 2016 [22]
	CYP39A1	Global protein quantification (LC–MS/MS), quantified using AMT database	Post mortem, hepatic microsomes	Stable expression	Fetus n = 30 Neonates n = 2 Infants n = 2 Children n = 10 Adolescents n = 1 Adults n = 9	Sadler et al., 2016 [22]
	CYP51A1	Global protein quantification (LC–MS/MS), quantified using AMT database	Post mortem, hepatic microsomes	Higher expression in fetus vs. postnatal samples	Fetus n = 30 Neonates n = 2 Infants n = 2 Children n = 10 Adolescents n = 1 Adults n = 9	Sadler et al., 2016 [22]
	FMO1	Targeted protein quantification (LC–MS/MS), nmol/ng protein	Post mortem, hepatic microsomes	Only detected in fetus	Fetus n = 7 Children n = 16 Adults n = 10	Zane et al., 2018 [23]
	FMO3	Targeted protein quantification (LC–MS/MS), nmol/ng protein	Post mortem, hepatic microsomes	Lower expression in fetus vs. adults	Fetus n = 7 Children n = 16 Adults n = 10	Zane et al., 2018 [23]
	FMO5	Targeted protein quantification (UPLC-MRM), pmol/mg protein	Post mortem, hepatic microsomes	Stable expression	Fetus n = 7 Children n = 16 Adults n = 10	Chen et al., 2016 [28]
	SULT1A1	Targeted protein quantification (LC–MS/MS), pmol/mg cytosol protein	Post mortem, hepatic cytosol	Non-linear expression, lower expression in neonatal vs. early child/middle child, infants vs. early child, higher expression in early child vs. adolescents/ adults	Neonates n = 4 Infants n = 17 Early child n = 29 Middle child n = 37 Adolescents n = 46 Adults n = 57	Ladumor et al., 2019 [26]
		Targeted protein abundance (TSQ Vantage mass spectrometer), fmol/mg cytosolic protein *Gene expression[a] (RT-qPCR)* *Gene expression[a] (RNAseq)*	Post mortem and surgical tissue, liver cytosol	Stable expression *Stable expression* *Stable expression*	Fetus n = 60 Neonates n = 76 Infants n = 9 Early child n = 14 Middle child n = 16 Adolescents n = 16	Dubaisi et al., 2019 [29]
	SULT1A2	Targeted protein abundance (TSQ Vantage mass spectrometer), fmol/mg cytosolic protein *Gene expression[a] (RT-qPCR)* *Gene expression[a] (RNAseq)*	Post mortem and surgical tissue, liver cytosol	Lower expression in fetus vs. adolescents *Lower expression in infants vs. adults* *Stable expression*	Fetus n = 40 Neonates n = 54 Infants n = 9 Early child n = 5 Middle child n = 12 Adolescents n = 14	Dubaisi et al., 2019 [29]

Table 2 (*Continued*)

Organ	Enzyme	Method	Tissue details	Relation with age	Population	Study
	SULT1A3	Targeted protein quantification (LC–MS/MS), pmol/mg cytosol protein	Post mortem, hepatic cytosol	Stable expression	Neonates n = 4 Infants n = 17 Early child n = 29 Middle child n = 37 Adolescents n = 46 Adults n = 57	Ladumor et al., 2019 [26]
		Targeted protein abundance (TSQ Vantage mass spectrometer), fmol/mg cytosolic protein *Gene expression[a] (RNAseq)*	Post mortem and surgical tissue, liver cytosol	Higher expression in fetus vs. neonates/early child/middle child/adolescents *Higher expression in fetus vs. early child*	Fetus n = 37 Neonates n = 12 Infants n = 9 Early child n = 2 Middle child n = 2 Adolescents n = 2	Dubaisi et al., 2019 [29]
	SULT1B1	Targeted protein quantification (LC–MS/MS), pmol/mg cytosol protein	Post mortem, hepatic cytosol	Lower expression in neonatal vs. early child/middle child/adults, infants vs. early child/middle child/adolescents/adults	Neonates n = 3 Infants n = 17 Early child n = 30 Middle child n = 37 Adolescents n = 47 Adults n = 57	Ladumor et al., 2019 [26]
		Targeted protein abundance (TSQ Vantage mass spectrometer), fmol/mg cytosolic protein *Gene expression[a] (RT-qPCR)* *Gene expression[a] (RNAseq)*	Post mortem and surgical tissue, liver cytosol	Lower expression in fetus vs. early child/adolescents *Lower expression in fetus vs. adults* *Higher expression in fetus vs early child*	Fetus n = 13 Neonates n = 27 Infants n = 9 Early child n = 3 Middle child n = 9 Adolescents n = 12	Dubaisi et al., 2019 [29]
	SULT1C2	Targeted protein abundance (TSQ Vantage mass spectrometer), fmol/mg cytosolic protein *Gene expression[a] (RT-qPCR)* *Gene expression[a] (RNAseq)*	Post mortem and surgical tissue, liver cytosol	Higher expression in fetus vs. early child/middle child/adolescents *Higher expression in fetus/infants vs. adults* *Higher expression in fetus vs. early child*	Fetus n = 61 Neonates n = 76 Infants n = 9 Early child n = 11 Middle child n = 12 Adolescents n = 15	Dubaisi et al., 2019 [29]
	SULT1C4	Targeted protein abundance (TSQ Vantage mass spectrometer), fmol/mg cytosolic protein *Gene expression[a] (RT-qPCR)* *Gene expression[a] (RNAseq)*	Post mortem and surgical tissue, liver cytosol	No expression pattern defined *Higher expression in fetus vs. infants/adults* *Higher expression in fetus vs. early child*	Fetus n = 17 Neonates n = 1 Infants n = 4 Early child n = 0 Middle child n = 0 Adolescents n = 0	Dubaisi et al., 2019 [29]
	SULT1E1	Targeted protein abundance (TSQ Vantage mass spectrometer), fmol/mg cytosolic protein *Gene expression[a] (RT-qPCR)* *Gene expression[a] (RNAseq)*	Post mortem and surgical tissue, liver cytosol	Higher expression in fetus vs. neonates/middle child/adolescents *Stable expression* *Higher expression in fetus vs. neonates*	Fetus n = 62 Neonates n = 76 Infants n = 9 Early child n = 14 Middle child n = 16 Adolescents n = 16	Dubaisi et al., 2019 [29]
	SULT2A1	Targeted protein quantification (LC–MS/MS), pmol/mg cytosol protein	Post mortem, hepatic cytosol	Non-linear expression, lower expression neonatal vs. early child, higher expression in early child vs. adolescents	Neonates n = 4 Infants n = 17 Early child n = 29 Middle child n = 37 Adolescents n = 42 Adults n = 54	Ladumor et al., 2019 [26]
		Targeted protein abundance (TSQ Vantage mass spectrometer), fmol/mg cytosolic protein *Gene expression[a] (RT-qPCR)* *Gene expression[a] (RNAseq)*	Post mortem and surgical tissue, liver cytosol	Lower expression in fetus vs. neonates *Lower expression in fetus vs. infants/adults* *Lower expression in fetus vs. early child*	Fetus n = 56 Neonates n = 70 Infants n = 9 Early child n = 11 Middle child n = 14 Adolescents n = 14	Dubaisi et al., 2019 [29]

Table 2 (*Continued*)

Organ	Enzyme	Method	Tissue details	Relation with age	Population	Study
	UGT1A1	Targeted protein quantification (LC–MS/MS), pmol/mg microsomal protein	Post mortem, liver microsomes	Lower expression in neonates vs. early child, neonates/infants/early child/middle child/adolescents vs. adults	Neonates n = 4 Infants n = 17 Early child n = 30 Middle child n = 38 Adolescents n = 48 Adults n = 35	Bhatt et al., 2019 [24]
	UGT1A4	Targeted protein quantification (LC–MS/MS), pmol/mg microsomal protein	Post mortem, liver microsomes	Lower expression in neonates vs. early child/middle child, infants vs. early child/adolescents, neonates/infants/early child/adolescents vs. adults	Neonates n = 4 Infants n = 17 Early child n = 30 Middle child n = 38 Adolescents n = 48 Adults n = 35	Bhatt et al., 2019 [24]
	UGT1A6	Targeted protein quantification (LC–MS/MS), pmol/mg microsomal protein	Post mortem, liver microsomes	Lower expression in fetus vs. middle child, neonates vs. early child/middle child/adolescents, all groups vs. adults	Neonates n = 4 Infants n = 17 Early child n = 30 Middle child n = 38 Adolescents n = 48 Adults n = 35	Bhatt et al., 2019 [24]
	UGT1A9	Targeted protein quantification (LC–MS/MS), pmol/mg microsomal protein	Post mortem, liver microsomes	Lower expression in fetus vs. middle child, all groups vs. adults	Neonates n = 4 Infants n = 17 Early child n = 30 Middle child n = 38 Adolescents n = 48 Adults n = 35	Bhatt et al., 2019 [24]
	UGT2B7	Targeted protein quantification (LC–MS/MS), pmol/mg microsomal protein	Post mortem, liver microsomes	Lower expression in fetus/neonates vs. adolescents, all groups vs. adults	Neonates n = 4 Infants n = 17 Early child n = 30 Middle child n = 38 Adolescents n = 48 Adults n = 35	Bhatt et al., 2019 [24]
	UGT2B15	Targeted protein quantification (LC–MS/MS), pmol/mg microsomal protein	Post mortem, liver microsomes	Lower expression in fetus/neonates/early child/middle child vs. adults	Neonates n = 4 Infants n = 17 Early child n = 30 Middle child n = 38 Adolescents n = 48 Adults n = 35	Bhatt et al., 2019 [24]
	UGT2B17	Targeted protein quantification (LC–MS/MS), pmol/mg microsomal protein *Transporter activity*[b] *(Metabolism rate)*	Post mortem, liver microsomes	Lower expression in early child vs. adolescents, infants/early child/middle child vs. adults *Lower activity in neonates/infants/early child/middle child vs. adolescents/adults*	Neonates n = 3 Infants n = 23 Early child n = 39 Middle child n = 46 Adolescents n = 63 Adults n = 185	Bhatt et al., 2018 [27]

[a] Gene expression performed by Dubaisi et al., 2019 was obtained in a different study population: RT-qPCR: fetus: n = 10, infants n = 10, adults n = 10. RNAseq: fetus: n = 10, pediatric n = 52.
[b] Transporter activity performed by Bhatt et al., 2018 was obtained in a different study population: neonates n = 3, infants n = 19, early child n = 29, middle child n = 35, adolescents n = 54, adults n = 127.

these studies are described, including the applied methodology, age ranges studied and the impact of age. We here present a summary of the main results.

Drug transporters

For the liver, we identified 4 studies presenting DT expression across the pediatric age. As all samples from the study by Mooij et al., 2016 were included in the larger cohort of the study by van Groen et al., 2018, we did not include these data in the table, to avoid duplicate reporting [16,17]. When combining the results of the three remaining studies, broadly three developmental patterns arise [17–19]. Most hepatic DTs show lower protein expression in the fetal population compared to later age stages, i.e. for example for P-gp, MRP2, MRP3 and OCT1. GLUT1 and OATP1B1 show the opposite pattern, with higher expression in fetal compared to neonatal and older age groups. BCRP, MATE1, MCT1, OATP2B1, OATP1B3 expression remains stable across the different age groups. Age-related transporter expression patterns are also summarized in Fig. 1.

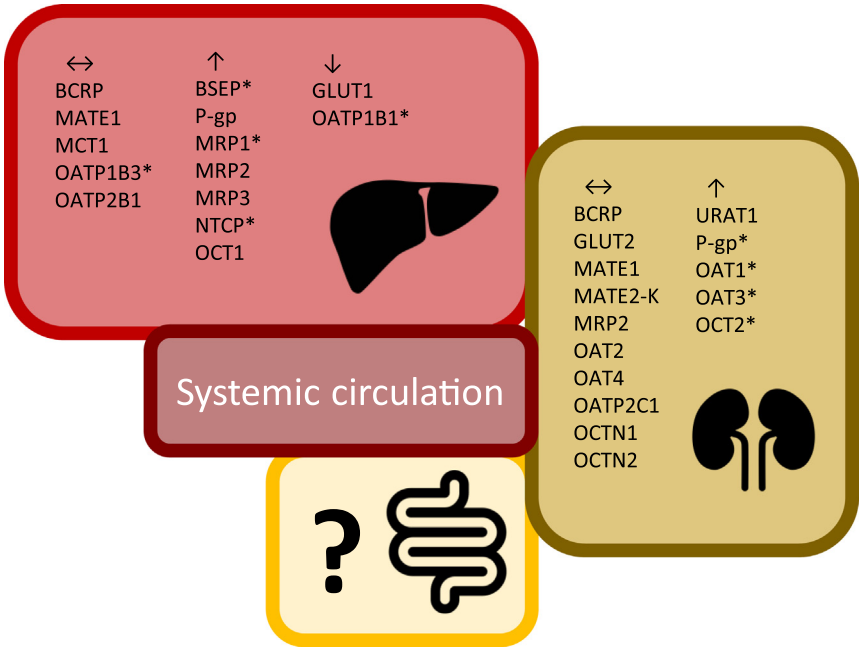

Fig. 1. Maturational patterns of drug transporters in the liver, kidney and intestine. ↔: stable maturational pattern; ↑: higher expression in older age groups; ↓: lower expression in older age groups; *: result shown based on the most recent study [17,20], previous study results vary [18,19,21].

The interpretation of descriptive developmental patterns should be done carefully. At first sight the developmental changes for BSEP, MRP1, NTCP, OATP1B1 and OATP1B3 appear to be different between van Groen et al. and Prasad et al. [17,19]. But the lack of developmental variation seen by Prasad et al. can be likely explained by the older age range studied. A close look at these data demonstrates the importance to consider the age ranges and number of patients studied when interpreting the published data. The studies on liver DT by Prasad et al. included 110 patients of which only 4 of neonatal age (0–1 months) [19]. In contrast, in the study by van Groen et al., where age-related changes in protein expression were identified, samples from 36 fetal, 12 preterm neonates and 10 term neonates were analyzed, most pronounced differences were found between fetus/neonates and infants/older children and adults [17]. This indicates that a sufficient representation of younger age groups is important to study developmental patterns.

Two studies presented renal DT protein expression data. Lower DT protein expression in neonates and infants compared to older age groups was found for P-gp, OAT1, OAT2 and URAT1 [20]. One study performed by Li et al., did not find an age-related pattern for OCT2 comparing 12 children (1–12 years) to adolescents and adults, whereas Cheung et al. reported lower OCT2 expression in term neonates (n = 11) and infants (n = 60) compared to children (n = 30) and adults. Similar to the liver data, this difference is probably also caused by variation in the age ranges study population [20,21].

Next to renal DT expression, Li et al. studied the inter-tissue correlation between renal and hepatic DT expression levels in unique samples from the same donor. No correlation was found between protein expression of the same transporter in the two organs and furthermore only a modest correlation ($r = 0.49$, $p < 0.05$) was found between renal OCTN1 and hepatic OCT1 expression [21].

Drug metabolizing enzymes

We found only one study reporting protein abundance for phase 1 DMEs. In 54 postmortem samples from 30 fetuses (lowest gestational age 87 days), 2 neonates, 2 infants, 10 children, 1 adolescent and 9 adults, out of 28 CYP450 enzymes, for only 4, protein abundance was similar across the age groups. Protein expression of 20 DMEs increased from fetus to adult, of 4 DMEs expression decreased [22], and for 1 DME a non-linear pattern was found [23]. Emphasizing the importance of also studying the maturational changes in hepatic drug metabolism.

Very recently, the hepatic abundance of the phase II DMEs UGTs and SULTs was studied in a pediatric population. The results for six hepatic microsomal UGTs in postmortem samples showed a lower protein expression in neonates (<1 month) compared to adults, reaching 50% of adult expression in childhood (2–<12 year) for UGT1A4, UGT1A6, UGT1A9, and UGT2B7 [24]. Lower DME expression was

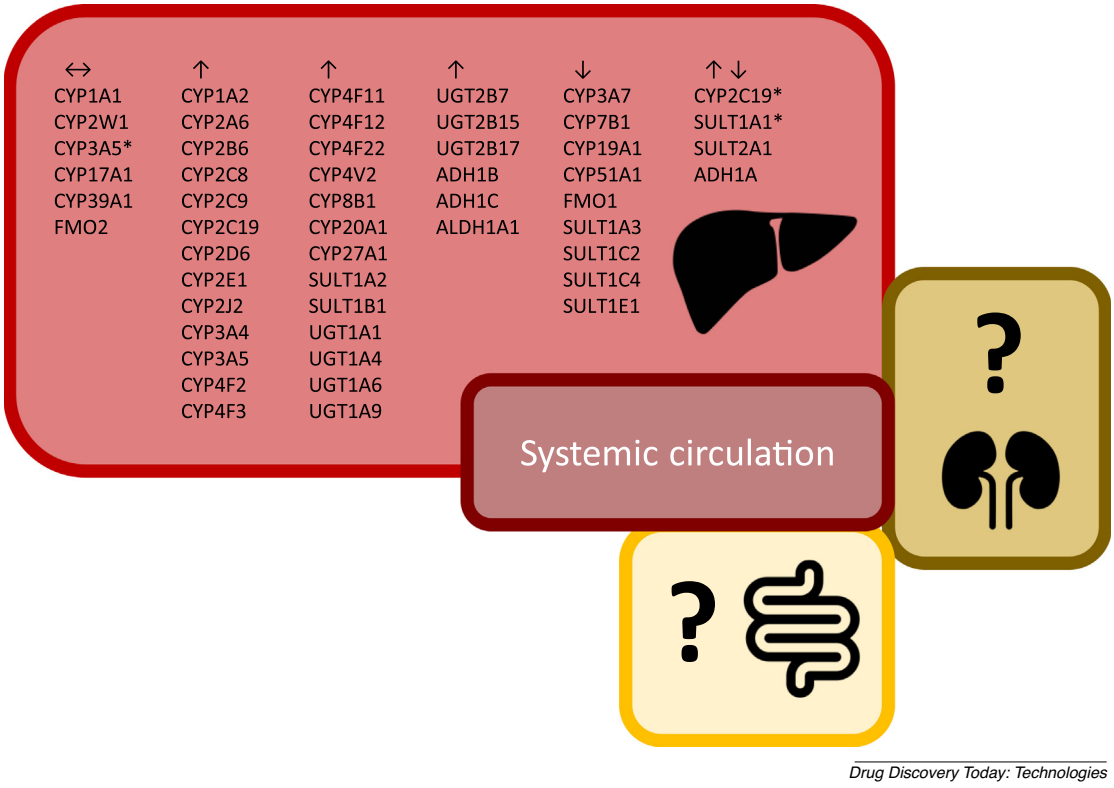

Fig. 2. Maturational patterns of drug metabolizing enzymes in the liver, kidney and intestine. ↔: stable maturational pattern; ↑: higher expression in older age groups; ↓: lower expression in older age groups; *: result shown based on the most recent study [23,26], previous study results vary [22–29].

associated with decreased protein activity for UGT2B17-mediated glucuronidation, which can result in lower drug clearance activity from the body [25]. Liver cytosolic SULTs showed a different developmental pattern than most CYP and UGT DMEs. Highest concentration of SULTs were found in early childhood (prenatal – <6 year), decreasing about 40% towards adolescence and adulthood for SULT1A1 and SULT2A1 [26].

These results emphasize the large variation in maturation of ADME genes, with clear developmental changes mainly from low to high for phase I DMEs, but this pattern is less prevalent for the phase 2 DMEs and DTs. The generalization of low to high expression across childhood, as is often used in reviews and presentations on developmental pharmacology needs, in our opinion, more nuance.

In contrast to the liver, a knowledge gap remains for age-related changes in renal and intestinal protein expression data on DMEs (Fig. 2).

Translation of proteomic data to pharmacokinetics

The major advantage of quantitative proteomics data is that they can be used in physiologically based pharmacokinetic models (PBPK) models. A PBPK model represents the body's physiology using multiple equations to predict drug disposition by ADME processes combining system and drug specific parameters [30]. Creating a pediatric PBPK model often starts with a solid adult model, the adult model is adjusted with pediatric specific input such as blood flow, weight and DT and DME expression data. By including pediatric specific data, PBPK models can be used to translate data to the pediatric situation to better design pediatric drug dosing guidelines and understand age-related in vivo variation [31]. The increased availability of pediatric proteomic data aids in developing of adequate models that allow reliable extrapolation of drug transport and metabolism to this understudied population.

To indicate the importance of ontogeny data, a case study was performed by Cheung et al., 2019. Developmental kidney OAT1 and OAT3 patterns derived from a proteomic study were used in a pediatric PBPK model for the antibiotic Tazobactam. Renal secretion of tazobactam is high (80%) and is mediated by glomerular filtration and excretion via OAT1 and OAT3. The pediatric PBPK model was built for three age groups; 0–3 months, 3 months-2 years and 2–7 years old with and without incorporation of the kidney DT proteomic ontogeny data. The addition of DT ontogeny to glomular filtration rate maturation significantly improved the model outcomes, now within 1.5 fold of previously observed clinical data [20].

Moreover, these proteomic data have recently been used to simulate the influence of DT maturation in drug disposition in children in a PBPK-based framework. A pediatric PBPK

model was based on a published adult model, adjusted for the pediatric age range. The model showed that excluding DT maturation from the model results in unacceptable clearance predictions, which could lead to overexposure and eventually toxicity when used. For instance, clearance predictions for pediatric patients versus adults supported slower maturation of OAT1 and OAT3 to adult levels compared to OCT1 and P-gp and is in line with the available proteomic data [20,32]. These examples illustrate the importance of age-related protein expression research and how these ontogeny data can increase the predictive power of PBPK models and thereby optimize dose selection.

Challenges and future directions to proteomics DME/DT studies in children

While we have made great progress in our understanding of DME and DT ontogeny by the availability of proteomics, some challenges still remain, as discussed here.

To begin with, extrapolation and/or combination of proteomic data can be difficult due to variability in data caused by the lack of harmonized methodologies between laboratories [24,33]. Methodologies used for LC–MS/MS analysis often differ in sample delivery, sample preparation, scaling factor differences, inconsistent unit use and absolute or relative quantification [34]. The lack of consensus contributes to the high variability in reported proteomics data. A white paper was published by Prasad et al. which encouraged harmonization of LC–MS/MS methodology [15]. This will help to compare and combine data from various laboratories, resulting in better PK prediction and PBPK modelling [15]. Next to the White Paper, a group set up a repository database of 55 DMEs and 104 membrane transporters visualizing the high interlaboratory variation. With this database they intended to emphasize that the variability affects PBPK modelling in healthy and special populations [12].

Another important practical limitation is that tissues available from children are scarce and often very small, especially from living donors. The tiny pieces of tissue complicate data collection using multiple methods, such as proteomics, IHC, gene expression and activity research. Therefore, there is a definite need for in vitro or ex vivo models, which require less or easily accessible material.

Innovative methods to overcome this latter limitation are the use of exosomes and organoids derived from fresh tissues [35,36]. One possibility could be to look into exosomes. Exosomes are extracellular vesicles excreted by organs in the circulatory system that contain their mRNA and proteins, and which can be isolated from body liquids as blood or urine. Such a 'liquid biopsy' makes it possible to quantify DT and DME protein expression from its organ of origin [37]. Proteomic analysis in exosomes has been successfully applied for the identification of cancer types and to quantify protein expression of DTs and DMEs in adults [37–39]. This less invasive and easy to obtain sampling makes these 'liquid biopsies' from different organs more accessible for research. Therefore, this method is promising and can potentially be very valuable tool in pediatric research [40]. However, validation for this method is required to confirm their value to optimize drug dosing guidelines in various sub groups, e.g. by comparing exosome derived DME and/or DT abundance with tissue abundance and functionality by analyzing pharmacokinetic data from marker substrates.

An alternative approach could be the use of tissue-derived organoids in proteomic research. Organoids are so called 'mini-organs' representing 3D morphology and different cell types of the tissue derived in vivo and can be isolated from stem cells of an organ of interest like intestine, kidney or liver [41]. Recently, the feasibility to study protein expression of DT and DME in organoids was shown in adults [42,43]. Next to proteomics, organoids could have great potential to be used in transporter protein functionality research. By culturing organoids in 2D, vectoral drug transport and drug metabolism studies can be performed in the organ of interest [44]. However, further research is needed to explore the feasibility for this approach in pediatrics, while data on DNA methylation suggest stable expression during multiple organoid passages, it is yet unknown if pediatric organoids will retain their age-specific DT and DME protein expression levels and functionality during repeated passages [45]. Obviously, these properties need to be preserved to use the methods in DT and DME ontogeny research.

Furthermore, a knowledge gap exists in DT and DME maturation proteomic data for the intestine, blood–brain barrier, lung, eye and skin tissues. The role of the intestine in drug metabolism and transport has been underestimated compared to the role of the liver and kidney [33]. Limited ex vivo models using fresh tissue are available to study protein functionality in tissues like the gut. Especially for the pediatric population tissue is very scarce, hampering the use of available models, such as precision cut tissue slices or the Ussing chamber [36,46]. Combining ontogeny expression and functionality data from different organs can provide us with better PK prediction for this vulnerable population.

Importantly, the inter-group variability regarding proteomics appears to be very high in the pediatric population. This results in inconsistent outcomes, especially in understudied age groups such as the fetal and neonatal age range (<0–2 years) [47]. Next to that, left over tissues available from surgeries is only available from sick pediatric patients, which may influence protein expression [48]. Besides, protein expression can be influenced by many other factors apart from age and disease. Not much is known about the interplay of age with inflammation, medication or environmental toxicants. As mentioned above, gene expression does not always correlate with protein expression or functionality [49]. Therefore, expression data must be used carefully to predict protein

activity [17]. Furthermore, genomic variation plays a role in gene and protein expression, resulting in variable protein functionality [50,51]. These examples implicate the complexity of predicting new dosages with the use of PBPK modelling, including genotype and disease.

In brief, the introduction of LC–MS/MS methods to quantify protein expression has greatly expanded our knowledge on pediatric DME and DT expression. The first application of these data in PBPK models shows the large protentional to adapt and even individualize pediatric dosing. Several challenges exist, including the lack of data, data variability and tissue availability. All studies reviewed so far suffer from high intra-age group variability, especially for the pre-term and neonatal age groups. Therefore, more large-scale studies are needed to lower variability. At the same time, innovative methodologies present a bright future for developmental pharmacology research by providing better access to (models of) pediatric tissue. Despite current challenges, valuable results have been generated and more knowledge is within our reach to improve pediatric drug dosing.

Declaration of interest

None.

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