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Ontogeny of Hepatic Transporters and Drug-Metabolizing Enzymes in Humans and in Nonclinical Species^S

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ABBREVIATIONS: ABC, ATP-binding cassette; ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; BCRP, breast cancer resistance protein; BSEP, bile salt export pump; CES, carboxylesterase; COMT, catechol-O-methyltransferase; CNT, concentrative nucleoside transporter; DHEAS, dehydroepiandrosterone sulfate; DME, drug-metabolizing enzyme; DT, drug transporter; ENT, equilibrative nucleoside transporter; FMO, flavin-containing monooxygenase; GSH, glutathione; GST, glutathione-S-transferase; LC, liquid chromatography; MATE1, multidrug and toxin extrusion 1; MRP, multidrug resistance-associated protein; MS, mass spectrometry; NAT, N-acetyltransferase; NPT1, sodium-dependent phosphate transporter 1; NTCP, sodium taurocholate cotransporting polypeptide; OATP, organic anion-transporting polypeptide; OCT1, organic cation transporter 1; OCTN, organic cation/carnitine transporter; PBPK, physiologically based pharmacokinetic; PK, pharmacokinetics; PXR, pregnane X receptor; SNAT, system A amino acid transporter; SULT, sulfotransferase; UGT, uridine 5-diphosphoglucuronic acid glucuronyltransferase.

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Abstract—The liver represents a major eliminating and detoxifying organ, determining exposure to endogenous compounds, drugs, and other xenobiotics. Drug transporters (DTs) and drug-metabolizing enzymes (DMEs) are key determinants of disposition, efficacy, and toxicity of drugs. Changes in their mRNA and protein expression levels and associated functional activity between the perinatal period until adulthood impact drug disposition. However, high-resolution ontogeny profiles for hepatic DTs and DMEs in nonclinical species and humans are lacking. Meanwhile, increasing use of physiologically based pharmacokinetic (PBPK) models necessitates availability of underlying ontogeny profiles to reliably predict drug exposure in children. In addition, understanding of species similarities and differences in DT/DME ontogeny is crucial for selecting the most appropriate animal species when studying the impact of development on pharmacokinetics. Cross-species ontogeny mapping is also required for adequate translation of drug disposition data in developing nonclinical species to humans. This review presents a quantitative cross-species compilation of the ontogeny of DTs and DMEs relevant to hepatic drug disposition. A comprehensive literature search

was conducted on PubMed Central: Tables and graphs (often after digitization) in original manuscripts were used to extract ontogeny data. Data from independent studies were standardized and normalized before being compiled in graphs and tables for further interpretation. New insights gained from these high-resolution ontogeny profiles will be indispensable to understand cross-species differences in maturation of hepatic DTs and DMEs. Integration of these ontogeny data into PBPK models will support improved predictions of pediatric hepatic drug disposition processes.

Significance Statement—Hepatic drug transporters (DTs) and drug-metabolizing enzymes (DMEs) play pivotal roles in hepatic drug disposition. Developmental changes in expression levels and activities of these proteins drive age-dependent pharmacokinetics. This review compiles the currently available ontogeny profiles of DTs and DMEs expressed in livers of humans and nonclinical species, enabling robust interpretation of age-related changes in drug disposition and ultimately optimization of pediatric drug therapy.

I. Introduction

Hepatic drug transporters (DTs) and drug-metabolizing enzymes (DMEs) are key players in the disposition of endogenous compounds, xenobiotics including drugs and their metabolites in human as well as in nonclinical species (Shi and Li, 2014). The importance of DMEs has been recognized for many decades (Yamazaki, 2014). The impact of DTs has more recently received both scientific and regulatory attention, highlighting the increasing knowledge on their significance in drug disposition and on efficacy and safety (European Medicines Agency; Food and Drug Administration, 2020; International Council for Harmonisation, 2000; Ministry of Labor and Welfare, 2018; Petzinger and Geyer, 2006).

Numerous DTs are located on the apical and basolateral membranes of the hepatocyte and facilitate active transport of substrates into as well as out of hepatocytes to the bile canaliculi or blood

compartment (i.e., uptake DTs and efflux DTs, respectively) (Fig. 1) (Giacomini and Huang, 2013). Once a substrate enters the hepatocyte, it becomes available for metabolism by DMEs. DMEs are divided into two broad classes (i.e., phase I and phase II). Phase I enzymes catalyze oxidation, hydrolysis, and reduction reactions, whereas phase II enzymes carry out conjugation reactions (Lyubimov and Ortiz de Montellano, 2011).

Consequently, age-dependent variation in expression levels and activities of DTs and DMEs is one of the factors underlying variability in functional activities of DTs and DMEs and will influence homeostatic processes of endogenous substrates as well as pharmacokinetics (PK) and indirectly pharmacodynamics of drug substrates (Morrissey et al., 2013). A classic example is the case of fatal cardiovascular collapse (i.e., gray baby syndrome) due to toxic exposure to chloramphenicol in neonates as a result of underdevelopment of the phase II enzyme uridine 5-diphosphoglucuronic

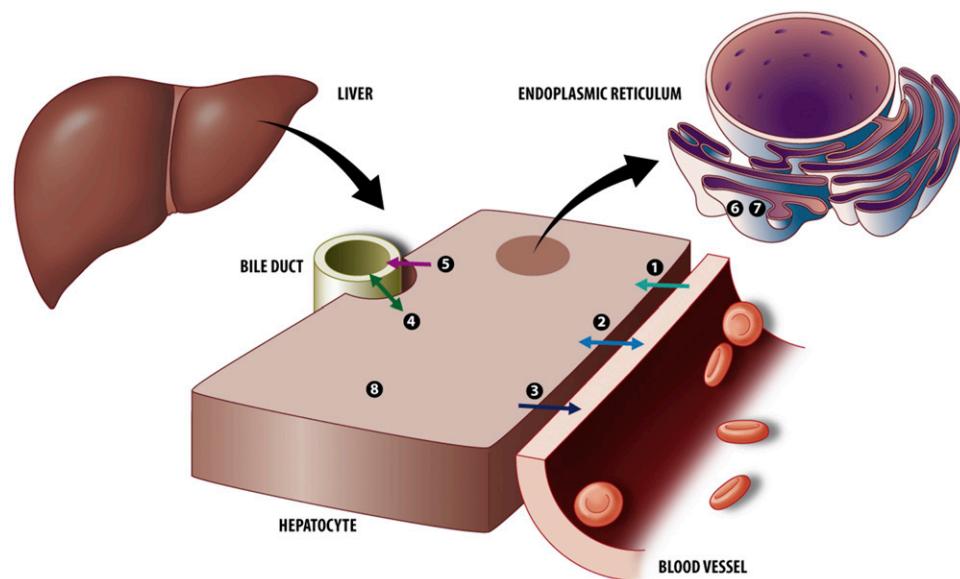


Fig. 1. Overview of localization of drug membrane transporters and drug-metabolizing enzymes in the hepatocyte. 1) Influx transporter (blood to hepatocyte), 2) bidirectional transporter (blood-hepatocyte), 3) efflux transporter (hepatocyte to blood), 4) canalicular bidirectional transporter (hepatocyte-bile), 5) canalicular efflux transporter (hepatocyte to bile), 6) phase I and 7) phase II drug-metabolizing enzymes, 8) cytosolic enzymes.

acid glucuronyltransferase (UGT) 2B7, mediating chloramphenicol glucuronidation (Weiss et al., 1960). More recently, DTs and their interplay with DMEs received attention in terms of drug disposition in children (Cheung et al., 2019). This is exemplified by the reduced hepatic clearance of the opioid morphine in newborns and young infants (Knibbe et al., 2009). Morphine is actively transported by the organic cation transporter 1 (OCT 1) (*SLC22A1*) and subsequently metabolized by UGT2B7. Significantly lower hepatic expression and activity of both OCT1 and UGT2B7 are reported in pediatric populations versus adults, which partly explains the relatively lower hepatic clearance of morphine in newborns and infants (Lu and Rosenbaum, 2014; Prasad et al., 2016; Hahn et al., 2019). Developmental changes in hepatic DTs and DMEs and their impact on drug disposition and toxicity have also been reported for nonclinical species. For instance, neonatal rat hepatocytes are less sensitive to hepatotoxicity of phalloidin than adult rat hepatocytes (Petzinger et al., 1979; Meier-Abt et al., 2004; Fattah et al., 2015). Phalloidin is a substrate for organic anion–transporting polypeptide (OATP) 1B2 (*SLCO1B2* gene) (Csanaky et al., 2011). Because expression of OATP1B2 is lower in neonatal than in adult rat hepatocytes, there is relatively lower uptake of phalloidin in neonatal hepatocytes, leading to reduced sensitivity to phalloidin hepatotoxicity (Belknap et al., 1981).

These examples show how age-related changes in DT and DME activities can impact drug disposition. At present, to determine many pediatric dosing regimens, still the standard approach is to linearly adjust the adult dose to that of a child based on the

child's body weight (Mahmood, 2016). However, this approach does not incorporate information on developmental physiology like age-related changes in DT and DME expression levels or activity and could therefore result in subtherapeutic or supratherapeutic doses. On the other hand, in silico methodologies, such as physiologically based pharmacokinetic (PBPK) models, allow integration of developmental changes of various aspects of PK and may improve prediction of pediatric drug disposition. The use of these in silico models has received much research interest in recent years (Johnson et al., 2014; Maharaj and Edginton, 2014), especially to better understand the effects of growth and maturation on drug disposition (Johnson et al., 2006; Krekels et al., 2012). However, the predictive performance is highly dependent on the availability and quality of the ontogeny profiles that are incorporated in these models (Zhou et al., 2018). Also, in terms of safety evaluation, extrapolation of drug disposition from nonclinical species to humans is common practice during drug development (Chen et al., 2012). This extrapolation relies heavily on our understanding of potential interspecies differences in ontogeny profiles of all pharmacological processes. More specifically, insights in the ontogeny profiles of DTs and DMEs across nonclinical species and humans may assist in selecting the appropriate juvenile animal model(s) for pediatric safety testing and to improve prediction of drug exposure in children.

Over the years, knowledge on developmental changes in hepatic DTs and DMEs in terms of mRNA expression levels, protein abundance, and functional activity has increased significantly (Cheung et al., 2019).

Currently, the data that describe the developmental patterns of hepatic DTs and DMEs are dispersed across individual publications with small sample sizes, largely because of scarcity of pediatric samples. Hence, the reported insights are limited and fragmented. Descriptive reviews are available in literature yet are limited to qualitative description of developmental patterns and include limited information on nonclinical species (Brouwer et al., 2015; Elmorsi et al., 2016). The process of compiling available quantitative information on maturation profiles of hepatic DTs and DMEs in nonclinical species and humans and incorporating this into PBPK models is expected to increase the predictive performance of PBPK models for age-dependent hepatic drug disposition. Therefore, we aimed to compile the hepatic ontogeny profiles of individual DTs and DMEs in human as well as nonclinical species from literature based on search results of available *in vitro* data of these proteins at the level of mRNA expression, protein expression, and activity.

II. Methods

The workflow of the employed methodology is outlined in Fig. 2. The subsequent steps are explained in more detail below.

A. Search Methods and Selection of Literature

PubMed was searched by appropriate search terms with Medical Subject Headings and free text terms (see

Supplemental Material 1) since creation up to June 2019. All articles were retained when they contained *in vitro* ontogeny data on DTs and DMEs, first based on the title or abstract and second based on the full text.

B. Raw Data Extraction, Normalization, and Pooling

We extracted the following data from the selected papers: age and the corresponding expression/activity levels, units of expression/activity level, method of quantification/semiquantification, race, sex, and substrate used to determine activity. The raw data were extracted from the selected articles and summarized in tables for individual isoforms. The data were subdivided by mRNA expression, protein abundance, and activity. Nonquantitative data (e.g., data obtained by immunoblotting) were also included in the raw data tables. If the raw data were not published in the article but only presented as graphs, they were extracted using Plotdigitizer. Data for individual studies were normalized to adult values, in which adult values were defined as 100%. This was followed by pooling data of the various studies.

C. Graphs

Based on the pooled raw data tables, graphs were generated for each isoform (mRNA expression, protein abundance, and/or activity). If multiple values were obtained for the same age, average \pm S.D. values were used. Data from various publications were only pooled

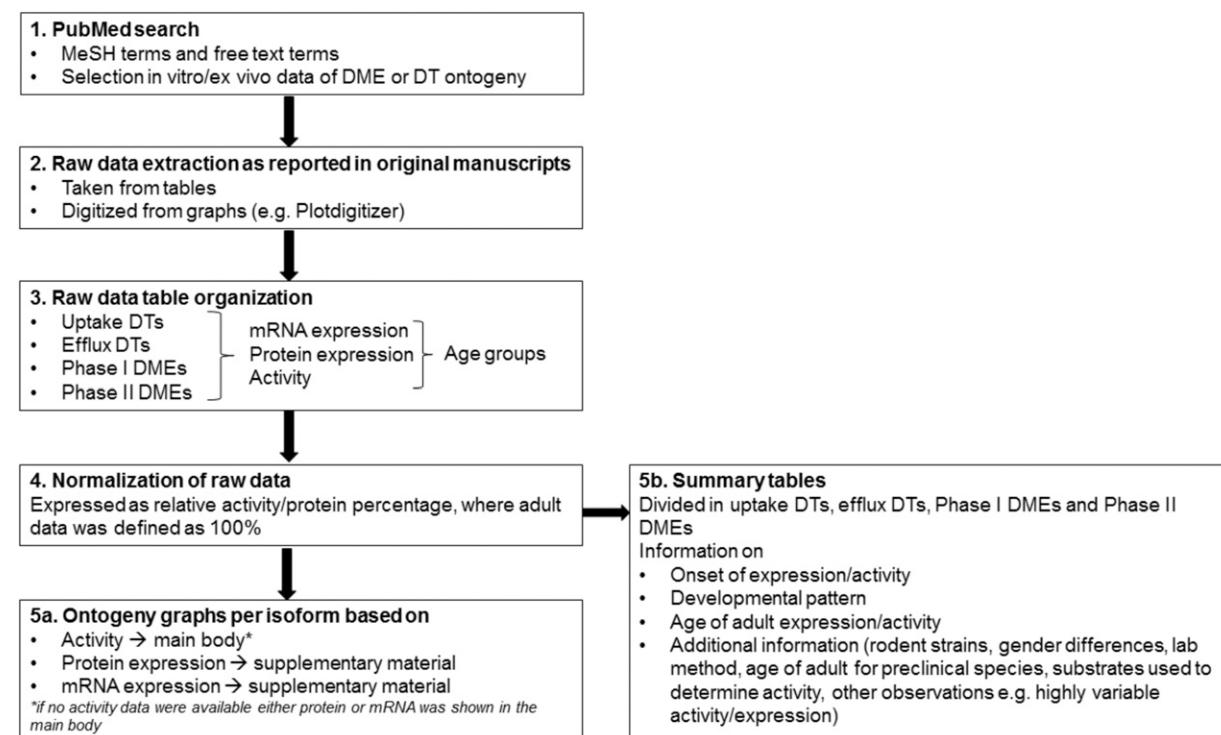


Fig. 2. Workflow and methodology of the search strategy.

when a similar developmental pattern was seen, and otherwise, individual developmental patterns obtained from separate publications were presented separately in the same graph. Graphs on the level of activity for a specific DT or DME are presented in the main body of the manuscript, and protein and mRNA expression graphs are included in the Supplemental Materials. In absence of activity data, protein expression graphs were presented in the main manuscript body as an alternative. In absence of both activity and protein expression data, mRNA expression graphs were presented in the main manuscript body.

D. Summary Tables

Based on the raw data tables containing quantitative and nonquantitative data and the graphs, a summary text table was created describing the onset of DT and DME expression/activity, the age at which adult expression was reached, and a description of the developmental pattern along with comments and references.

When feasible and depending on data availability, human pediatric samples were divided into subsets as defined by the International Conference on Harmonization E11 guidance (International Council for Harmonisation, 2000) as follows: neonates (birth to <28 days), infants (28 days to <2 years), young children (2 to <6 years), old children (6 to <12 years), adolescents (12–18 years), and adults (>18–65 years). For nonclinical species, age was presented on a continuous scale.

E. Results Section Text

Throughout the *Results* section, ontogeny profiles in the summary tables are classified in the following order: 1) age-related increase in expression/activities, 2) age-related decrease in expression/activities, 3) no age-related changes, or 4) a more complex ontogeny pattern in activity/expression. References to the individual studies are provided in the summary tables and are not included in the *Results* section. Also, because the tables provide supporting information for the individual graphs, graphs and tables should be used together. The figure legends contain references to the corresponding tables.

F. Nomenclature

Throughout the manuscript upper-case protein names of DTs and DMEs have been consistently used; they have also been used when mRNA expression levels of the DTs and DMEs are discussed. This approach has been adopted for humans as well as for rodent and nonrodent animal species. Supplemental Table 1 provides an overview of all included DT isoforms with their corresponding gene names.

III. Results

A. Uptake Transporters

1. Human. The results (including references) are included in Fig. 3, Supplemental Fig. 1, and Table 1.

a. Age-related increase in activity/expression. Sodium taurocholate cotransporting polypeptide (NTCP) showed a pronounced increase early in life. In fetal tissue, mRNA and protein expression levels were 3% and 6% of adult, respectively, reaching full maturation at the age of 28 days. OCT1, on the other hand, showed a more gradual increase in its expression, with 54% of adult protein expression levels in fetal tissue and adult values from adolescent age onward.

b. Age-related decrease in activity/expression. Three transporters showed high abundance in fetal tissue, with a subsequent decrease with increasing age. The glucose transporter 1 transporter showed the most distinct decrease, with 45-fold higher protein expression in fetal tissue than in adult tissue, whereas organic cation/carnitine transporter (OCTN) 2 expression appeared 2-fold higher in fetal tissue and decreased slowly toward adult levels during childhood. OATP1B1 is the third transporter that showed an overall decline in protein expression. However, the results on OATP1B1 protein expression were conflicting, as fetal values corresponding to 50%–165% of adult values were reported.

c. No age-related differences in activity/expression. Protein expression of the transporters monocarboxylate transporter 1, OATP2B1, and OATP1B3 did not show age-related changes. However, data on mRNA expression of OATP1B3 are conflicting, as one study reported no age-related changes, whereas another study found 5% in fetal tissue and 1% in infant tissue compared with adult values.

2. Rat. The results are depicted in Fig. 4, Supplemental Figs. 2 and S3, and Table 2.

a. Age-related increase in activity/expression. The literature contained activity data for four hepatic uptake transporter(s) (families) [i.e., OATP, NTCP, OCT1, and concentrative nucleoside transporter (CNT) 1/2] in juvenile rats. The ontogeny of other hepatic uptake transporters was established based on either protein expression levels [i.e., system A amino acid transporter (SNAT) 1] or mRNA expression levels (i.e., OATP1A1, OATP1A4/OATP2, OATP1A5, OATP1B2/OATP4, OATP2, OATP2B1, and OATP4A1)].

OATP and NTCP activity levels rose progressively from birth (10%–50%) to achieve maximal activity levels at 21 and 29 days of age in male rats based on the uptake activity levels using sodium fluorescein and taurocholate (1–200 μ M), respectively.

The ontogeny profiles of distinct OATP transporters were characterized based on protein and mRNA expression levels. Percentage of maximal

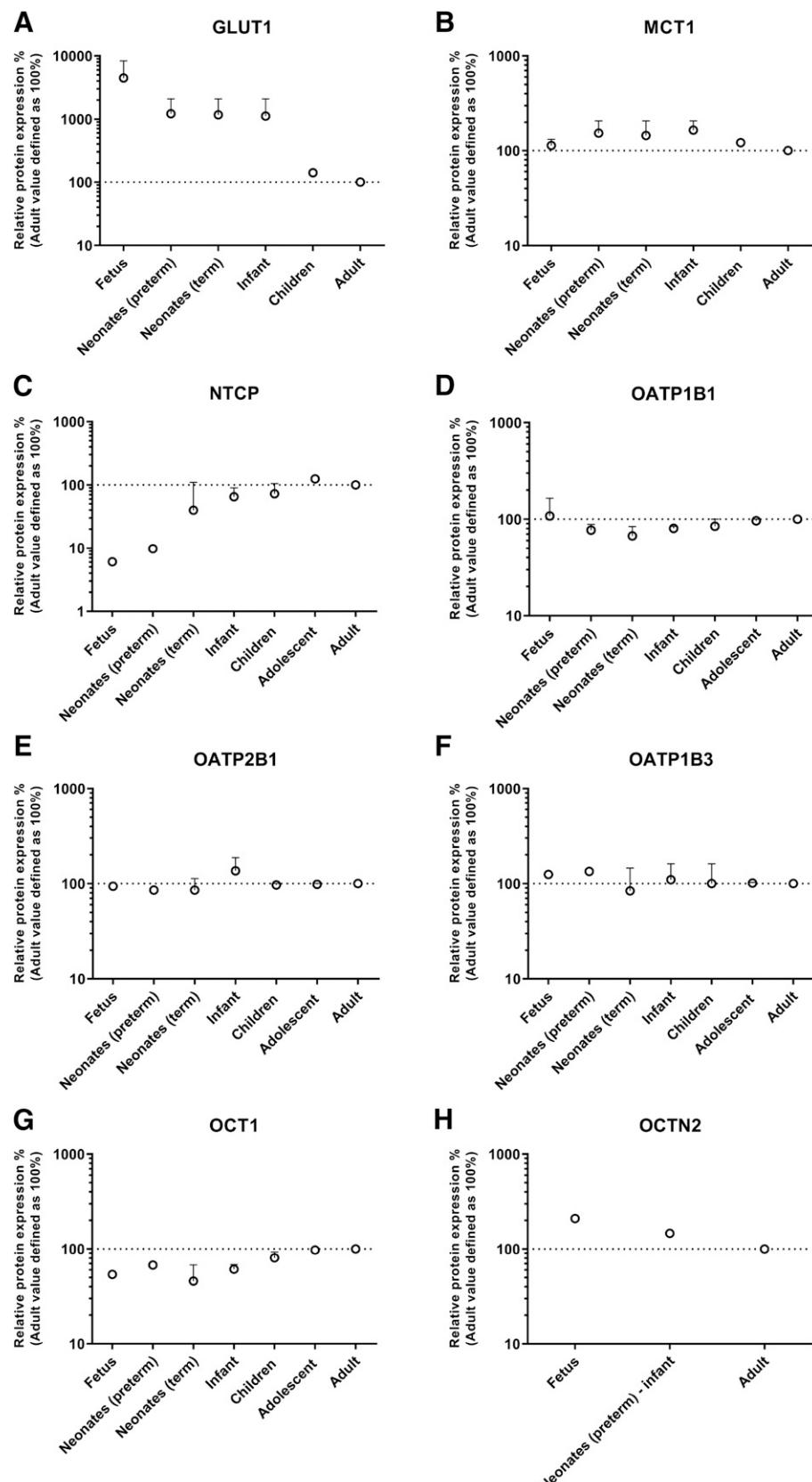


Fig. 3. Pooled literature data on the ontogeny of protein expression of hepatic uptake transporters in humans: GLUT1 (A), MCT1 (B), NTCP (C), OATP1B1 (D), OATP2B1 (E), OATP1B3 (F), OCT1 (G), and OCTN2 (H). The symbols represent the relative protein expression in each age group and the dotted line indicates the adult value defined as 100%. If multiple values were obtained for the same age group, the symbols represent the average relative protein expression, and the error bars show the S.D. See Table 1 for explanation on the ontogeny profiles and literature references. GLUT1, glucose transporter 1; MCT1, monocarboxylate transporter 1.

TABLE 1
Ontogeny profile of hepatic uptake transporters in humans based on protein expression and mRNA expression levels
Percentages represent expression/activity relative to adult levels.

Transporters	Onset of Expression/ Activity	Adult Levels Reached	Age-Related Changes (% of Adult) in Expression/Activity after Birth	Comments	References
GLUT1 Protein	Fetal development (4500%)	4 yr	Decreased progressively	Methods: LC-MS/MS mixed	Mooij et al. (2016); van Groen et al. (2018)
MCT1 Protein	Fetal development (100%)		No changes	Methods: LC-MS/MS	Mooij et al. (2016); van Groen et al. (2018)
NTCP Protein	Fetal development (6%)	28 days	Increased rapidly	Reported neonatal values are highly variable. Methods: Western blot (Yanni et al., 2011; Prasad et al., 2016; van Groen et al., 2018)	Yanni et al. (2011); Prasad et al. (2016); van Groen et al. (2018)
mRNA	Fetal development (3%)	NR	NR	Methods: RT-PCR	Chen et al. (2005); Sharma et al. (2013)
OATP1B1 Protein	Fetal development (50%–165%)	6–12 yr	Decreased slowly	The results are conflicting, as fetal values of 50%–165% of adult values were reported. Methods: LC-MS/MS (Mooij et al., 2016; Prasad et al., 2016; van Groen et al., 2018) and Western blot (Yanni et al., 2011; Thomson et al., 2016)	Yanni et al. (2011); Prasad et al. (2014, 2016); Mooij et al. (2016); van Groen et al. (2018)
mRNA OATP1B3 Protein	Fetal development (100%)	NR	Inconsistent pattern	Methods: RT-PCR	Sharma et al. (2013); Mooij et al. (2014)
mRNA	Fetal development (5%–100%)	NR (0–7 yr: 1%)	Decreased rapidly, increased rapidly	Methods: RT-PCR	Yanni et al. (2011); Prasad et al. (2014, 2016); van Groen et al., 2018) and Western blot (Yanni et al., 2011; Thomson et al., 2016)
OATP2B1 Protein	Fetal development (100%)		No changes	Methods: LC-MS/MS	Prasad et al. (2014, 2016); Mooij et al. (2016); van Groen et al. (2018)
OCT1 Protein	Fetal development (54%)	12–16 yr	Increased slowly	Methods: LC-MS/MS (Prasad et al., 2016; van Groen et al., 2018) and Western blot (Hahn et al., 2017)	Prasad et al. (2016); Hahn et al. (2017); van Groen et al. (2018)
OCTN2 Protein	Fetal development (210%)	NR	Decreased slowly	Methods: LC-MS/MS	Mooij et al. (2016)

GLUT1, glucose transporter 1; MCT1, monocarboxylate transporter 1; NR, not reported; RT-PCR, reverse-transcriptase polymerase chain reaction.

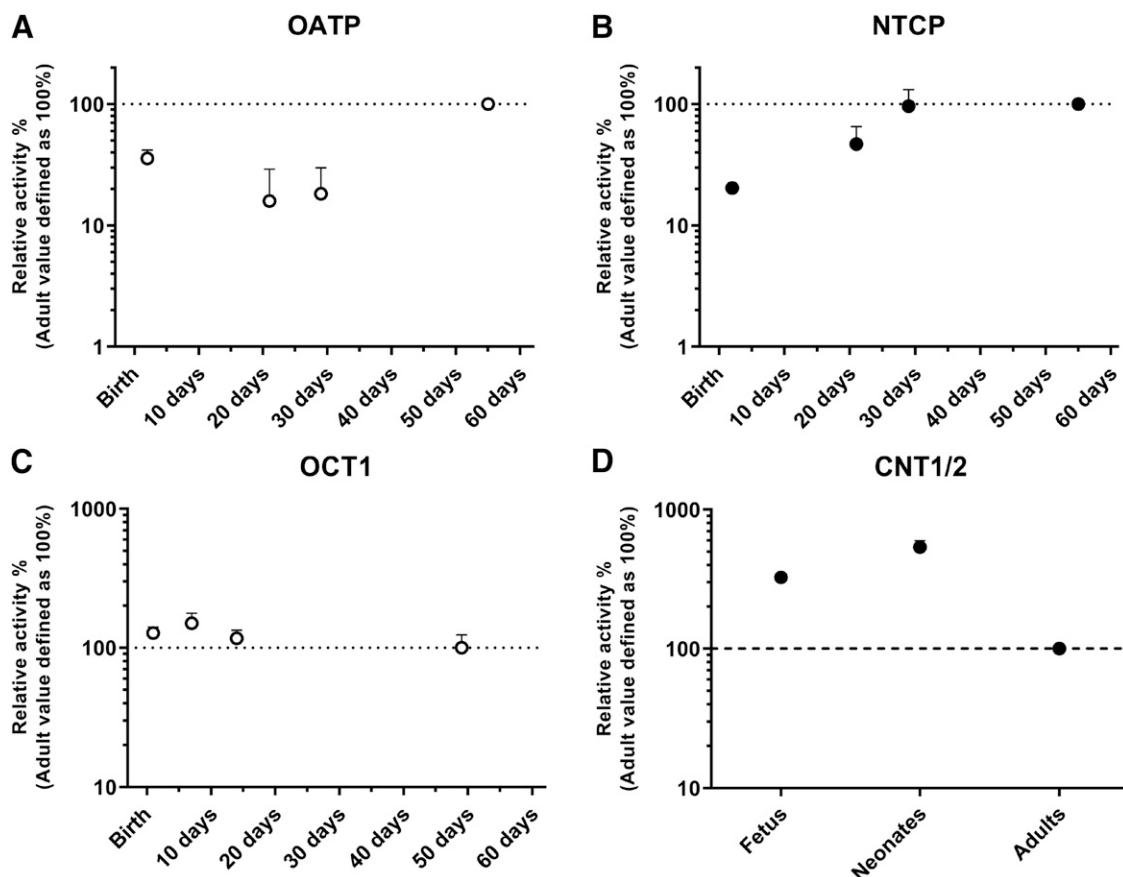


Fig. 4. Pooled literature data on activity levels of hepatic uptake transporters in rats: OATP (A), NTCP (B), OCT1 (C), and CNT1/2 (D). The symbols represent the relative activity in each age group, and the dotted line indicates the adult value defined as 100%. See Table 2 for explanation on the ontogeny profiles and literature references.

mRNA levels during fetal development compared with adults represented <10% for OATP1A1, OATP1A4, and OATP1B2; up to 30% for OATP1A5 and OATP2B1; and 100% for OATP4A1. Based on one study, OATP2 (OATP1A4) adult values were reached at 25 days (male) and 30 days (female). For the other OATP transporters, adult values were reached only at adulthood. Notably, a 55% decrease in protein abundances of OATP1A1, OATP1A4, and OATP1B2 in elderly rats was observed in comparison with those of adult levels.

b. Age-related decrease in activity/expression. The developmental patterns of CNT1/2 and SNAT1 suggested that CNT1/2 and SNAT1 are fetal uptake transporters. Maximal uptake activity levels of uridine mediated by CNT1/2 were achieved during fetal development (300% of adult levels) and then rapidly decreased in neonates (200% of adult levels). Similarly, fetal protein expression levels of SNAT1 were up to 2.5-fold and 5-fold greater than those reported in very young and adult rats, respectively.

c. No age-related differences in activity/expression. OCT1 activity was stable from birth to adulthood. This is supported by maximal uptake activity levels of OCT1 that were rapidly reached at 1 day after

birth using 1-methyl-4-phenylpyridinium as test substrate.

3. Mouse. The results are depicted in Fig. 5, Supplemental Fig. 4, and Table 3 and are further explained below.

a. Age-related increase in activity/expression. The majority of the uptake transporters showed a lower expression in younger versus older age groups. Most data were available for mRNA expression of the OATP family. OATP1A1 and OATP1A4 showed a slow rise in expression over age, whereas OATP1A6, OATP1B2, OATP2A1, and OATP2B1 increased more rapidly. The amount in fetal tissue was transporter-dependent (e.g., only 1% of adult values was detected for OATP1B2 compared with 32%–57% of adult values for OATP1A6). The OCT1 and apical sodium-dependent bile acid transporters both showed a very low expression in fetal tissue, with adult values reached at day 22 and at adult age, respectively.

b. Age-related decrease in activity/expression. The transporters that showed a decrease in expression all declined very rapidly from fetal age. OAT3 showed very high fetal levels, and adult levels were reached between day 25 and day 30. OCTN1 showed

TABLE 2
Ontogeny profile of hepatic uptake transporters in rats based on uptake activity, protein expression, and mRNA expression levels
Percentages represent expression/activity relative to adult levels.

NTCP	Onset of Activity and/or Expression	Adult Levels Reached	Age-Related Changes in Activity/Expression	Comments	References	
OCT1	Uptake activity mRNA expression	2 days: 10%–20% Fetal development: <36%	29 days Birth: 75% Increased rapidly during fetal development	Increased progressively	Adult age: 55 days. Fetal development: NR Substrate: ^3H -taurocholate. Strain and sex: Wistar (M). Matrix: hepatocytes in suspension. Methods: liquid scintillation counting Adult age: NR. Birth–adult: NR. Strain and sex: Wistar (F). Methods: RT-PCR	Fattah et al. (2015)
CNT1	Uptake activity Protein expression	1 day: 130% Fetal development: 35%–50%	1 days 21 days: 70% Peaked at 7 days (150%)	Increased slowly	Adult age: 49 days. Fetal development: NR. Substrate: $[^3\text{H}]$ -1-methyl-4-phenylpyridinium. Strain and sex: Wistar (M/F). Matrix: liver slices. Methods: liquid scintillation counting	Martel et al. (1998)
CNT2	Protein expression	Fetal development: 40%–70%	21 days: 70% Increased slowly	Increased slowly	Adult age: NR. Strain and sex: Wistar (F). Methods: Western blotting	del Santo et al. (2001)
CNT1/2	mRNA expression	Fetal development: 20%	45 days Increased slowly	Increased slowly	Adult age: NR. Substrate: uridine. Strain and sex: Wistar (F). Methods: Northern blotting	del Santo et al. (2001)
SNAT1	Uptake activity Protein expression	Fetal development: 300%	Fetal development Decreased rapidly in neonates (200%)	Decreased rapidly in neonates (200%)	Adult age: NR. Substrate: uridine. Strain and sex: Wistar (F). Matrix: hepatocytes in suspension. Methods: Radioactivity immunoblotting	del Santo et al. (2001)
OATPLA1	Protein expression	Fetal development: <2%	Fetal development: 300%–500%	NR Decreased in elderly M: 800 days (40%). F: 800 days (10%) Increased slowly then decreased in elderly	Adult age: 60 days. Fetal development–adult: NR. Strain and sex: Sprague Dawley (M/F). Adult age: 56–70 days. Strain and sex: Sprague Dawley (M/F). Methods: RT-PCR	Hou et al. (2014)
OATPLA4	Protein expression	Birth: 15%–40%	28 days: 40%; M: 25 days; F: 30 days	Increased slowly then decreased in elderly or increased progressively with no changes after 25 days	Adult age: 60 days. Strain and sex: Sprague Dawley (M/F). Methods: Western blotting	Guo et al. (2002); Hou et al. (2014)
mRNA expression	Fetal development: <2%; Birth: 30%–76%	M: 10–35 days (65%). F: 25–35 days (20%)	Interstrain variability: increased slowly then decreased in elderly, or increased rapidly (M) v. progressively (F)	Adult age: 45–60 days. Strain and sex: Sprague Dawley (M/F). Methods: RT-PCR, branched DNA signal amplification assay	Guo et al. (2002); St-Pierre et al. (2004); Macias et al. (2011); Hou et al. (2014)	
OATPLA5	mRNA expression	Fetal development: <30%	35 days: 65%–70%	Increased slowly then decreased in elderly	Adult age: 60 days. Strain and sex: Sprague Dawley (M/F). Methods: RT-PCR	Hou et al. (2014)
OATP1B2	Uptake activity	2 days: 25%	55 days	Some decrease between birth and weaning (10% at 3 wk) then rapid increase to adult values	Adult age: 55 days. Fetal development: NR. Substrate: sodium fluorescein. Strain and sex: Wistar (M). Matrix: hepatocytes in suspension. Methods: fluorescence spectrometry	Fattah et al. (2015)
Protein expression	mRNA expression	NR Fetal development: <10%; birth: 20%	M: 28 days (50%). F: 28 days (70%) M: 35 days (78%). F: 60 days	Increased slowly then decreased in elderly M: Increased progressively. F: Increased slowly. Decreased in elderly	Adult age: 60 days. Fetal development – 28 days: NR Strain and sex: Sprague Dawley (M/F) Adult age: 45–60 days. Strain and sex: Sprague Dawley and Wistar (M/F). Methods: RT-PCR, branched DNA signal amplification assay	Li et al. (2002); St-Pierre et al. (2004); Macias et al. (2011); Hou et al. (2014)

(continued)

TABLE 2—Continued

	Onset of Activity and/or Expression	Adult Levels Reached	Age-Related Changes in Activity/Expression	Comments	References
OATP2B1 mRNA expression	Fetal development: <16%	Birth: 20%	NR	Adult age: NR. Birth–adult: NR. Strain and sex: Wistar (M/F), St-Pierre et al. (2004) Methods: RT-PCR	St-Pierre et al. (2004)
OATP4A1 mRNA expression	Fetal development: 255%	Fetal development	Increased rapidly at birth (310%)	Adult age: NR. Birth–adult: NR. Strain and sex: Wistar (M/F), St-Pierre et al. (2004) Methods: RT-PCR	St-Pierre et al. (2004)

F, female; M, male; NR, not reported; RT-PCR, reverse-transcriptase polymerase chain reaction.

similarly high fetal values, which were followed by a more gradual decrease toward adult values.

c. *Complex and/or inconsistent ontogeny pattern in activity/expression.* For NTCP, mRNA expression data from four studies as well as protein expression data from one study were available. mRNA expression was clearly present in fetal tissue. No distinct ontogeny profile could be defined, as the studies reported varying results. The transporters equilibrative nucleoside transporter (ENT) 1, OAT2, and OCTN2 fluctuated between age groups because they had an overall increased developmental pattern from fetal age onward but also showed a decrease in expression between various age groups. ENT3 had relatively high fetal levels, and adult levels were reached at day 25.

4. *Nonrodents.* Data on ontogeny profiles of hepatic uptake transporters were lacking for nonrodent species, including Beagle dog, cynomolgus monkey, Göttingen Minipig, and the domestic pig.

B. Efflux Transporters

1. *Human.* The results are depicted in Fig. 6, Supplemental Fig. 5, and Table 4 and are further explained below.

a. *Age-related increase in activity/expression.* Most efflux transporters showed a developmental pattern with rise in expression with increasing age. For all studies that included fetal tissue, the transporters were detectable at fetal age, yet maturation rates differed. For example, bile salt export pump (BSEP), multidrug resistance-associated protein (MRP) 3, and multi-resistance protein (MDR) 1 (or P-glycoprotein) showed a rapid increase from fetal tissue to neonatal tissue. In contrast, MRP1 and MRP2 increased more gradually, with 50% of adult values in neonates and 30%–100% of adult values in infants, respectively. Data on MDR3 were scarce, but fetal tissue showed 6% of adult values.

b. *Complex and/or inconsistent ontogeny pattern in activity/expression.* The breast cancer resistance protein (BCRP) transporter ontogeny is well described in literature, yet results are inconsistent. Fetal values were reported to be between 94% and 235%, and adult values were likely reached at neonatal age. However, higher values than those of adults were detected at infant age, which decrease again thereafter to adult values.

2. *Rat.* The results are depicted in Fig. 7, Supplemental Fig. 6, and Table 5 and are further explained below.

a. *Age-related increase in activity/expression.* No activity data for hepatic efflux transporters in juvenile rats were found in literature. The ontogeny of hepatic efflux transporters was established based on either protein expression levels (MRP2 and BSEP) or mRNA expression levels (MRPs, BCRP, and BSEP).

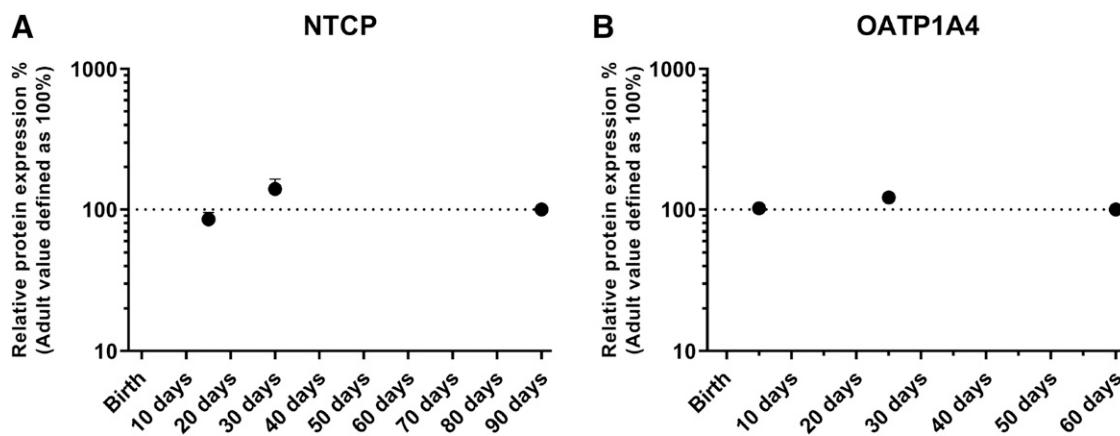


Fig. 5. Pooled literature data on the ontogeny of protein expression levels of hepatic uptake transporters in mice: NTCP (A) and OATP1A4 (B). The symbols represent the relative protein expression in each age group, and the dotted line indicates the adult value defined as 100%. If multiple values were obtained for the same age group, the symbols represent the average relative protein expression, and the error bars show the S.D. See Table 3 for explanation on the ontogeny profiles and literature references.

Protein and mRNA expression levels of BSEP represented <10% of adult levels during fetal development, suggesting an onset of expression after birth. Maturation of BSEP rapidly increased after birth, with 50% of maximal protein expression levels achieved at 1 day and maximal levels achieved at 21 days in rats. Onset of MRP2 and MRP3 expression started during fetal development as fetal mRNA expression levels reached up to 60% of maximal levels in female and male rats. However, maximal levels of MRP2 were achieved at different maturational age: within the first week in male rats and at adulthood in female rats.

b. Age-related decrease in activity/expression. Adult mRNA expression levels of MRP1 and BCRP were high during the fetal development and decreased progressively to adult levels after birth.

c. Complex and/or inconsistent ontogeny pattern in activity/expression. mRNA expression levels of MRP6 corresponded to 20% and 40% of adult levels during fetal development and at birth, respectively. mRNA expression levels of MRP4 rose progressively to reach adult levels at 28 days in male rats, whereas no changes in mRNA expression levels were observed in female rats. Fetal expression levels of MRP4 ranged from 30% in male rats to more than 200% in female rats.

3. Mouse. The results are depicted in Fig. 8, Supplemental Fig. 7, and Table 6 and are further explained below.

a. Age-related increase in activity/expression. The mRNA expression of BSEP and MRP2 is well studied compared with other transporters. Both transporters showed 30% expression in fetal tissue compared with adults and an increase in expression up to birth, with higher expression than adult levels for BSEP and similar expression to adult levels for MRP2. After birth, the expression of both transporters fluctuated until adult values were reached at adult age, with 46%–153% for BSEP and 40%–90% for MRP2.

Interestingly, not only BSEP and MRP2 had $\pm 30\%$ expression at fetal age, as this was also observed for MRP3 and MRP6. MRP3 expression showed a gradual increase from fetal to adult levels, whereas MRP6 mean adult levels were reached at day 3.

In one study, mRNA expression of MDR2/3, sodium-dependent phosphate transporter 1 (NPT1), and multidrug and toxin extrusion 1 (MATE1) was measured by RNA sequencing (Cui et al., 2012a). Both MATE1 and NPT1 showed a steep increase in expression from birth to day 1 with a more gradual increase thereafter.

b. Age-related decrease in activity/expression. The mRNA expression of MRP1 and MRP5 was measured by RNA sequencing in one study (Cui et al., 2012a) and showed high expression in fetal tissue (558%–1200% of adult values), which was followed by an overall decrease in expression. The age at which adult values were reached was transporter-dependent and varied between day 25 and day 30.

For MRP4, a high mRNA expression in fetal tissue (200%) was captured, and a more rapid decrease in expression was observed, reaching adult values at day 10. Interestingly, there was a slightly higher expression in females than in males.

c. No age-related differences in activity/expression. Protein and mRNA expression of MDR1b showed no age-related changes.

d. Complex and/or inconsistent ontogeny pattern in activity/expression. MDR1 [ATP-binding cassette (ABCB1)] expression was higher in fetal tissue than at birth, and the overall developmental pattern showed an increase from birth up to 15 days of age (160%–250%) with a subsequent decrease up to adult age. In addition, for MDR2/3 a complex pattern was observed. Onset of expression was in fetal tissue and increased to 156% at birth. At day 5, a steep decrease to 48% was observed, and this was followed by an increase to adult levels at day 30. For the ABCG5 and the ABCG8 transporter,

Ontogeny of Hepatic Transport and Drug Metabolism

TABLE 3
Ontogeny profile of hepatic uptake transporters in mice based on protein expression and mRNA expression levels

Uptake Transporters	Onset of Expression/Activity	Adult Levels Reached	Age-Related Changes (% of Adult) in Expression/Activity after Birth	Comments	References
ASBT mRNA expression	Fetal development (0.17%)	NR	Increased rapidly	Adult age: 60 days. Strain: C57BL/6J (M). Methods: RNA-seq	Cui et al. (2012a)
ENT1 mRNA expression	Fetal development (>100%)	15 days	Inconsistent pattern	Statistical differences in expression between CV and GF strains (Selwyn et al., 2015). Adult age: 60 days (Cui et al., 2012a) and 90d ⁴³ . Strain: C57BL/6J (Cui et al., 2012a) and CV + GF (Selwyn et al., 2015) (M). Methods: RNA-seq (Cui et al., 2012a) and RT-PCR ⁴³	Cui et al. (2012a); Selwyn et al. (2015)
ENT3 mRNA expression	Fetal development (23%)	25 days	Inconsistent pattern	Adult age: 60 days. Strain: C57BL/6J (M). Methods: RNA-seq	Cui et al. (2012a)
NTCP mRNA expression	Fetal development (17%)	F: 30 days. M: 0 days	Inconsistent pattern	Adult age: 56 (Cheng et al., 2007), 60 days (Cui et al., 2012a), 90d ⁴³ . Strain: C57BL/6J (Cheng et al., 2007; Cui et al., 2012a) (mixed) and CV + GF (Selwyn et al., 2015) (M). Methods: bDNA (Cheng et al., 2007), RNA-seq (Cui et al., 2012a) and RT-PCR ⁴³ .	Cheng et al. (2007); Cui et al. (2012a); Selwyn et al. (2015)
Protein expression	NR (15 days: CV 95%; GF 75%)	NR	Inconsistent pattern	Statistical differences in expression between CV and GF strains (Selwyn et al., 2015). Adult age: 90 days. Strain: CV + GF (M). Methods: Western blotting	Selwyn et al., (2015)
OAT2 mRNA expression	1 days (0.6%)	60 days	Increased progressively	Adult age: 60 days. Strain: C57BL/6J (M). Methods: RNA-seq	Cui et al. (2012a)
OAT3 mRNA expression	Fetal development (48372%)	25–30 days	Decreased progressively	Adult age: 60 days. Strain: C57BL/6J (M). Methods: RNA-seq	Cui et al. (2012a)
OATP1A1 mRNA expression	Fetal development (0.01%–0.77%)	30–40 days	Increased slowly	Adult age: 45 days (Cheng et al., 2005), 60 days (Cui et al., 2012a), 90 days (Selwyn et al., 2015). Strain: C57BL/6J (Cheng et al., 2005; Cui et al., 2012a) (mixed) and CV + GF (Selwyn et al., 2015) (mixed). Methods: RNA-seq (Cui et al., 2012a) and RT-PCR (Cheng et al., 2005; Cui et al., 2012a) (Selwyn et al., 2015)	Cheng et al. (2005); Cui et al. (2012a); Selwyn et al. (2015)
OATP1A4 mRNA expression	M: fetal development (35%). F: fetal development (4%)	M: 5–10 days. F: 23 days	Increased slowly	Adult age: 45 days (Cheng et al., 2005) and 60 days (Cui et al., 2012a); Li et al., 2016. Strain: C57BL/6 (mixed). Methods: bDNA (Cheng et al., 2005; RNA-seq (Cui et al., 2012a), and RT-PCR (Cui et al., 2012a; Li et al., 2016).	Cheng et al. (2005); Cui et al. (2012a); Li et al. (2016)
Protein expression	NR	5 days	NR	Adult age: 60 days. Strain: C57BL/6 (M). Methods: Western blotting	Li et al. (2016)
OATP1A6 mRNA expression	M: fetal development (57%). F: fetal development (32%)	M: 5 days. F: 10–15 days	Increased slowly	Adult age: 45 days. Strain: C57BL/6 (mixed). Methods: bDNA	Cheng et al. (2005)
OATP1B2 mRNA expression	Fetal development (1%)	60 days	Increased slowly	Adult age: 45 days (Cui et al., 2012a) and 90d ⁴³ . Strain: C57BL/6J (Cui et al., 2012a) and CV + GF (Selwyn et al., 2015) (M). Methods: RNA-seq (Cui et al., 2012a) and RT-PCR ⁴³	Cui et al. (2012a); Selwyn et al. (2015)
OATP2A1 mRNA expression	Fetal development (21%–135%)	23 days	Increased slowly	Adult age: 45 days (Cheng et al., 2005), 60 days (Cui et al., 2012a). Strain: C57BL/6J (Cheng et al., 2005; Cui et al., 2012a) (mixed). Methods: RNA-seq (Cui et al., 2012a) and RT-PCR (Cheng et al., 2005; Cui et al., 2012a)	Cheng et al. (2005); Cui et al. (2012a)
OATP2B1 mRNA expression	Low expression in fetal tissue	23 days	Increased slowly	Adult age: 45 days (Cheng et al., 2005), 60 days (Cui et al., 2012a), 90 days (Selwyn et al., 2015). Strain: C57BL/6J (Cheng et al., 2005; Cui et al., 2012a) (mixed) and CV + GF (Selwyn et al., 2015) (mixed). Methods: RNA-seq (Cui et al., 2012a) and RT-PCR (Cui et al., 2012a), (Cheng et al., 2005) (Selwyn et al., 2015)	Cheng et al. (2005); Cui et al. (2012a); Selwyn et al. (2015)

(continued)

TABLE 3—Continued

Uptake Transporters	Onset of Expression/Activity	Adult Levels Reached	Age-Related Changes (% of Adult) in Expression/Activity after Birth	Comments	References
OCT1 mRNA	M: fetal development (0.05%); F: ND	M: 15–22 days; F: 22–30 days	Increased progressively	Adult age: 45 days (Anoniti et al., 2006), 60 days (Cui et al., 2012a). Strain: C57BL/6J (mixed). Methods: RNA-seq (Cui et al., 2012a) and RT-PCR (Ahnouti et al., 2006; Cui et al., 2012a)	Ahnouti et al. (2006); Cui et al. (2012a)
OCTN1 mRNA	Fetal development expression (1578%–1975%)	60 days	Decreased progressively	Adult age: 60 days (Cui et al., 2012a) and 90 days (Selwyn et al., 2015). Strain: C57BL/6J (Cui et al., 2012a) and CV + GF (Selwyn et al., 2015) (M). Methods: RNA-seq (Cui et al., 2012a) and RT-PCR (Selwyn et al., 2015)	Cui et al. (2015)
OCTN2 mRNA	Fetal development expression (20%–50%)	1 days	Inconsistent pattern	Adult age: 60 days. Strain: C57BL/6J (M). Methods: RNA-seq	Cui et al. (2012a)

ASBT, apical sodium-dependent bile acid transporter; bDNA, branched DNA signal amplification assay; F, female; M, male; ND, not detectable; NR, not reported; RNA-seq, RNA sequencing; RT-PCR, reverse-transcriptase polymerase chain reaction.

protein expression levels were available for mice at days 15, 30, and 90, and statistical difference in expression was found between the strains. For both transporters, protein expression was very high at day 15, and a distinct decrease was captured thereafter. This was supported by mRNA expression data that also showed a decrease in expression from day 15 onward. Interestingly, mRNA expression was available from younger mice, and an increase was seen from fetal tissue up to day 15. The mRNA expression of the copper-transporting P-type ATPase (ATP) 7B and BCRP (ABCG2) showed high expression in fetal tissue (280% of adult values) followed by an overall decrease in expression. The age at which adult values were reached was transporter-dependent and varied between day 25 and adult age.

4. *Nonrodents.* Data on ontogeny profiles of hepatic efflux transporters were lacking for the Beagle dog, cynomolgus, monkey and domestic pig. For the Göttingen minipig, a semiquantitative assessment of P-glycoprotein showed no difference between livers from 84 days of gestation versus adult animals (1.5–3 years of age).

C. Phase I Drug-Metabolizing Enzymes

1. *Human.* The results are depicted in Fig. 9, Supplemental Figs. 9 and S10, and Table 7 and are further explained below.

a. *Age-related increase in activity/expression.* Except cytochrome P450 (CYP) enzymes, very few data about ontogeny of phase I enzymes in human liver could be found in literature. Among the non-CYP phase I enzymes, all showed a lower expression in neonates and pediatrics than in adults (ADH1A, ADH1B, ADH1C, ALDH1A1, CES1, CES2, and FMO3). Activity was reported only for CES1 and was lower in neonates and pediatrics than in adults. For most of the CYP enzymes, data on catalytic activity in various age groups were available. The isoforms CYP2D6, CYP2E1, and CYP1A2 had low activity during fetal age <30 weeks of gestation and reached adult levels between neonatal and infant age. For CYP2C18, only data on fetal age >30 weeks of gestation were available, showing a low mRNA expression that reached adult levels between neonatal and infant age. The best-characterized CYP enzyme in terms of ontogeny is CYP2D6, for which onset of expression and activity is captured during fetal life, with a rapid increase during neonatal development. The patterns for CYP2D6 activity, protein expression, and mRNA expression lack similarity other than that they all increase with increasing age. Similar to CYP2D6, CYP1A2 showed very low activity and protein/mRNA expression in fetuses. Adult values of activity and protein expression were reached at 5–15 years and 1–5 years of age, respectively. The catalytic activity of CYP2E1 showed low activity in fetuses <30 weeks of gestation (3%–20% of adult values) and increased to

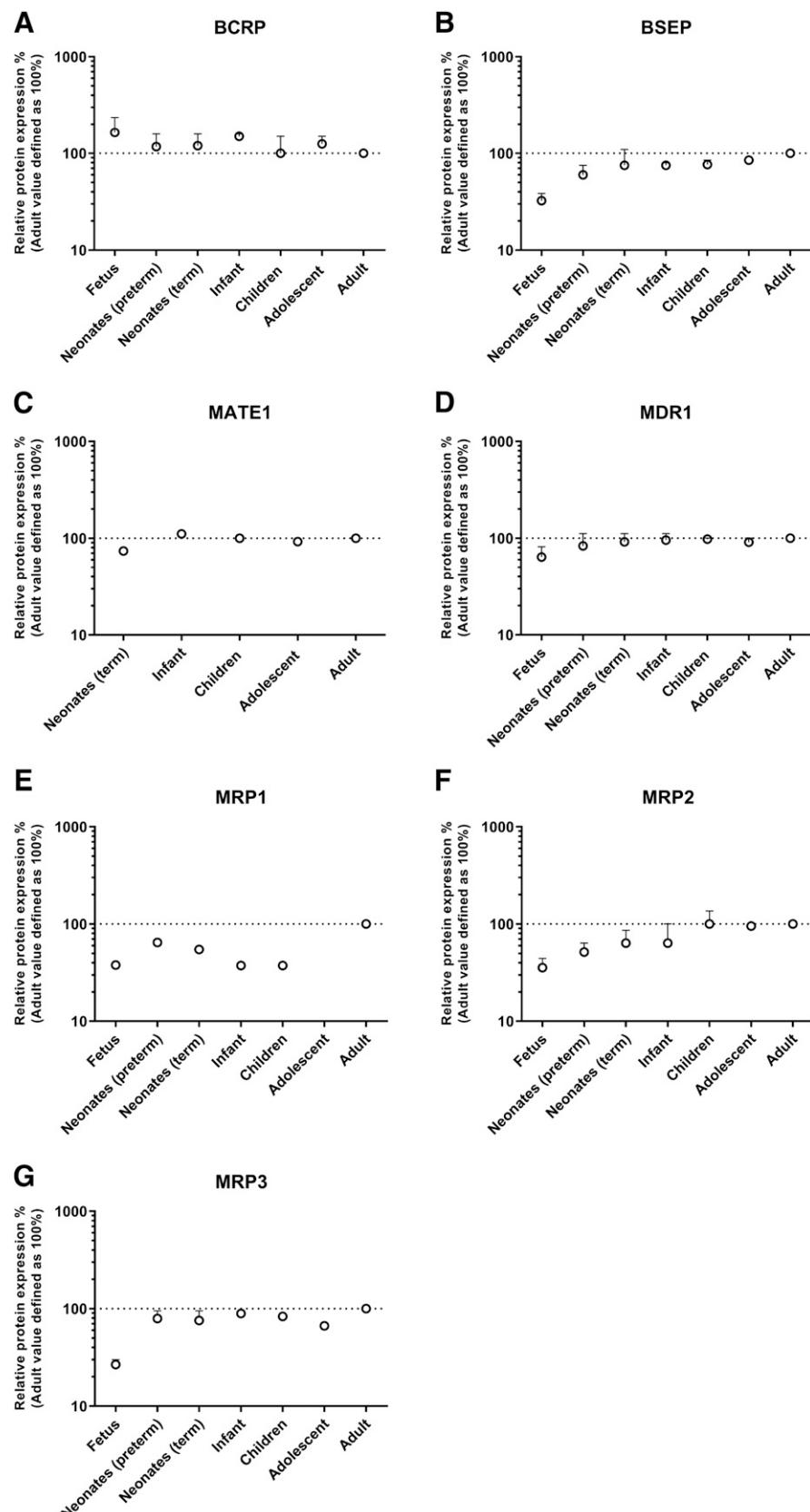


Fig. 6. Pooled literature data on the ontogeny of protein expression of hepatic efflux transporters in humans: BCRP (A), BSEP (B), MATE1 (C), MDR1 (D), MRP1 (E), MRP2 (F), and MRP3 (G). The symbols represent the relative protein expression in each age group, and the dotted line indicates the adult value defined as 100%. If multiple values were obtained for the same age group, the symbols represent the average relative protein expression, and the error bars show the S.D. See Table 4 for explanation on the ontogeny profiles and literature references.

TABLE 4
Ontogeny profile of hepatic efflux transporters in humans based on protein and mRNA expression levels

Transporters	Onset of Expression/Activity	Adult Levels Reached	Age-Related Changes (% of Adult) in Expression/Activity	Comments	References
MATE1 Protein	NR (28 days: 74%)	29 days–1yr	Increased slowly	Methods: LC-MS/MS	Prasad et al. (2016)
MDR1 Protein	Fetal development (46%–81%)	28 days	Increased slowly	Wide variety in values from various publications. Methods: LC-MS/MS (Prasad et al., 2014, 2016; Mooij et al., 2016; van Groen et al., 2018), immunohistochemistry (Cizkova et al., 2005; Konieczna et al., 2011; Abanda et al., 2017), and Western blot (Tang, 2007; Konieczna et al., 2011; Abanda et al., 2017)	Cizkova et al. (2005); Tang (2007); Konieczna et al. (2011); Prasad et al. (2014, 2016); Mooij et al. (2016); van Groen et al. (2018); Abanda et al. (2017); van Kalken et al. (1992); Fakhoury et al. (2009); Sharma et al. (2013); Mooij et al. (2014); Abanda et al. (2017)
mRNA	Fetal development (4%–64%)	NR (0–17 yr: 51%)	Increased slowly	Wide variety in values from various publications. Methods: RT-PCR (Fakhoury et al., 2009; Sharma et al., 2013; Mooij et al., 2014; Abanda et al., 2017) and immunohistochemistry (van Kalken et al., 1992)	van Kalken et al. (1992); Fakhoury et al. (2009); Sharma et al. (2013); Mooij et al. (2014); Abanda et al. (2017)
MRP1 Protein	Fetal development (38%)		Increased slowly	Methods: LC-MS/MS ^{b4} and Western blot ⁷¹	Konieczna et al. (2011); van Groen et al. (2018)
MRP2 Protein	Fetal development (27%–44%)	29 days–1 yr	Increased slowly	Methods: LC-MS/MS (Deo et al., 2012; Mooij et al., 2016; Prasad et al., 2016; van Groen et al., 2018), immunohistochemistry (Chen et al., 2005; Cizkova et al., 2005), and Western blot (Tang, 2007)	Chen et al. (2005); Cizkova et al. (2005); Deo et al. (2012); Mooij et al. (2016); van Groen et al. (2018)
mRNA	Fetal development (3%–50%)	NR	Inconsistent pattern	Methods: RT-PCR (Chen et al., 2005; Sharma et al., 2013; Mooij et al., 2014)	Prasad et al. (2016); Klaassen and Aleksunes (2010); Chen et al. (2005); Klaassen and Aleksunes (2010); Sharma et al. (2013); Mooij et al. (2014)
MRP3 Protein	Fetal development (30%)	28 days	Increased rapidly	Methods: LC-MS/MS (Mooij et al., 2016; Prasad et al., 2016; van Groen et al., 2018) and Western blot (Yanni et al., 2011)	Chen et al. (2005); Klaassen and Aleksunes (2010); Sharma et al. (2013)
BSEP Protein	Fetal development (30%)	28 days	Increased progressively	Methods: LC-MS/MS (Mooij et al., 2016; Prasad et al., 2016; van Groen et al., 2018), Western blot (Yanni et al., 2011), and immunohistochemistry	Yanni et al. (2011); Mooij et al. (2011); Chen et al. (2005); Yanni et al. (2011); Mooij et al. (2016); Prasad et al. (2016); van Groen et al. (2018)
mRNA	Fetal development (40%)	NR	NR	Methods: RT-PCR (Chen et al., 2005; Sharma et al., 2013)	Chen et al. (2005); Klaassen and Aleksunes (2010); Sharma et al. (2013)
BCRP Protein	Fetal development (94%–255%)	28 days	Decreased progressively	Methods: LC-MS/MS (Prasad et al., 2013; Mooij et al., 2016; Prasad et al., 2016; van Groen et al., 2018), Western blot (Yanni et al., 2011), and immunohistochemistry (Konieczna et al., 2011)	Konieczna et al. (2011); Yanni et al. (2011); Prasad et al. (2013); Mooij et al. (2016); Prasad et al. (2016); van Groen et al. (2018)
MDR3 mRNA	Fetal development (6%)	NR	NR	Methods: RT-PCR	Chen et al. (2005); Klaassen and Aleksunes (2010)

NR, not reported; RT-PCR, reverse-transcriptase polymerase chain reaction.

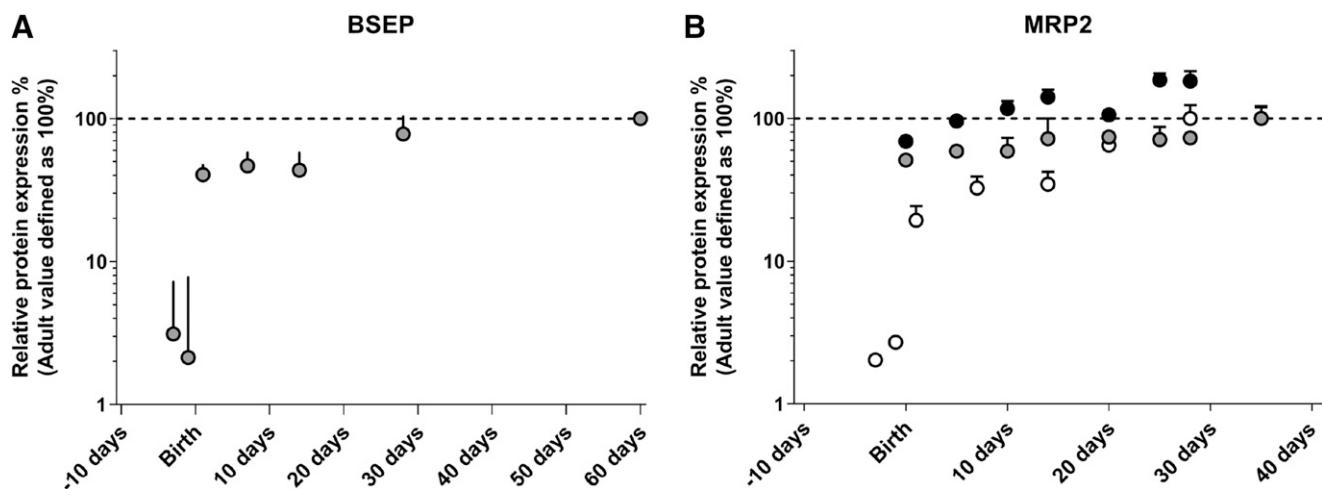


Fig. 7. Pooled literature data on the ontogeny of protein expression levels of hepatic efflux transporters in rats: BSEP (A) and MRP2 (B). The symbols represent the relative protein expression in mixed-sex rat population (open), male rats (gray), and female rats (black). If multiple values were obtained for the same age group, the symbols represent the average relative protein expression, and the error bars show the S.D. See Table 5 for explanation on the ontogeny profiles and literature references.

50% activity of adult values in infants. The results on protein expression show a wide interstudy range in fetal CYP2E1 expression (1.5%–70% of adult values), and adult values were reached at 1–5 years of age. The pattern of low activity/protein expression in fetuses is

also seen for mRNA expression, with concordant expression of 50% in infants.

For the isoforms CYP3A4, CYP2A6, and CYP2C8, a more gradual increase in activity and expression was seen. The CYP3A family consists of the isoforms

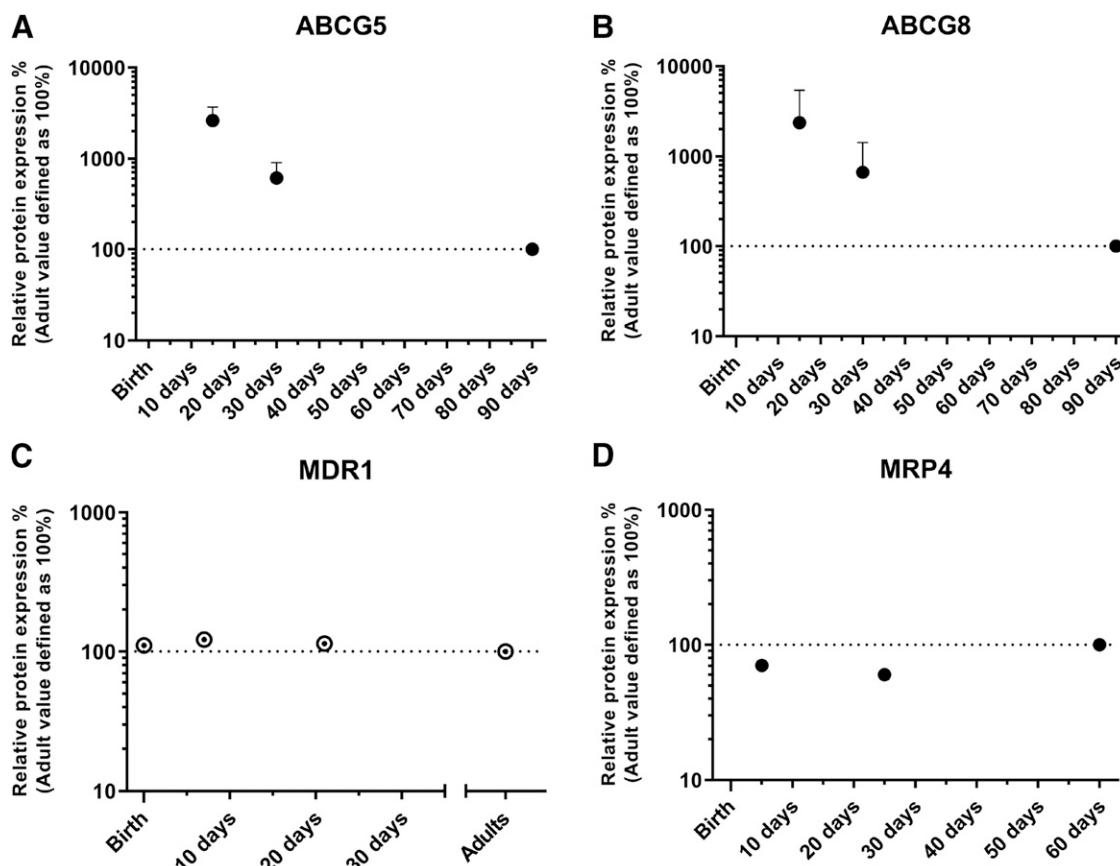


Fig. 8. Pooled literature data on the ontogeny of protein expression levels of hepatic efflux transporters in mice: ABCG5 (A), ABCG8 (B), MDR1 (C), and MRP4 (D). The symbols represent the relative protein expression in each age group, and the dotted line indicates the adult value defined as 100%. If multiple values were obtained for the same age group, the symbols represent the average relative protein expression, and the error bars show the S.D. See Table 6 for explanation on the ontogeny profiles and literature references.

TABLE 5
Ontogeny profile of hepatic efflux transporters in rats based on efflux activity, protein expression, and mRNA expression levels

Percentages represent expression/activity relative to adult levels.

	Onset of Activity and/or Expression	Adult Levels Reached	Age-Related Changes in Activity/Expression	Comments	References
BSEP	Fetal development: $<3\%$	28 days: 78%	Increased slowly	Adult age: NR. Strain and sex: Sprague Dawley (F). Methods: Tomer et al. (2003); Western blotting	
Protein expression	Fetal development: $<10\%$	21 days	Increased progressively	Adult age: 60 days. Strain and sex: Sprague Dawley (F) and Wistar (M/F). Methods: RT-PCR, Northern blotting	Zinchuk et al. (2002); Tomer et al. (2003); St-Pierre et al. (2004)
mRNA expression					
MRP1	Fetal development: $300\%-500\%$	Fetal development	Decreased rapidly (14 days: 200%)	Adult age: 60 days. Strain and sex: Sprague Dawley and Wistar (M/F). Methods: RT-PCR	St-Pierre et al. (2004); Zhu et al. (2017)
Protein expression	Birth (50%–60%)	M: 5 days; F: 35 days (73%)	M: increased rapidly; F: increased slowly	Adult age: 35 days. Fetal development: NR. Strain and sex: Sprague Dawley (M/F). Methods: Western blotting	Johnson et al. (2002); Tomer et al. (2003)
mRNA expression	Fetal development (M: 60%, F: 100%)	M: 1 days; F: fetal development	Inconsistent pattern	Adult age: 60–70 days. Strain and sex: Sprague Dawley and Wistar (M/F). Methods: RT-PCR, Northern blotting, branched DNA signal amplification assay	Johnson et al. (2002); Zinchuk et al. (2002); Tomer et al. (2003); St-Pierre et al. (2004); Zhu et al. (2017)
MRP3	Fetal development (M: 60%, F: 10%)	M: 1 days; F: 35 days	M: no changes; F: increased progressively. Decreased in elderly	Adult age: 60 days. Strain and sex: Sprague Dawley and Wistar (M/F). Methods: RT-PCR	St-Pierre et al. (2004); Zhu et al. (2017)
Protein expression					
MRP4	Fetal development (M: <30%, F: 200%)	M: 28 days; F: fetal development	M: decreased in elderly (540 days: 40%); F: no changes	Adult age: 60 days. Strain and sex: Sprague Dawley (M/F). Methods: RT-PCR	Zhu et al. (2017)
mRNA expression	Fetal development: $<20\%$	Birth: 40%	NR	Birth–adult: NR. Adult age: NR. Strain and sex: Wistar (M/F). Methods: RT-PCR	St-Pierre et al. (2004)
MRP6					
Protein expression					
BCRP	mRNA expression	Fetal development (M: 200%, F: 100%)	Inconsistent pattern	Adult age: 60–70 days. Strain and sex: Sprague Dawley (M/F) and Wistar (M). Methods: RT-PCR	Kawase et al. (2015); Zhu et al. (2017)

F, female; M, male; NR, not reported; RT-PCR, reverse-transcriptase polymerase chain reaction.

TABLE 6
Ontogeny profile of hepatic efflux transporters in mice based on protein expression and mRNA expression levels

Efflux Transporter	Onset of Expression/Activity	Adult Levels Reached	Age-Related Changes (% of Adult) in Expression/Activity after Birth	Comments	References
Percentages represent expression/activity relative to adult levels.					
CERP mRNA expression	Fetal development (97%–179%)	0 days	Inconsistent pattern	Adult age: 60 days. Strain: C57BL/6J (M). Methods: RNA-seq and RT-PCR	Cui et al. (2012a)
MDR1B mRNA expression	Fetal development (100%)		No changes	Adult age: 45 days. Strain: C57BL/6J (mixed). Methods: RNA-seq	Cui et al. (2009)
Protein expression	NR	NR	No changes	Adult age: NR. Strain: FVB (NR). Methods: Western blotting	Mahmood et al. (2001)
MDR2 mRNA expression	Fetal development (~43%–44% at day –2)	1 days	Peaks 1.1–1.5-fold adult levels immediately after birth in M and F, falling back to ~50% adult levels between days 5–45	Adult age: 60 days. Strain: C57BL/6J (M/mixed). Methods: RNA-seq	Cui et al. (2009, 2012a)
ABCG5 mRNA expression	Fetal development (1.3%)	5 days	Inconsistent pattern	Statistical difference in expression between CV and GF strain (Selwyn et al., 2015). Adult age: 60 days (Cui et al., 2012a) and 90 days (Selwyn et al., 2015). Strain: C57BL/6J (Cui et al., 2012a) and CV + GF (Selwyn et al., 2015) (M). Methods: RNA-seq (Cui et al., 2012a) and RT-PCR (Selwyn et al., 2015)	Cui et al. (2012a); Selwyn et al. (2015)
Protein expression	NR (15 days: 1867%–3371%)	NR	Decreased progressively	Statistical difference in expression between CV and GF strain. Adult age: 90 days. Strain: CV + GF (M). Methods: Western blotting	Selwyn et al. (2015)
ABCG8 mRNA expression	Low expression in fetal tissue	5 days	Inconsistent pattern	Adult age: 60 days (Cui et al., 2012a) and 90 days (Selwyn et al., 2015). Strain: C57BL/6J (Cui et al., 2012a) and CV + GF (Selwyn et al., 2015) (M). Methods: RNA-seq (Cui et al., 2012a) and RT-PCR (Selwyn et al., 2015)	Cui et al. (2012a); Selwyn et al. (2015)
Protein expression	NR (15 days: 208%–4520%)	NR	Decreased progressively	Statistical difference in expression between CV and GF strain. Adult age: 90 days. Strain: CV + GF (M). Methods: Western blotting	Selwyn et al. (2015)
ATP7B mRNA expression	Fetal development (280%)	25 days	Inconsistent pattern	Adult age: 60 days. Strain: C57BL/6J (M). Methods: RNA-seq	Cui et al. (2012a)
BCRP mRNA expression	Fetal development (271%) as measured by RNA sequencing	60 days	Increased slowly	Adult age: 60 days. Strain: C57BL/6J (M). Methods: RNA-seq	Cui et al. (2012a)
BSEP mRNA expression	Fetal development (30%)	5 days	Inconsistent pattern	Adult age: 56 days (Cheng et al., 2007) and 60 days (Cui et al., 2012a). Strain: C57BL/6J (mixed). Methods: bDNA (Cheng et al., 2007) and RNA-seq (Cui et al., 2012a)	Cheng et al., et al., (2012a)
MATE1 mRNA expression	Fetal development (28%)	15 days	Increased rapidly	Adult age: 60 days. Strain: C57BL/6J (M). Methods: RNA-seq	Cui et al. (2012a)
MRP1 mRNA expression	Fetal development (55%)	25–30 days	Decreased rapidly	Adult age: 60 days. Strain: C57BL/6J (M). Methods: RNA-seq	Cui et al. (2012a)
MRP2					

(continued)

TABLE 6—Continued

Efflux Transporter	Onset of Expression/Activity	Adult Levels Reached	Age-Related Changes (% of Adult) in Expression/Activity after Birth	Comments	References
mRNA expression	Fetal development (30%)	10 days	Increased slowly		
MRP3 mRNA expression	Fetal development (26%)	30 days	Increased slowly	Adult age: 45 days (Maher et al., 2005), 60 days (Cui et al., 2012a; Li et al., 2016), and 90 days (Selwyn et al., 2015). Strain: C57BL/6J (Maher et al., 2005; Cui et al., 2012a; Selwyn et al., 2015; Li et al., 2016) (mixed) and CV + GF (Selwyn et al., 2015) (M). Methods: RNA-seq (Cui et al., 2012a), RT-PCR (Selwyn et al., 2015; Li et al., 2016), and bDNA ⁴⁴	Maher et al. (2005); Cui et al. (2012a); Selwyn et al. (2015); Li et al. (2016)
MRP4 mRNA expression	Fetal development (200%)	10 days	Decreased slowly	Adult age: 45 days (Maher et al., 2005) and 60 days (Cui et al., 2012a; Li et al., 2016). Strain: C57BL/6J (Maher et al., 2005; Cui et al., 2012a; Selwyn et al., 2015; Li et al., 2016) (mixed). Methods: RNA-seq (Cui et al., 2012a), RT-PCR (Selwyn et al., 2015; Li et al., 2016), and bDNA (Maher et al., 2005)	Maher et al. (2005); Cui et al. (2012a); Li et al. (2016)
Protein expression	NR	NR	Increased slowly	Adult age: 45 days (Maher et al., 2005) and 60 days (Cui et al., 2012a; Li et al., 2016). Strain: C57BL/6J (Maher et al., 2005; Cui et al., 2012a; Selwyn et al., 2015; Li et al., 2016) (mixed). Methods: RNA-seq (Cui et al., 2012a), RT-PCR (Selwyn et al., 2015; Li et al., 2016), and bDNA ⁴⁴	Maher et al. (2005); Cui et al. (2012a); Li et al. (2016)
MRP5 mRNA expression	Fetal development (1200%)	25–30 days	Decreased rapidly	Adult age: 60 days. Strain: C57BL/6J (M). Methods: RNA-seq	Cui et al. (2012a)
MRP6 mRNA expression	Fetal development (0%–30%)	3 days	Increased rapidly	Adult age: 45 days (Maher et al., 2005) and 60 days (Cui et al., 2012a). Strain: C57BL/6J (mixed). Methods: RNA-seq (Cui et al., 2012a) and bDNA (Maher et al., 2005)	Maher et al. (2005); Cui et al. (2012a)
NPT1 mRNA expression	Fetal development (3%)	20 days	Increased progressively	Adult age: 60 days. Strain: C57BL/6J (M). Methods: RNA-seq	Cui et al. (2012a)

bDNA, branched DNA signal amplification assay; CERP, cholesterol efflux regulatory protein; M, male; NR, not reported; RT-PCR, reverse-transcriptase polymerase chain reaction; RNA-seq, RNA sequencing.

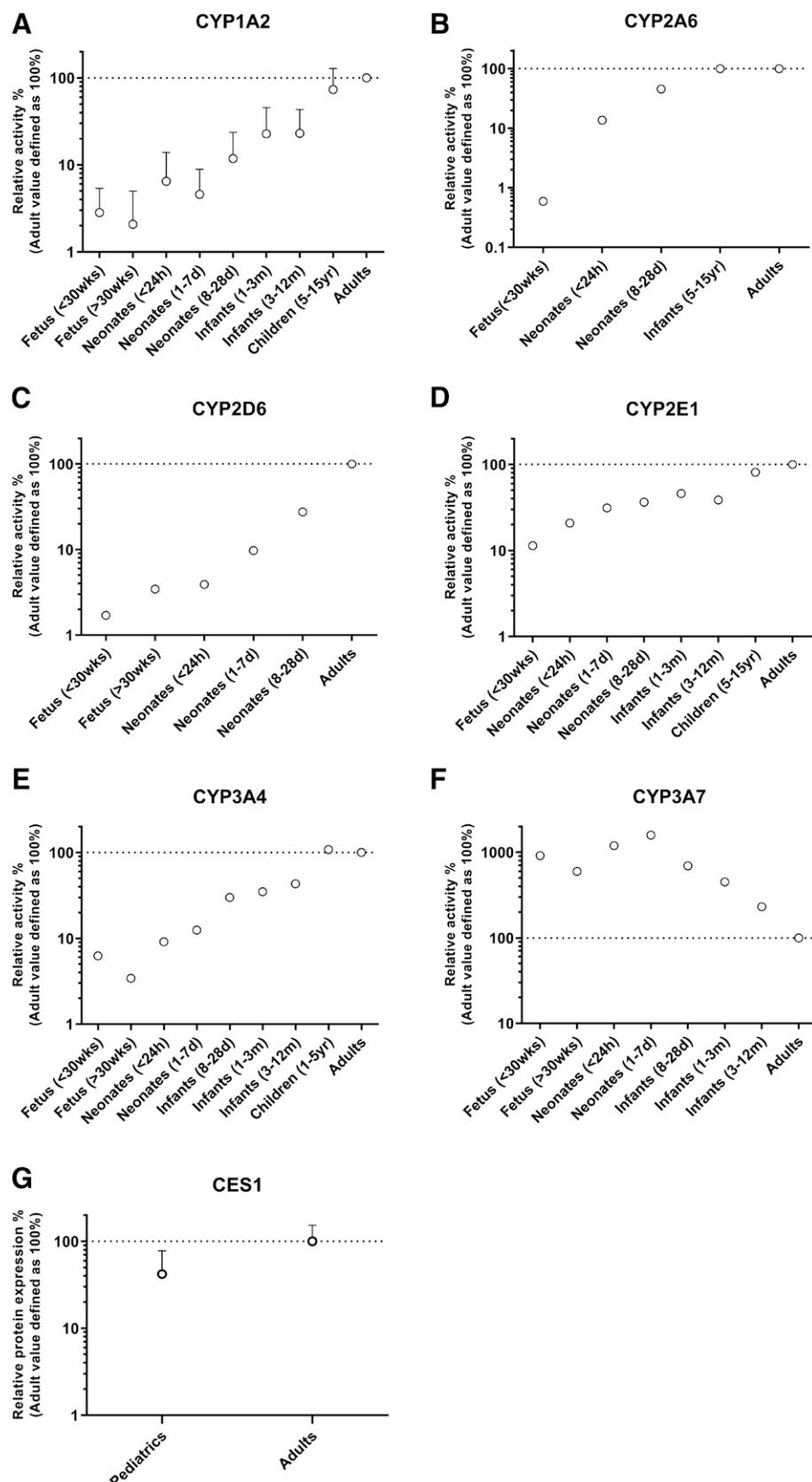


Fig. 9. Pooled literature data on the ontogeny of metabolic activity of hepatic phase I enzymes in humans: CYP1A2 (A), CYP2A6 (B), CYP2D6 (C), CYP2E1 (D), CYP3A4 (E), CYP3A7 (F), and CES1 (G). The symbols represent the relative activity in each age group, and the dotted line indicates the adult value defined as 100%. If multiple values were obtained for the same age group, the symbols represent the average relative activity, and the error bars show the S.D. See Table 7 for explanation on the ontogeny profiles and literature references.

TABLE 7 Ontogeny profile of hepatic phase I enzymes in humans based on metabolic activity, protein expression, and mRNA expression levels
Percentages represent expression/activity relative to adult levels.

	Onset of Expression/ Activity	Adult Levels Reached	Age-Related Changes (% of Adult) in Expression/ Activity after Birth	Comments	References
ADH1A Protein expression	Birth: 37.0%	1–6 yr	Increased progressively	Age at which 50% of maximum protein expression was reached: 10.1 mo. Adult age: 18 yr. Methods: LC-MS/MS	Bhatt et al. (2017)
ADH1B Protein expression	Birth: 12.6%	1–6 yr	Increased progressively	Age at which 50% of maximum protein expression was reached: 9.3 mo. Adult age: 18 yr. Methods: LC-MS/MS	Bhatt et al. (2017)
ADH1C Protein expression	Birth: 2.7%	1–6 yr	Increased progressively	Age at which 50% of maximum protein expression was reached: 12.3 mo. Adult age: 18 yr. Methods: LC-MS/MS	Bhatt et al. (2017)
ALDH1A1 Protein expression	Birth: 36.5%	1–6 yr	Increased progressively	Age at which 50% of maximum protein expression was reached: 10.9 mo. Adult age: 18 yr. Methods: LC-MS/MS	Bhatt et al., (2017)
CES1 Catalytic activity	Pediatric: 42.0%	NR	Increased	Adult age: 18 yr. Substrate: oseltamivir. Methods: depletion assay.	Boberg et al. (2017)
Protein expression	Birth: 19%–34%	3 wk to 6 yr (Boberg et al., 2017); 18 yr (Hines et al., 2016)	Increased rapidly	Adult age: 18 yr. Methods: LC-MS/MS (Boberg et al., 2017), Western blotting (Hines et al., 2016)	Hines et al. (2016); Boberg et al. (2017)
CES2 Protein expression	Birth: 34%–43%	3 wk to 6 yr	Increased progressively	Adult age: 18 yr. Methods: LC-MS/MS (Boberg et al., 2017), Western blotting (Hines et al., 2016)	Hines et al. (2016); Boberg et al. (2017)
FMO3 Protein expression	Birth: 46.4%	6–12 yr	Increased slowly	Adult age: 18 yr. Methods: LC-MS/MS	Xu et al. (2017)
CYP total Protein	Fetal development (57%–21%)	NR	Inconsistent pattern	Methods: Western blot, immunoblot	Cazeneuve et al. (1994); Shimada et al. (1994, 1996); Tateishi et al. (1997); Treliyer et al. (1997)
mRNA	Fetal development (40%)	NR	NR	Methods: RNase protection assay	Shephard et al. (1992)
CYP1A2 Catalytic activity	Fetal development (4%)	5–15 yr	Increased slowly	Substrates: ECOD (Mäenpää et al., 1993), Imipramine, Methoxyresorufin (Sonnier and Cresteil, 1998), caffeine (Cazeneuve et al., 1994). Matrix: microsomes. Methods: spectrophotometry (Mäenpää et al., 1993), radioactivity (Sonnier and Cresteil, 1998), fluorimetry (Sonnier and Cresteil, 1998), HPLC (Cazeneuve et al., 1994)	Mäenpää et al. (1993); Cazeneuve et al. (1994); Sonnier and Cresteil (1998)
Protein	Fetal development (0%–13%)	1–5 yr: 50%–100%	Increased slowly	Methods: immunoblot, Western blot	Berthou et al. (1988); Ratanasavanh et al. (1991); Shimada et al., (1994); Tateshi et al. (1997); Sonnier and Cresteil, (1998); Song et al. (2017)
mRNA	NR (ND in fetal tissue)	NR	NR (D in adult tissue)	Methods: PCR, RNA blot	Ratanasavanh et al. (1991); Hakkola et al. (1994); Yang et al. (1995)
CYP2A6 Catalytic activity	Fetal development (0.6%)	5–15 yr	Increased progressively	Substrate: coumarin, nicotine. Matrix: microsomes. Methods: NR Shimada et al. (1996); Hakkola et al. (1998); Upreti and Wahlistrom, (2016)	Shimada et al. (1994); Shimada et al. (1996); Tateishi et al. (1997); Upreti and Wahlistrom (2016)
Protein	Fetal development (T3: 65%)	1–15 yr	Inconsistent pattern	Methods: Western blot, immunoblot	
mRNA	NR (ND in fetal tissue)	NR	NR (D in adult tissue)	Methods: PCR	
CYP2C					

(continued)

Ontogeny of Hepatic Transport and Drug Metabolism

TABLE 7—Continued

	Onset of Expression/ Activity	Adult Levels Reached	Age-Related Changes (% of Adult) in Expression/ Activity after Birth	Comments	References
Protein	Fetal development (<1%)	NR (3–12 mo: 34%)	Increased rapidly	Methods: Western blot, immunoblot	Shimada et al. (1994, 1996); Treluyer et al. (1997)
mRNA	Fetal development (6%–60%)	NR (1–5 yr: 87%)	Increased slowly	Methods: Northern blot, RNA blot	Ratanasavanh et al. (1991); Treluyer et al. (1996); Treluyer et al. (1997)
CYP2C8 Protein	Fetal development (28%)	NR (>7 yr: 100%)	NR (increased)	Methods: Western blot, immunoblot	Tateishi et al. (1997); Naraharisetti et al. (2010); Song et al. (2017)
mRNA	Fetal development (D)	NR (>7 yr: 100%)	NR (no changes > 7 yr)	Methods: PCR	Hakkola et al. (1994); Naraharisetti et al. (2010)
CYP2C9 Protein	Fetal development (100%)	No changes	No changes	Methods: Western blot, immunoblot	Tateishi et al. (1997); Koukouritaki et al. (2004)
mRNA	Fetal development (T1-2: ND and T3: 13%)	28 days	Increased progressively	Methods: Northern blot	Treluyer et al. (1997)
CYP2C18 mRNA	Fetal development (40%)	28 days	Inconsistent pattern	Methods: Northern blot	Treluyer et al. (1997)
CYP2D6 Catalytic activity Protein	Fetal development (1.2%–3.7%)	NR (28 days: 27.5%)	Increased rapidly	Substrate: dextromethorphan. Matrix: microsomes. Methods: HPLC	Treluyer et al. (1991); Jacqz-Aigrain and Cresteil (1992); Stevens et al. (2008)
	Fetal development (8%–82%)	1–5 yr	Increase rapidly	Methods: Western blotting (Tateishi et al., 1997; Stevens et al., 2008), immunoblotting (Treluyer et al., 1991; Jacqz-Aigrain and Cresteil, 1992; Shimada et al., 1996)	Treluyer et al. (1991); Jacqz-Aigrain and Cresteil (1992); Stevens et al. (2008)
mRNA	Fetal development (50%–90%)	1 days	Non-linear pattern	Methods: slot blot (Treluyer et al., 1991; Jacqz-Aigrain and Cresteil, 1992), PCR (Hakkola et al., 1994)	Treluyer et al. (1991); Jacqz-Aigrain and Cresteil (1992); Hakkola et al. (1994)
CYP2E1 Catalytic activity Protein	Fetal development (3%–20%)	NR (5–15 yr: 81%)	Increased slowly	Substrate: EtOH (Carpenter et al., 1996; Miller et al., 1996), chlorzoxazone (Vieira et al., 1996). Matrix: microsomes.	Carpenter et al. (1996); Miller et al. (1996); Vieira et al. (1996)
	Fetal development (1.5%–70%)	1–5 yr	Increased slowly	Methods: immunoblot	Vieira et al. (1996)
mRNA	Fetal development (2%–6%)	NR (3–12 mo: 40%)	Increased slowly	Methods: Western blot, immunoblot	Shimada et al. (1994, 1996); Miller et al. (1996); Tateishi et al. (1997); Johnsrud et al. (2003)
CYP4A Protein	Fetal development (200%)	NR (1–5 yr: 169%)	Decreased slowly	Methods: Slot blot	Hakkola et al. (1994); Vieira et al. (1996)
CYP total Protein	Fetal development (57%–21%)	NR	Inconsistent pattern	Methods: Western blot, immunoblot	Cazenave et al. (1994); Shimada et al. (1994, 1996); Tateishi et al. (1997); Treluyer et al. (1997)
mRNA	Fetal development (40%)	NR	NR	Methods: RNase protection assay	Shephard et al. (1992)
CYP3A Protein	Fetal development (65%–80%)	1–5 yr	Increased slowly	Methods: Western blot, immunoblot	Ratanasavanh et al. (1991); Shimada et al. (1994, 1996); Lacroix et al. (1997); Tateishi et al. (1997)
CYP3A4 Catalytic activity Protein	Fetal development (6%–3%)	1–5 yr	Increased slowly	Substrate: testosterone. Matrix: microsomes. Methods: radioactivity (Lacroix et al., 1997)	Shimada et al. (1996); Lacroix et al. (1997); Leeder et al. (2005)
	Fetal development (T1-2: 12.5% and T3: 33%)	NR (5–15 yr: 33%)	Increased slowly	Methods: immunoblot	Stevens et al. (2003); Pope et al. (2005)

(continued)

TABLE 7—Continued

	Onset of Expression/ Activity	Adult Levels Reached	$\Delta_{\text{Age-Related}} \text{Changes (\% ofAdult) in Expression/}$ $\text{Activity after Birth}$	Comments	References
mRNA	Fetal development (T1-2: 10% and T3: 20%)	3–12 mo	Increased slowly	Methods: PCR, slot blot, RT-PCR	Yang et al. (1994); Lacroix et al. (1997); Leeder et al. (2005)
CYP3A5 Protein	Fetal development (42%)	NR (9 yr: 196%)	Inconsistent pattern	Methods: immunoblot	Wrighton et al. (1990); Stevens et al. (2003)
mRNA	Fetal development (1)	NR	NR	Methods: PCR, Northern blot	Schuetz et al. (1994); Yang et al. (1994)
CYP3A7 Catalytic activity Protein	Fetal development (T1-2: 915%)	NR (3–12 mo: 232%)	Decreased progressively	Substrate: DHEAS. Matrix: microsomes. Methods: radioactivity, HPLC	Lacroix et al. (1997); Tateishi et al. (1997); Stevens et al. (2003)
	Fetal development (T1-2: 2000%)	1–5 yr	Decreased progressively	Methods: Western blot, immunoblot	Hakkola et al. (1994); Schuetz et al. (1994); Leeder et al. (2005)
mRNA	Fetal development (185%)	NR	NR	Methods: PCR, RT-PCR, Northern blot	

D, day; ECOD, 7-ethoxycoumarin O-deethylase; HPLC, high-performance LC; ND, not detectable; NR, not reported; PCR, polymerase chain reaction; RT-PCR, reverse-transcriptase polymerase chain reaction.

CYP3A4, CYP3A5, and CYP3A7. The total CYP3A protein expression was 65%–80% in fetuses but appeared to be relatively constant across other age groups. CYP3A4 is an extensively studied enzyme that shows low activity in fetuses, with 50% of adult levels in children up to 1 year old. Adult levels are reached in children between 1 and 5 years old. Protein expression data showed an increase, but activity data showed a decrease during fetal life. The activity of CYP2A6 is very low during fetal age (<1% of adult values) but reaches 50% activity of adult values at neonatal age. This is also reported for protein expression of CYP2A6. Activity and protein expression both reach adult values between 1 and 15 years of age. For CYP2C8, activity data are missing, but the maturational pattern is delayed compared with CYP2A6. Protein expression of CYP2C8 in fetuses shows 28% of adult values, with adult values reached in children >7 years.

b. Age-related decrease in activity/expression. The CYP3A family consist of various isoforms as noted before, of which CYP3A7 is known as the fetal isoform. This is confirmed by literature data, as CYP3A7 is highly active in fetuses. Also, the protein expression is 2000% of adult expression. However, mRNA expression in fetuses was found to be only 185% of adult values. For CYP4A, only data on protein expression were available. From neonates to children, protein expression was higher than in adults (130%–200% of adult values). Expression data for age groups between children (1–5 years) and adults were lacking.

c. Complex ontogeny pattern in activity/expression. Total CYP enzyme protein levels and mRNA expression in fetuses were 21%–57% and 40% of adult values, respectively. Adult values are not yet reached at infant age, and data were lacking between infant and adult age. The CYP2C9 maturational pattern was discrepant between protein and mRNA expression. Protein expression did not show age-related differences. In contrast, mRNA expression was not detected in fetuses and showed neonatal development with 60% of adult values 1 week postnatally. Adult values were reached 1 month postnatally. For the enzyme CYP2C18, only mRNA expression is available, with 40% in fetuses, and rapid neonatal development to adult levels decreasing to 87% of adult levels 1 week postnatally. CYP3A5 is polymorphic and is only expressed in 23% of the adult population. The expression shows no clear developmental pattern.

2. Rat. The results are depicted in Fig. 10, Supplemental Fig. 11 and S12, and Table 8 and are further explained below.

a. Age-related increase in activity/expression. Metabolic activity gradually increased with age, reaching maximal activity levels from 3 to 4 days (CYP2E and CYP2B1/2), 7 days (CYP4A1), and 21 days of age (CYP1A and CYP3A). For CYP2C11, a sex-related pattern was

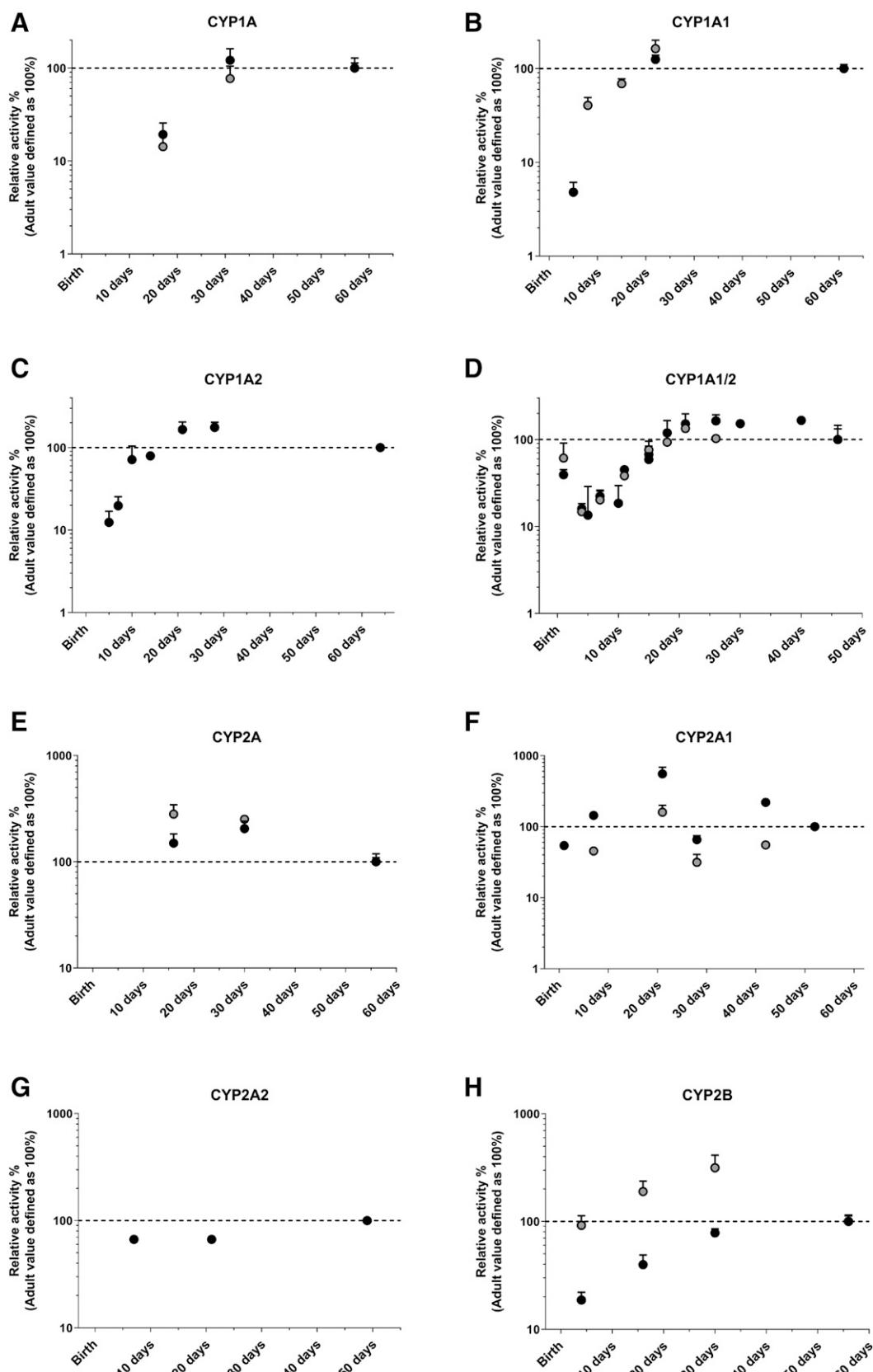


Fig. 10. Pooled literature data on the ontogeny of metabolic activity of hepatic CYP enzymes in rats: CYP1A (A), CYP1A1 (B), CYP1A2 (C), CYP1A1/2 (D), CYP2A (E), CYP2A1 (F), CYP2A2 (G), CYP2B (H), CYP2B1 (I), CYP2B1/2 (J), CYP2C (K), CYP2C6 (L), CYP2C11 (M), CYP2D (N), CYP2E1 (O), CYP3A (P), CYP3A1 (Q), CYP3A2 (R), CYP3A1/2 (S), and CYP4A1 (T). The symbols represent the relative activity in each age group, and the dotted line indicates the adult value defined as 100%. If multiple values were obtained for the same age group, the symbols represent the average relative activity, and the error bars show the S.D. See Table 8 for explanation on the ontogeny profiles and literature references.

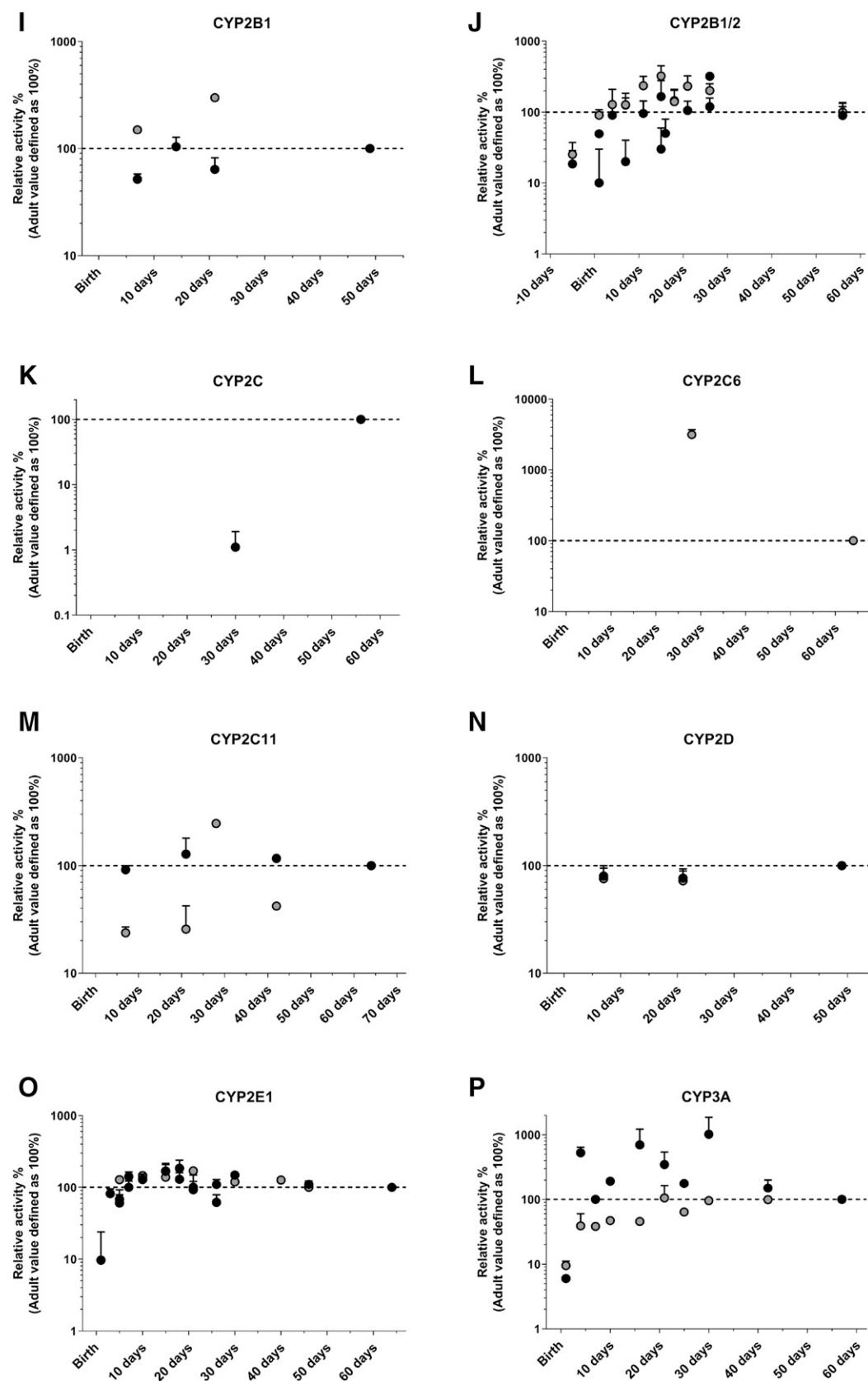


Fig. 10. Continued.

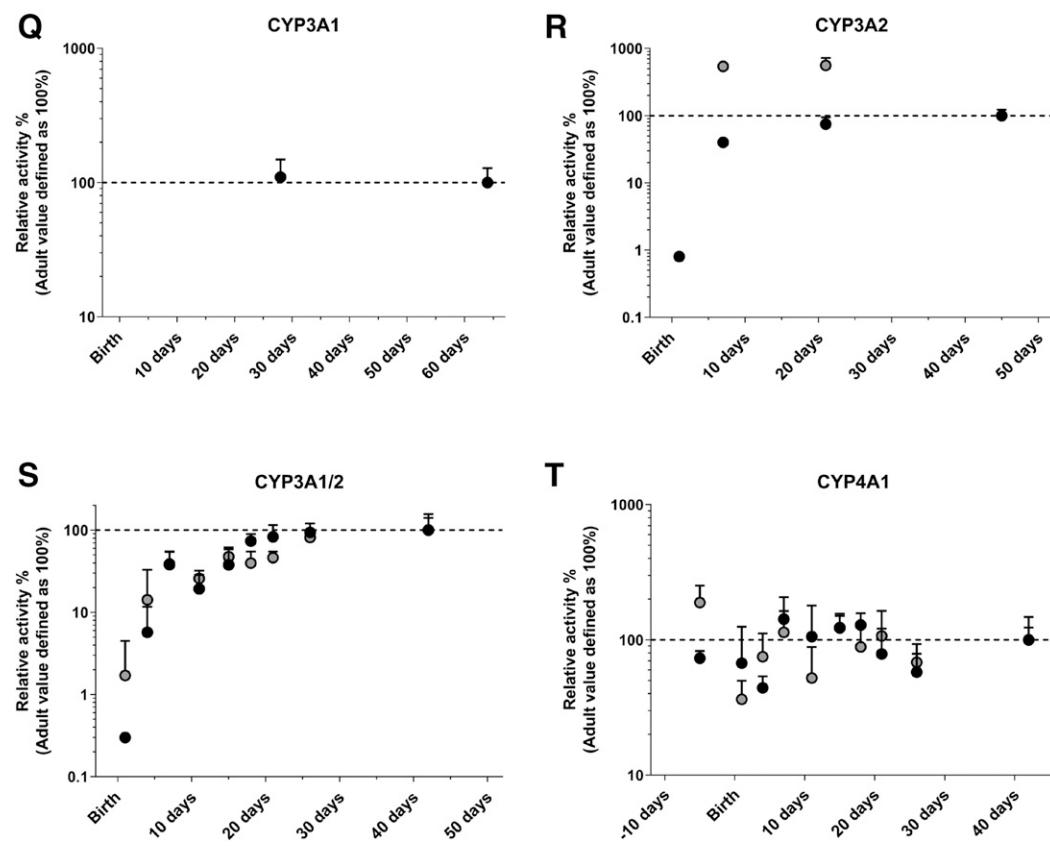


Fig. 10. Continued.

observed, reaching adult levels of activity at 7 days for female rats and 21 days for male rats.

Activity of CYP4A2 and CYP4A3 rose from 20% at birth until adulthood, except for female rats showing >100% of adult CYP4A2 activity at 7 days after birth.

Age- and sex-dependent increase in mRNA expression levels of CYP3A9, CYP3A11, CYP3A18, CYP3A23, CYP2D3, CYP7A1, CYP8B1, and CYP27 was reported. Maximal mRNA expression levels were achieved after 7 days of age for CYP3A11 and CYP3A23 or during adulthood for CYP3A9. Interstudy discrepancies exist with regard to the ontogeny of CYP3A18 reaching maximal mRNA expression levels at various ages, from 7 days in both female and male rats to 4 days in female rats and adulthood in male rats.

b. Age-related decrease in activity/expression. The ontogeny of CYP2C6 activity levels was not reported in juvenile rats below 28 days of age, but a 50-fold decrease in activity from 28 days to adult rats was seen. However, CYP2C6 protein and mRNA expression levels were inconsistent with activity levels: The more detailed ontogeny of CYP2C6 mRNA/protein levels revealed an age-related increase in expression from birth until adulthood in both male and female rats.

c. No age-related changes in activity/expression. Little to no change in metabolic activity of CYP2A, CYP2C12, and CYP2D isoforms was observed with advancing age in rats. No apparent sex differences in

activity were associated with ontogeny of CYP2A, whereas only male rats were studied for other CYP isoforms.

d. Inconsistent ontogeny pattern of expression levels. Fetal mRNA values of CYP7A1 and CYP8B1 represented >30% of adult levels. However, their mRNA expression levels rapidly declined after birth (<25% of adult levels after 7–14 days) and then increased throughout adulthood and peaked in elderly rats (>300%). However, mRNA protein levels of CYP27 reached maximal values after birth and remained unchanged until adulthood.

3. Mouse. The results are depicted in Fig. 11 and Table 9 and are further explained below.

a. Age-related increase in mRNA expression. The majority of the phase I enzymes showed a lower mRNA expression in younger age groups than in older age groups, and an important number of these enzymes reached adult levels at 20 days postnatal, including CYP1A2, CYP2A4, CYP2A5, CYP2B26, CYP2C50, CYP2D22, CYP3A11, CYP3A25, CYP4F14, and ALDH1. All but CYP1A1, CYP1A2, CYP2B10, CYP2B13, CYP2B23, CYP2F2, CYP4A10, CYP4A32, FMO2, and alternative oxidase were expressed in fetal tissue.

b. Age-related decrease in mRNA expression. CYP3A16 was the only isoform that showed a clear decrease in expression with a slow decrease from fetal life until adult values were reached at 25 days.

TABLE 8
Ontogeny profile of hepatic CYP enzymes in rats based on metabolic activity, protein expression and mRNA expression levels

	Onset of Activity and/ or Expression	Adult Levels Reached	Age-Related Changes in Activity/ Expression	Comments	References
CYP1A1 Catalytic activity	4 days: UD	M: 30 days; F: 30 days (77%)	M: increased progressively; F: increased slowly	Adult age: 56 days. Fetal development–4 days: NR. Substrate: ethoxresorufin. Strain and sex: Sprague Dawley (M/F). Matrix: liver microsomes. Methods: HPLC	Asaoka et al. (2010)
CYP1A1 Catalytic activity	4 days: 5%	21 days	Increased progressively	Adult age: 49–60 days. Fetal development–4 days: NR. Substrate: ethoxresorufin. Strain and sex: Sprague Dawley and Long-Evans (M/F). Matrix: liver microsomes. Methods: HPLC	Gebremichael et al. (1995); Szabo et al. (2009)
Protein expression mRNA expression	7 days: 76% Fetal development: >100%	21 days	Increased progressively; no changes after 21 days	Fetal development–7 days: NR. Strain and sex: Wistar (M/F). Methods: Western blotting	Imaoka et al., (1991)
CYP1A2 Catalytic activity	5 days: 10%	21 days	Inconsistent pattern	Adult age: 42 days. Strain and sex: Sprague Dawley (M/F). Methods: qRT-PCR	de Zwart et al. (2008)
Protein expression mRNA expression	3 days: 35%	28 days: 78%	Increased progressively; decreased in elderly (112 days: 70%)	Adult age: 62–65 days. Substrate: methoxyresorufin. Fetal development–1 day: NR. Strain and sex: Sprague Dawley (M). Matrix: liver microsomes. Methods: HPLC	Alcorn et al. (2007)
CYP1A1/2 Catalytic activity	Fetal: <1% M: 1 day (40%); F: 1 day (60%)	11 days	Increased rapidly; peaked at 15 days (300%)	Adult age: 42–50 days. Fetal development–3 days: NR. Strain and sex: Sprague Dawley (M/F) and Wistar (M/F). Methods: Western blotting	Imaoka et al. (1991); Alcorn et al. (2007)
Protein expression CYP2A Catalytic activity	5 days: 45%	21 days	Increased progressively then decreased in elderly	Adult age: 42–112 days. Strain and sex: Sprague Dawley (M/F). Methods: qRT-PCR	de Zwart et al. (2008); Asaoka et al. (2010)
CYP2A1 Catalytic activity	4 days: UD	16 days	Increased rapidly	Adult age: 49–50 days. Fetal development–5 days: NR. Strain and sex: Sprague Dawley (M/F). Methods: Western blotting	McPhail et al. (2016)
Protein expression mRNA expression	M: 7 days (95%); F: 7 days (40%)	M: 21 days M: 4 days (100%); F: 4 days (78%)	M: peaked at 21 days (300%); F: no changes after 21 days No changes after 4 days	Adult age: 56 days. Fetal development–4 days: NR. Strain and sex: Sprague Dawley (M/F). Methods: Western blotting	McPhail et al. (2016)
CYP2A2 Catalytic activity	7 days: 55%	M: 7 days; F: 21 days	Increased rapidly (M); increased progressively (F)	Adult age: 49–65 days. Fetal development–1 days: NR. Substrates: testosterone, androstenedione. Strain and sex: Sprague Dawley and Wistar (M/F). Matrix: liver microsomes. Methods: HPLC	Imaoka et al. (1991); Wright et al. (1997); Cherala et al. (2007)
Protein expression mRNA expression	M: 4 days (100%); F: 4 days (>200%)	M: 4 days; F: 16 days	M: increased slowly; F: increased rapidly	Adult age: 49–50 days. Fetal development–7 days: NR. Strain and sex: Wistar (M/F). Methods: Western blotting	Imaoka et al. (1991)
CYP2B Catalytic activity	M: 4 days (20%); F: 4 days (90%)	M: 30 days (80%); F: 4 days (90%)	M: increased slowly; F: increased rapidly	Adult age: 56 days. Fetal development–4 days: NR. Strain and sex: Sprague Dawley (M/F). Methods: qRT-PCR	Asaoka et al. (2010)
				Adult age: 49–50 days. Fetal development–7 days: NR. Substrate: testosterone. Strain and sex: Wistar (M/F). Matrix: liver microsomes. Methods: HPLC	Asaoka et al. (1991)
				Adult age: 49–50 days. Fetal development–7 days: NR. Strain and sex: Wistar (M/F). Methods: Western blotting	Imaoka et al. (1991)
				Adult age: 56 days. Fetal development–4 days: NR. Strain and sex: Sprague Dawley (M/F). Methods: qRT-PCR	Asaoka et al. (2010)
				Adult age: 56 days. Fetal development–4 days: NR. Strain and sex: Sprague Dawley (M/F). Matrix: liver microsomes. Methods: HPLC	Asaoka et al. (2010)

(continued)

Ontogeny of Hepatic Transport and Drug Metabolism

TABLE 8—Continued

	Onset of Activity and/or Expression	Adult Levels Reached	Age-Related Changes in Activity/ Expression	Comments	References
CYP2B1 Catalytic activity	M: 7 days (50%); F: 7 days (>100%)	14 days	Increased rapidly then decreased in elderly	Adult age: 49–50 days. Fetal development–7 days: NR. Substrate: testosterone, pentoxysorufin. Strain and sex: Sprague Dawley (M) and Wistar (M/F). Matrix: liver microsomes. Methods: HPLC	Imaoka et al. (1991); Gebremichael et al. (1995)
Protein expression	M: 7 days (75%); F: 7 days (>120%)	M: 21 days (77%); F: 7 days	M: increased slowly; F: increased rapidly. Decreased in elderly	Adult age: 49–50 days. Fetal development–7 days: NR. Strain and sex: Wistar (M/F). Methods: Western blotting	Imaoka et al. (1991)
mRNA expression	M: 4 days (50%); F: 4 days (20%)	M: 21 days; F: 7 days	No changes after 4 days	Adult age: 56 days. Fetal development–4 days: NR. Strain and sex: Asaoka et al. (2010)	
CYP2B2 Protein expression	M: 7 days (70%); F: 7 days (40%)	M: 21 days; F: 21 days (80%)	M: increased progressively, decreased slowly	Adult age: 49–50 days. Fetal development–7 days: NR. Strain and sex: Wistar (M/F). Methods: Western blotting	Imaoka et al. (1991)
mRNA expression	M: 4 days (700%); F: 4 day (280%)	M: 4 days; F: 7 days	Decreased slowly	Adult age: 56 days. Fetal development–4 days: NR. Strain and sex: Sprague Dawley (M/F) and Long-Evans (M). Methods: qRT-PCR	Szabo et al. (2009); Asaoka et al. (2010)
CYP2B1I/2 Catalytic activity	Fetal development: <25%	M: 4 days; F: 7 days	Increased rapidly	Adult age: 56 days. Substrate: pentoxysorufin. Strain and sex: Sprague Dawley (M/F). Matrix: liver microsomes. Methods: spectrophotometry	Asaoka et al. (2008); McPhail et al. (2016)
Protein expression	5 days: 80%–90%	M: 21 days; F: 15 days	Increased progressively, increased slowly	Adult age: 49–50 days. Fetal development–5 days: NR. Strain and sex: Sprague Dawley (M/F). Methods: Western blotting	McPhail et al. (2016)
CYP2C Catalytic activity	4 days: UD	30 days: 1%		Adult age: 56 days. Fetal development–4 days: NR. Substrate: testosterone. Strain and sex: Sprague Dawley (M/F). Matrix: liver microsomes. Methods: HPLC	Asaoka et al. (2010)
CYP2C6 Catalytic activity	28 days: 3000%	NR	Decreased after 28 days	Adult age: 63–65 days. Fetal development–28 days: NR. Substrate: testosterone. Strain and sex: Sprague Dawley (F). Matrix: liver microsomes. Methods: HPLC	Cherrala et al. (2007)
Protein expression	7 days: <10%	21 days: 45%	Increased slowly	Adult age: 49 to 50 days. Fetal development–7 days: NR. Strain and sex: Wistar (M/F). Methods: Western blotting	Imaoka et al. (1991)
mRNA expression	4 days: 25%–30%	30 days	Increased progressively	Adult age: 56 days. Fetal development–4 days: NR. Strain and sex: Sprague Dawley (M/F). Methods: qRT-PCR	Asaoka et al. (2010)
CYP2C11 Catalytic activity	M: 7 days (20%); F: 7 days (90%)	M: 28 days; F: 7 days	M: increased progressively; F: increased rapidly	Adult age: 63–65 days. Fetal development–7 days: NR. Substrate: testosterone, androstenedione. Strain and sex: Sprague Dawley (M/F). Matrix: liver microsomes. Methods: HPLC	Imaoka et al. (1991); Wright et al. (1997); Cherala et al. (2007)
Protein expression	M: 7 days (6%); F: 7 days (100%)	M: 21 days (17%); F: 21 days (90%)	M: increased slowly; F: increased progressively	Adult age: 49–50 days. Fetal development–7 days: NR. Strain and sex: Wistar (M/F). Methods: Western blotting	Imaoka et al. (1991)
mRNA expression	4 days: <1%	30 days: 14%	Increased slowly	Adult age: 56 days. Fetal development–4 days: NR. Strain and sex: Sprague Dawley (M/F). Methods: qRT-PCR	Asaoka et al. (2010)
CYP2C12 Catalytic activity	1 day: UD	NR	Decreased in elderly (365 days: 85%)	Adult age: 40 days. Fetal development–1 day: NR. Substrate: testosterone. Strain and sex: Wistar (M). Matrix: liver slices.	Lupp et al. (2008)
Protein expression	M: 7 days (65%); F: 7 days (<4%)	M: 21 days; F: 21 days (16%)	M: increased progressively; F: inconsistent pattern	Adult age: 49 days. Fetal development–7 days: NR. Strain and sex: Wistar (M/F). Methods: Western blotting	Imaoka et al. (1991)
CYP2D Catalytic activity	7 days: 75%–80%	21 days: 75%	Increased slowly	Adult age: 49 days. Fetal development–7 days: NR. Substrate: bufuralol. Strain and sex: Wistar (M/F). Matrix: liver microsomes. Methods: HPLC	Chow et al. (1999)
Protein expression	7 days: 65%–75%	21 days: 65%	Increased slowly	Adult age: 49 days. Fetal development–7 days: NR. Strain and sex: Wistar (M/F). Methods: immunoblotting	Chow et al. (1999)
CYP2D1 mRNA expression	7 days: >80%	21 days	Increased progressively then decreased in elderly	Adult age: 49 days. Fetal development–7 days: NR. Strain and sex: Chow et al. (1999)	

(continued)

TABLE 8—Continued

	Onset of Activity and/or Expression	Adult Levels Reached	Age-Related Changes in Activity/ Expression	Comments	References
CYP2D2 mRNA expression	7 days: 10%	7 days	Increased rapidly. No changes after 7 days	Adult age: 49 days. Fetal development–7 days: NR. Strain and sex: Chow et al. (1999)	
CYP2D3 mRNA expression	M: 7 days (15%); F: 7 days (40%)	21 days: 30%–50%	Increased slowly	Adult age: 49 days. Fetal development–7 days: NR. Strain and sex: Chow et al. (1999)	
CYP2E1 Catalytic activity	M: 1 day (10%); F: 5 days (60%)	7 days	Increased rapidly	Adult age: 42–50 days. Fetal development–1 day: NR. Substrates: aniline, p-nitro-phenol. Strain and sex: Sprague Dawley (M/F). Matrix: liver microsomes. Methods: spectrophotometry	de Zwart et al. (2008); McPhail et al. (2016)
Protein expression	M: 3 days (40%); F: 5 days (180%)	M: 10 days; F: fetal development	M: increased rapidly. F: no changes after 5–10 days	Adult age: 42–112 days. Fetal development–3 days: NR. Strain and sex: Sprague Dawley and Wistar (M/F). Methods: Western blotting	Imaoka et al. (1991); Alcorn et al. (2007); McPhail et al. (2016)
mRNA expression	Fetal development (M: 9%; F: 20%)	M: 10 days; F: 7 days	Increased rapidly. Peaked at 15 days (F: 50%)	Adult age: 42–112 days. Strain and sex: Sprague Dawley (M/F). Methods: qRT-PCR	Elbarbry et al. (2007); de Zwart et al. (2008)
CYP3A Catalytic activity	1 day: <10%	M: 21 days; F: 4 days	M: increased progressively. F: increased rapidly.	Adult age: 50–65 days. Substrate: testosterone, androstenedione, benzoxyresorufin. Strain and sex: Sprague Dawley and Wistar (M/F) and Long-Evans (M). Matrix: HPLC	Imaoka et al. (1991); Johnson et al. (2000); Szabo et al. (2009); Asaoka et al. (2010); Wright et al. (2010)
Protein expression	M: 1 days (180%); F: 1 day; F: 10 days 1 day (70%)	M: 1 day; F: 10 days	Decreased in elderly Increased rapidly	Adult age: 60–63 days. Strain and sex: Wistar (M/F). Methods: Western blotting	Johnson et al. (2000)
CYP3A1/2 Catalytic activity	1 day: <2%	28 days: 80%–90%	Increased progressively	Adult age: 42 days. Substrate: ethoxresofurin. Fetal development–1 day: NR. Strain and sex: Sprague Dawley (M/F).	de Zwart et al. (2008)
CYP3A1 Catalytic activity	NR	NR	Inconsistent pattern	Matrix: liver microsomes. Methods: spectrophotometry	
Protein expression mRNA expression	NR	NR	Decreased in elderly (728 days; 45%)	Adult age: 42 days. Substrate: testosterone. Strain and sex: Sprague Dawley (M). Matrix: liver microsomes. Methods: HPLC	Cherala et al. (2007)
CYP3A2 Catalytic activity	1 day: <1%	M: 21 days (75%); F: 7 days	No changes	Adult age: NR. Fetal development–adult: NR. Strain and sex: Fischer-344 (M). Methods: Western blotting	Warrington et al. (2004)
Protein expression mRNA expression	4 days: 200%–600%	Fetal development		Adult age: 56–60 days. Strain and sex: Sprague Dawley and Wistar (M/F). Methods: qRT-PCR	Asaoka et al. (2010); Kawase et al. (2015)
CYP3A9 mRNA expression	4 days: 20% or 7 days: <3%	M: 30 days (36%); F: 30 days (80%)	M: increased slowly then decreased in elderly. F: peaked at 7 days (500%)	Fetal development–1 day: NR. Substrate: testosterone. Strain and sex: Wistar (M/F). Matrix: liver microsomes and liver slices. Methods: HPLC	Imaoka et al. (1991); Lupp et al. (2008)
CYP3A11 mRNA expression	Fetal development: <5%	7 days	M: increased slowly then decreased in elderly	Adults age: 42–63 days. Fetal development–7 days: NR. Strain and sex: Fischer-344 (M) and Wistar (M/F). Methods: Western blotting	Imaoka et al. (1991); Warrington et al. (2004)
CYP3A18 mRNA expression	M: 4 days (4%); F: 4 days (78%)	M: NR or 7 days; F: 7–16 days	M: increased slowly or rapidly. F: increased rapidly	Adults age: 42–60 days. Strain and sex: Sprague Dawley (M/F). Methods: qRT-PCR	Mahrke et al. (1997); de Zwart et al. (2008)
CYP3A23 mRNA expression	M: 4 days (20%); F: 4 days (55%)	7 days	Increased rapidly	Adult age: 49–56 days. Fetal development–4 days: NR. Strain and sex: Sprague Dawley (M/F). Methods: qRT-PCR	Mahrke et al. (1997); Asaoka et al. (2010)

(continued)

TABLE 8—Continued

	Onset of Activity and/or Expression	Adult Levels Reached	Age-Related Changes in Activity/ Expression	Comments	References
CYP4A1 Catalytic activity	Fetal development (M: 70%, F: 190%)	M: 7 days; F: fetal development	Inconsistent pattern	Adult age: 42 days. Substrate: lauric acid. Strain and sex: Sprague Dawley (M/F). Matrix: liver microsomes. Methods: spectrophotometry	de Zwart et al. (2008)
mRNA expression	Fetal development 100%	Fetal development	Inconsistent pattern	Adult age: 42 days. Strain and sex: Sprague Dawley (M/F). Methods: qRT-PCR	de Zwart et al. (2008)
CYP4A2 Protein expression	M: 7 days (20%); F: 7 days (100%)	M: 21 days (20%); F: 7 days	M: increased slowly; F: no changes after 7 days	Adult age: 49–50 days. Fetal development–7 days: NR. Strain and sex: Wistar (M/F). Methods: Western blotting	Imaoka et al. (1991)
CYP4A3 Protein expression	7 days: 20%–30%	21 days: 20%–30%	Increased slowly	Adult age: 49–50 days. Fetal development–7 days: NR. Strain and sex: Wistar (M/F). Methods: Western blotting	Imaoka et al. (1991)
CYP7A1 mRNA expression	Fetal development: 30%–130%	Fetal development	Inconsistent pattern	Adult age: 56 days. Strain and sex: Wistar (M). Methods: qRT-PCR	Cuesta de Juan et al. (2007)
CYP8B1 mRNA expression	Fetal development: 2%–40%	1 day	Inconsistent pattern	Adult age: 56 days. Strain and sex: Wistar (M). Methods: qRT-PCR	Cuesta de Juan et al. (2007)
CYP27 mRNA expression	Fetal development: 20%–40%	1 day	Increased rapidly	Adult age: 56 days. Strain and sex: Wistar (M). Methods: qRT-PCR	Cuesta de Juan et al. (2007)

F, female; HPLC, high-performance LC; M, male; NR, not reported; qRT-PCR, quantitative RT-PCR; RT-PCR, reverse-transcriptase polymerase chain reaction; UD, undetected.

c. No age-related changes in mRNA expression. CYP2D26 did not show age-related changes in its ontogeny profile.

d. Complex and/or inconsistent ontogeny pattern of mRNA expression. For the isoforms CYP2B13, CYP2B23, CYP2B9, CYP3A41B, CYP3A59, CYP4A31, CYP4A32, and CYP4F16, no clear developmental pattern could be distinguished. CYP2B13 and CYP2B23 were not expressed in fetal tissue. CYP2B13 and CYP2B9 expression was higher in male mice than in female mice. Expression of CYP2B23 increased from birth to 10 days and subsequently decreased.

4. Nonrodents.

a. *Göttingen minipig*. The results are depicted in Fig. 12, Supplemental Fig. 13, and Table 10. The metabolic activity of CYP1A2 and CYP2D6 showed a rapid increase, with adult levels reached at 28 days of age. CYP2C9 and CYP3A4 showed a more gradual increase with lower activity at 28 days of age compared with adults. All CYPs mentioned above showed detectable albeit very low (0.2%–3%) fetal activity.

Only CYP3A protein expression has been reported. It was low (about 20% of adult) during the late fetal stages and gradually increased postnatally with still a lower expression at 28 days of age compared with adults.

b. *Cynomolgus monkey*. Results are depicted in Fig. 13 and Table 11. For this species, only mRNA data are reported. Several CYPs show a rapid increase and normalize at adult values (CYP2A23, CYP2A24, CYP2B6, CYP2C9, CYP2C19, CYP2C76, CYP2D17, CYP2E1, CYP3A4, and CYP4F2). CYP2C18 mRNA levels, on the other hand, increase very slowly. Others show a more particular profile that either increases well above adult expression levels before declining (CYP1A1, CYP2C8, CYP2J2, CYP3A5, CYP4A11, CYP4F3, and CYP4F11) or starts above adult values to first fluctuate and eventually decrease toward adult age (CYP3A43). CYP4F12 was the only enzyme with a more or less stable profile from fetal to adult age. No sex differences were reported.

c. *Beagle dog*. The results are depicted in Fig. 14, Supplemental Fig. 14, and Table 12. The data need to be interpreted with caution because they are derived from animals of 7 days and older, and CYP activity already reached values around 50% of adult levels at this young age. CYP1A1 activity and metabolism mediated by CYP3A and CYP2B already showed adult levels in male Beagle dogs at 1 week of age, whereas CYP1A2 increased after birth and reached adult values at 30 weeks of age. Combined activity of CYP2C, CYP2E1, and CYP3A was higher at 3 weeks of age, whereas the older age groups showed similar levels as dogs at 1 week of age. Activity levels of especially CYP2C9 and also CYP2E1 increased from 7 days and valued well above adult values around 100 days, after which they steadily declined to adult values. CYP3A4/5 activity decreased between 7 days and 50 days, after which it increased

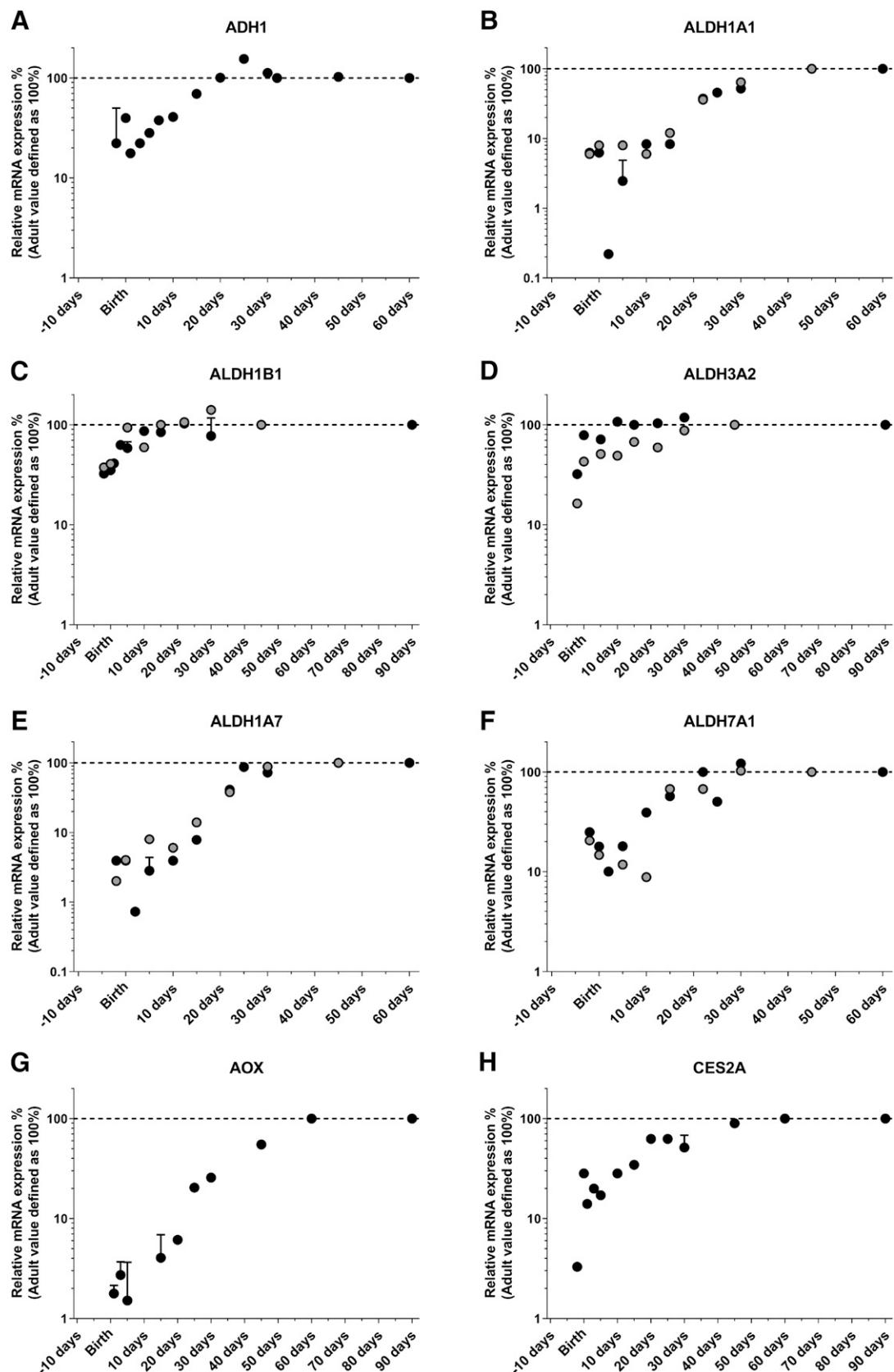


Fig. 11. Pooled literature data on the ontogeny of hepatic mRNA expression of phase I drug-metabolizing enzymes in mice: ADH1 (A), ALDH1A1 (B), ALDH1B1 (C), ALDH3A2 (D), ALDH1A7 (E), ALDH7A1 (F), AOX (G), CES2A (H), CYP1A2 (I), CYP2A4 (J), CYP2A5 (K), CYP2B10 (L), CYP2B13 (M), CYP2B23 (N), CYP2B9 (O), CYP2C29 (P), CYP2C37 (Q), CYP2C40 (R), CYP2C44 (S), CYP2C50 (T), CYP2C54 (U), CYP2C67 (V), CYP2C69 (W), CYP2C70 (X), CYP2D10 (Y), CYP2D22 (Z), CYP2D26 (AA), CYP2D9 (AB), CYP2E1 (AC), CYP2F2 (AD), CYP3A11 (AE), CYP3A13 (AF), CYP3A16 (AG), CYP3A25 (AI), CYP3A41A (AJ), CYP3A41B (AK), CYP3A44 (AL), CYP3A59 (AM), CYP4A10 (AN), CYP4A14 (AO), CYP4A31 (AP), CYP4A32

gradually to adult values. No mRNA or protein expression level data were available.

d. Domestic pig. The results are depicted in Fig. 15, Supplemental Figs. 15 and S16, and Table 13. Activity data were available from male and female animals 2, 28, 56, and 180 days of age. Female CYP2C as well as male/female CYP2E activity levels were high at birth ($\pm 50\%$) and gradually increased to adult values. Male CYP2E and male/female CYP3A activity levels were low at birth and rapidly increased to $\pm 75\%$ of adult values, which was followed by a gradual increase to adult values (180 days). Microsomal protein content was high at birth and gradually decreased to adult values at 30 days.

Protein expression levels as determined by LC-MS/MS were reported for male and female animals at 2, 28, 56, and 180 days of age. Expression levels of CYP1A2, CYP2C34, and CYP2C49 increased rapidly after birth. A sex difference was observed for CYP1A2, with female expression reaching well above adult levels before declining back to adult levels. A more gradual increase without sex differences was observed for CYP2A19, CYP2D6, CYP3A22, CYP3A46, CYP4A21, CYP20A1, and CYP51A1. Protein expression levels of CYP2B22, CYP2C33, male CYP2C36, and female CYP2E1 and CYP4A24 were stable postnatal until 56 days of age, and then this was followed by an increase to adult levels at 180 days of age. Female CYP2C36 and male CYP2E1 levels were stable from birth to adulthood.

D. Phase II Drug-Metabolizing Enzymes

1. Human. The results are depicted in Fig. 16, Supplemental Fig. 17 and S18, and Table 14 and are further explained below.

a. Age-related increase in activity/expression. Metabolic conjugating activity gradually increased with age, reaching maximal levels at various ages from infancy [<2 years, for *N*-acetyltransferase (NAT) and UGT1A9], early childhood [7 years, for glutathione-S-transferase (GST) ζ 1], or adulthood [>18 years for catechol-O-methyltransferase (COMT), sulfotransferase (SULT) 1E1, UGT2B7, UGT2B15, and UGT2B17]. Activity was readily apparent by either the fetal period for COMT, GSTZ1, NAT, UGT2B7, UGT2B17, and SULT1E1 or at least within a few days after birth for UGT1A9 and UGT2B15—there was no fetal activity data available.

The developmental pattern of several phase II enzymes has been characterized only based on either protein expression levels [GSTA2, GSTM, and SULT2A1] or mRNA expression levels (UGT2B4). Protein abundance levels of GSTA2, GSTM, and SULT2A1 gradually

increased with advancing age, reaching maximal levels within 2 years of age and with onset of protein expression started during fetal life. Although no data were reported below 6 months of age, 50% of adult mRNA expression levels of UGT2B4 was achieved after 6 months and remained unchanged until adulthood.

b. Age-related decrease in activity/expression. SULT1A3 activity levels and GSTP1 protein abundances decreased by 2.5- and 20-fold from fetal to neonatal population, respectively. Although no data were reported during childhood, SULT1A3 activity levels increased by 10-fold, whereas GSTP1 protein levels further decreased by 10-fold from neonates to adulthood.

c. No age-related changes in activity/expression. Little to no change in SULT1A1 activity levels was observed during fetal life, being more than 80% of adult levels. However, activity data were not reported between 6 months and adult age, with 3-fold higher activity at 6 months of age compared with adult levels. Similar results were observed in protein abundance levels of SULT1A1 and GSTA1.

2. Rat. The results are depicted in Fig. 17, Supplemental Figs. 19 and S20, and Table 15 and are further explained below.

a. Age-related increase in activity/expression. Activity of UGT1A1, UGT1A6, and UGT2B1 was present at 1 to 2 weeks before birth and rapidly increased to adult levels around 3 weeks postnatally. The mRNA profile of UGT2B2 followed a similar pattern. Although increasing, UGT1A6 activity was variable and fluctuated in three distinct ages, namely 0–15 days, 15–45 days, and 45–300 days. This was also observed for the mRNA maturation profile of UGT1A6. UGT1A1 mRNA expression showed a marked peak after birth that rapidly declined during the first week of life and gradually increased until 56 days.

SULT1A1 (activity and mRNA), SULT2A1 (activity and mRNA), SULT-40/41 (mRNA) bile-salt sulfotransferase (activity), and 3β -hydroxy-5-cholenoate sulfotransferase (activity) increased gradually after birth, reaching a maximum around 20 (SULT2A1, bile-salt sulfotransferase, and 3β -hydroxy-5-cholenoate) to 40 (SULT1A1) days postnatally. SULT1A1 activity and mRNA levels remained stable through adolescence and adulthood. However, for SULT2A1, bile-salt sulfotransferase, and 3β -hydroxy-5-cholenoate, activity in male livers decreased between day 20 and days 60–80 postnatally, whereas activity in female livers remained stable. The decrease in SULT2A1 levels was also observed in the corresponding mRNA levels (SULT-20/21). SULT-40/41 mRNA levels also showed a decrease after 20 days of age, which was observed in both sexes.

(AQ), CYP4F13 (AR), CYP4F14 (AS), CYP4F15 (AT), CYP4F16 (AU), FMO1 (AV), FMO2 (AW), FMO5 (AX), NQO1 (AY), and POR (AZ). The symbols represent the relative mRNA expression in each age group, and the dotted line indicates the adult value defined as 100%. See Table 9 for explanation on the ontogeny profiles and literature references. AOX, alternative oxidase; NOR, nitric oxide reductase; NQO1, NAD(P)H:quinone acceptor oxidoreductase.

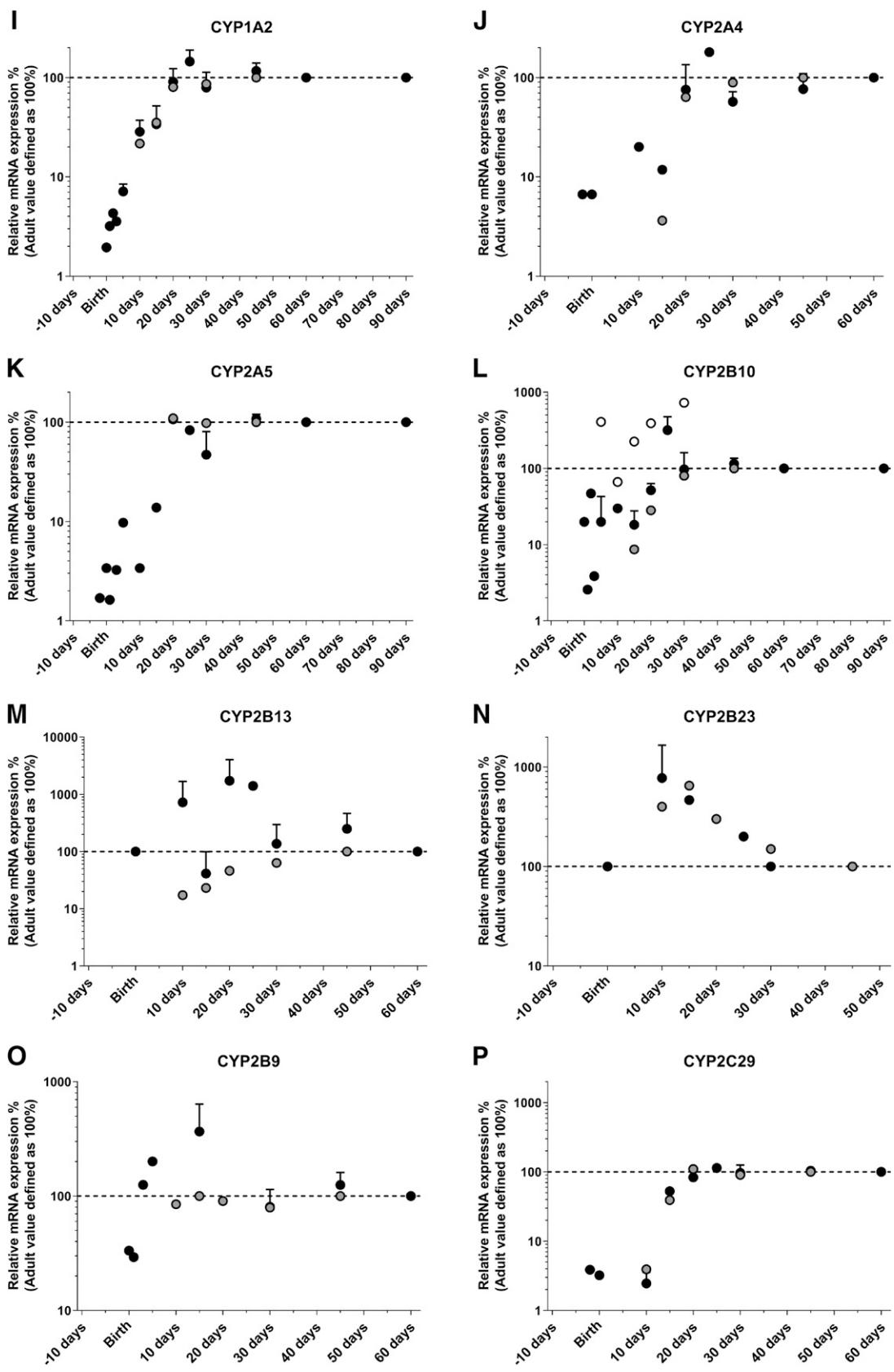


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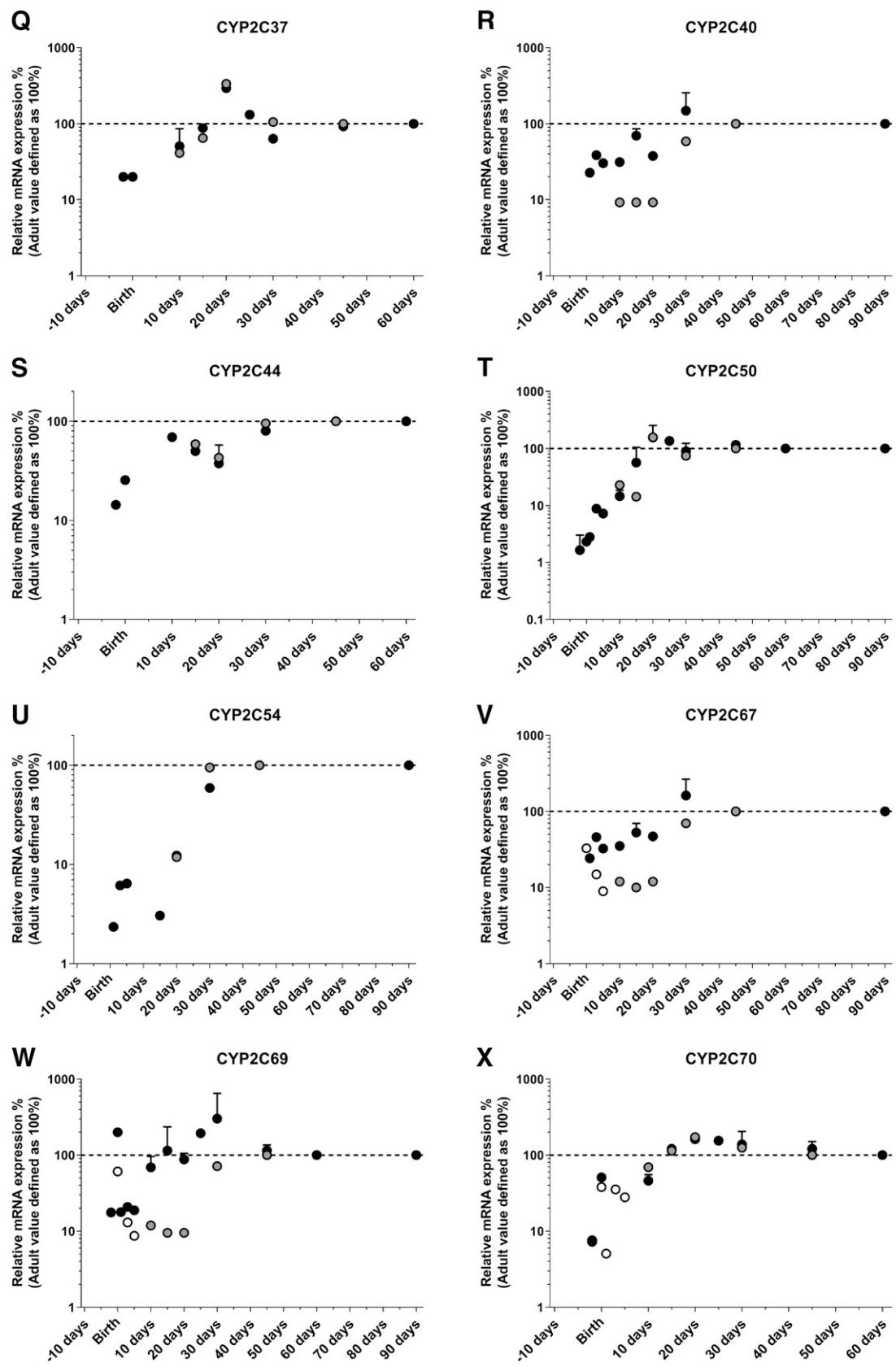


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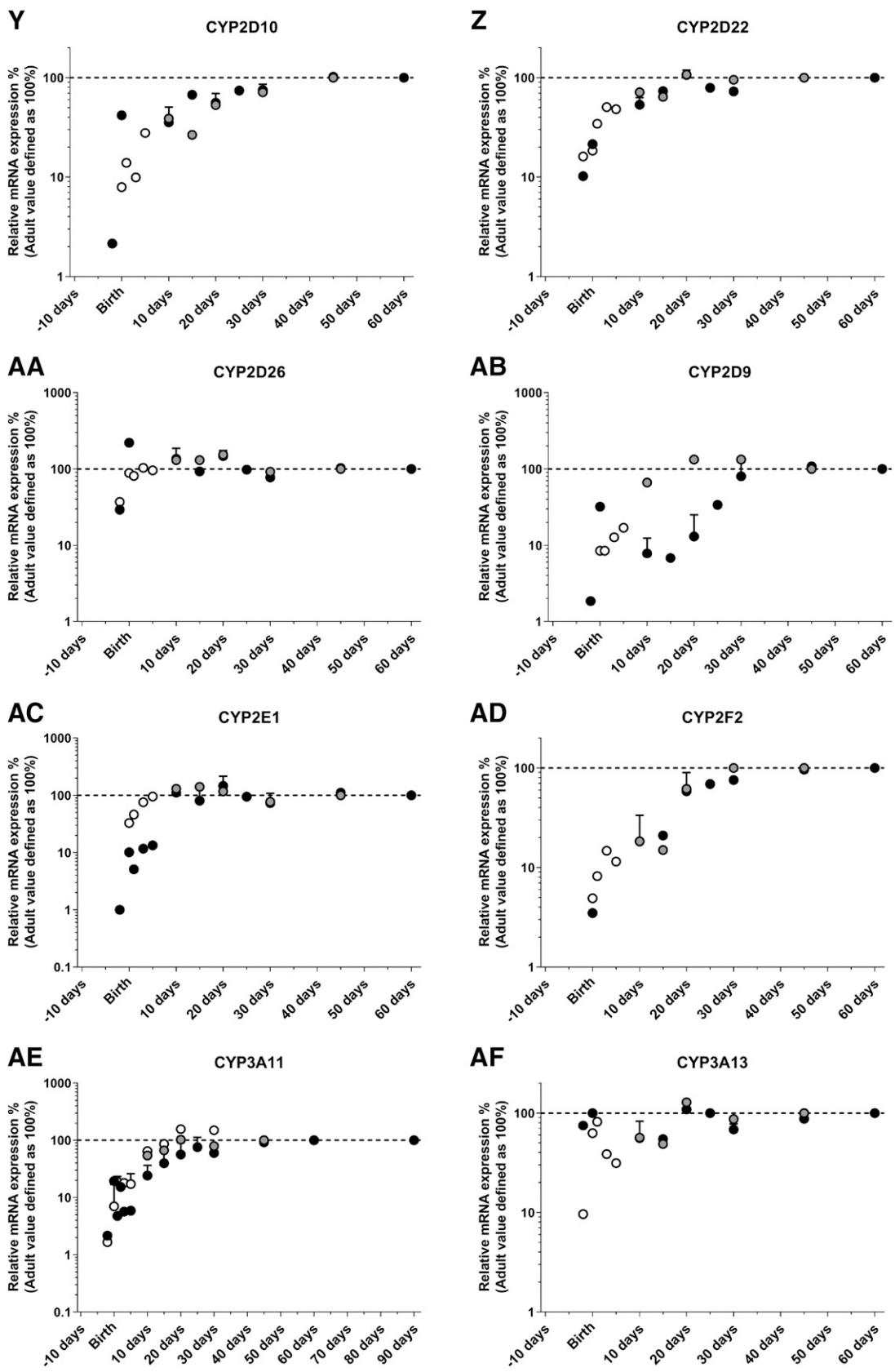


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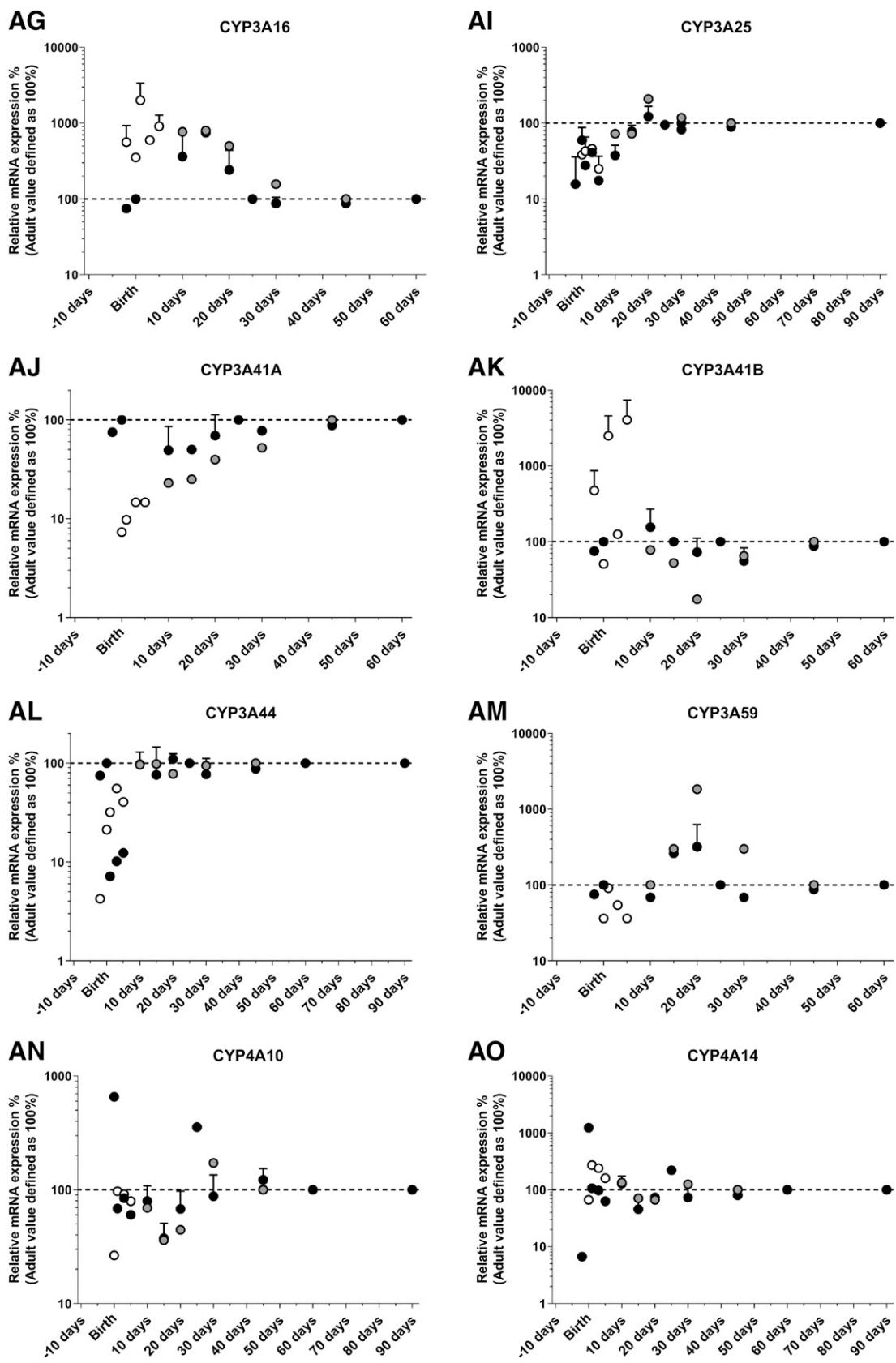


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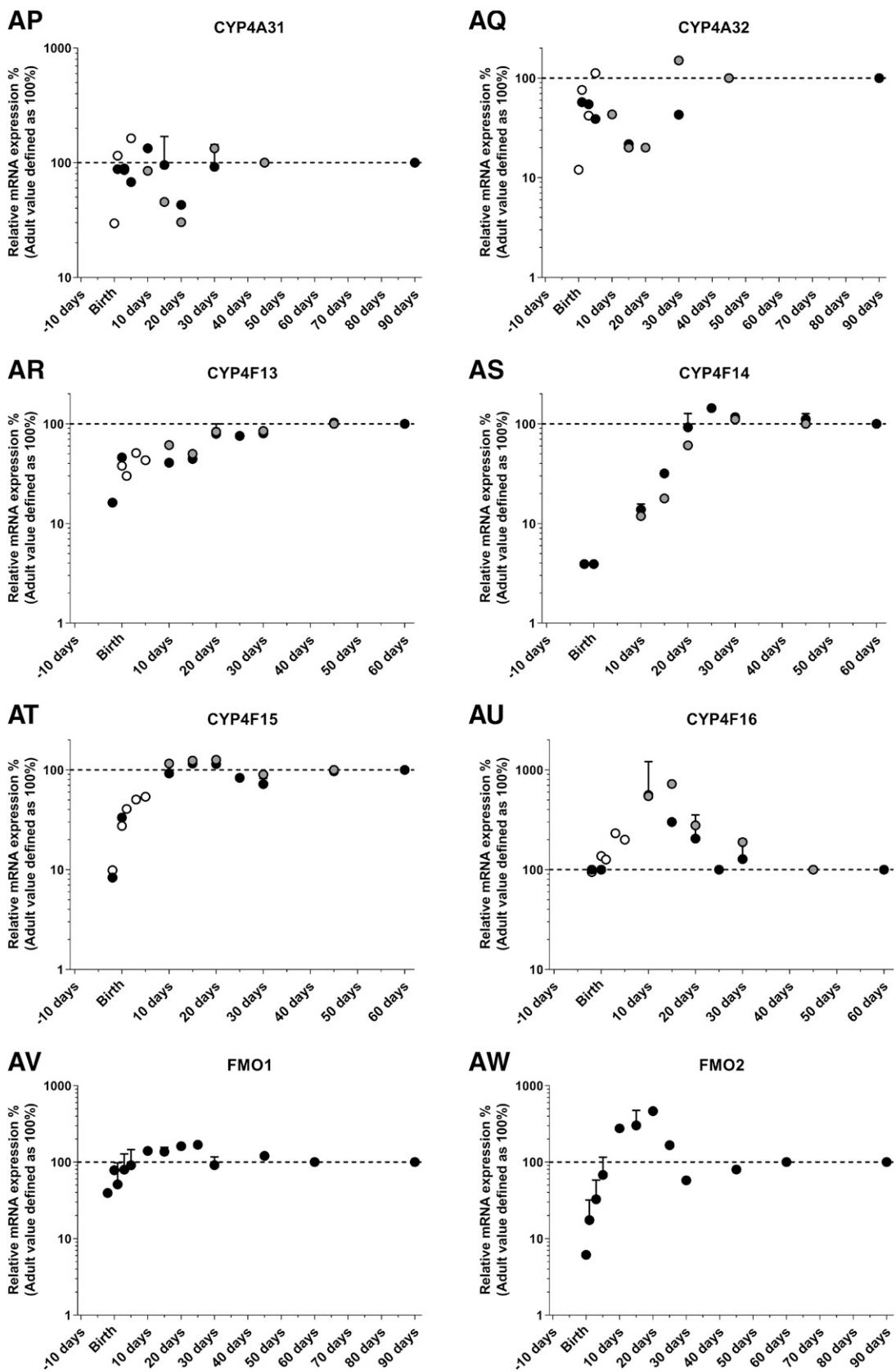


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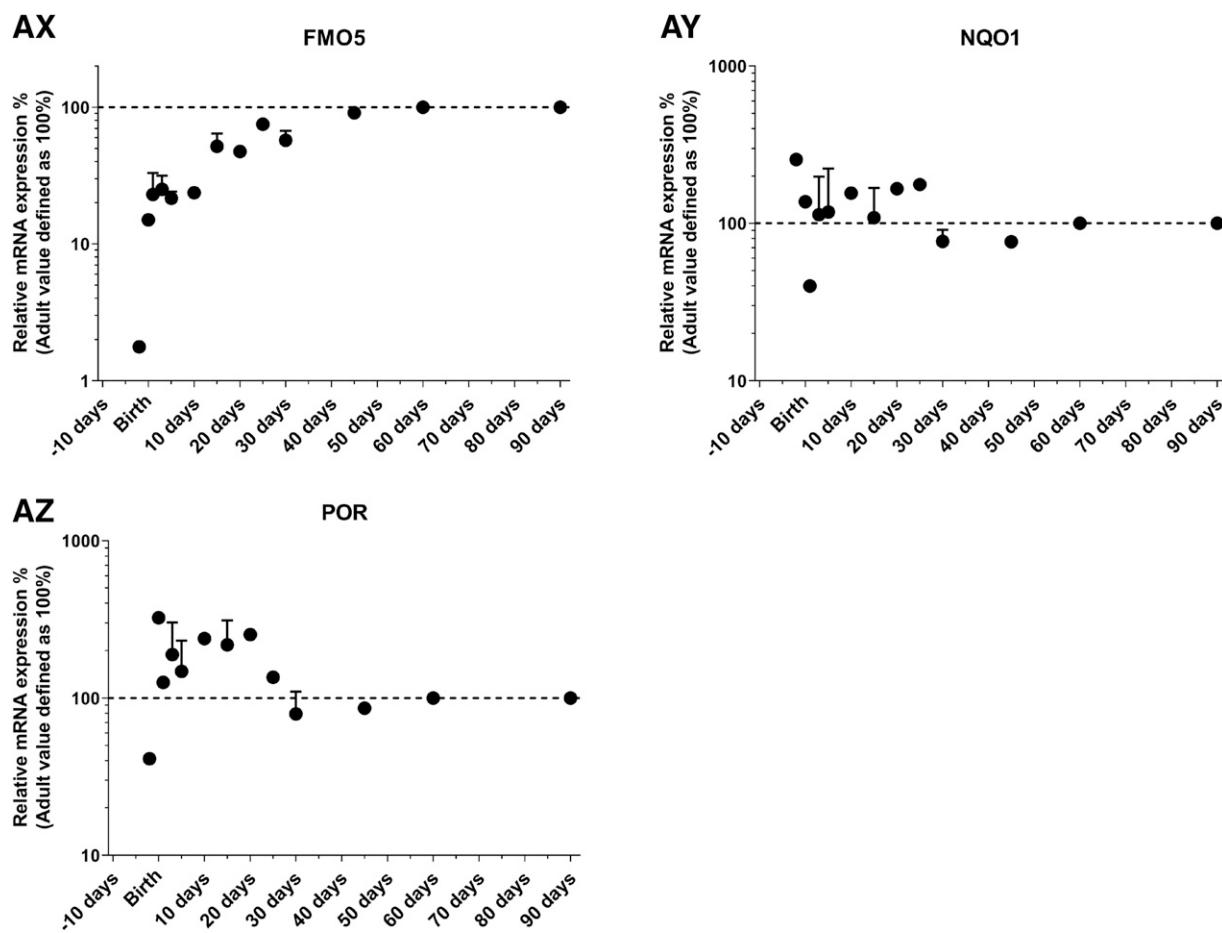


Fig. 11. Continued.

Glutathione-S-transferase, GSH peroxidase, and GSH reductase activity steadily increased from several days before birth and reached adult levels around 30 days of age, after which activity became variable. GSH reductase activity appeared to gradually decline between 60 and 800 days of age. Cytosolic GST activity comprised a conglomerate of GST activity of different classes of GST. For GSTA1, GSTM1, and GSTP1, both mRNA and protein expression levels were determined. GSTA1 and GSTP1 mRNA levels reached adult values a few days after birth and remained stable until 180 days postpartum, which was followed by a gradual increase until 800 days. GSTA1 and GSTP1 protein expression levels differed from mRNA expression levels in that they steadily increased from birth until 800 days. For GSTM1, the maturation profile of mRNA expression levels increased gradually from birth until 60 days, after which it steadily declined until reaching 30% of maximum levels. Protein expression levels followed a similar trend. GSTA4, GSTM3, GSTK1, GSTO1, GSTT1, and GSTT2 mRNA expression levels all showed a similar maturation profile with a start around birth, a steady increase until 60 days of age, and a gradual decline until 800 days. The latter decline did differ between different classes of GST.

b. Complex and/or inconsistent ontogeny pattern of activity/expression levels. UGT1A5 mRNA expression was observed at birth and persisted during the first 10 days of life, after which mRNA levels decreased until 30 days and remained stable until 56 days of age.

Rat hepatic SULT1B1 activity appeared stable between 2 days and 25 days of age with a gradual decline until 25 days. At 50 days, a 2–4-fold increase in activity was observed in liver samples from male and female rats, respectively. SULT1C1 and SULT1E1 mRNA profiles shared a similar pattern with low male and female levels at birth until 20 days of age. Subsequently, male levels increased markedly, reaching a maximum around 40 days of age. A comparable maturation profile was reported for male and female SULT-60 mRNA levels; however, the sex difference was inverted as compared with SULT1C1 and SULT1E1.

NAT1 activity was high during fetal development and gradually decreased until birth, after which it stabilized, whereas NAT1 mRNA levels increased suddenly at 15–20 days and remained stable.

c. No age-related changes in activity/expression. NAT2 (mRNA) levels were stable across all age ranges.

TABLE 9
Ontogeny profile of hepatic phase I enzymes in mice based on metabolic activity, protein expression, and mRNA expression levels
Percentages represent expression/activity relative to adult levels.

Onset of Expression/ Activity	Adult Levels Reached	Age-Related Changes (% of Adult) in Expression/Activity after Birth	Comments	References
CYP1A1 mRNA	NR (3 days: 35%)	10 days	Inconsistent pattern	Adult age: 45 days. Strain: C57BL/6 (mixed). Methods: bDNA and RT-PCR
CYP1A2 mRNA	0 days (2%)	20 days	Increased rapidly	Adult age: 45 days (Cui et al., 2012b); 60 days (Peng et al., 2012; Li et al., 2016); 90 days (Selwyn et al., 2015). Strain: C57BL/6 (mixed). Methods: RT-PCR (Cui et al., 2012b; Selwyn et al., 2015; Li et al., 2016), RNA-seq (Peng et al., 2012), bDNA (Cui et al., 2012b)
CYP2A4 mRNA	Fetal development (7%)	20 days	Increased slowly	Adult age: 45 days (Cui et al., 2012b); 60 days (Peng et al., 2012). Strain: C57BL/6 (mixed). Methods: RT-PCR (Cui et al., 2012b), RNA-seq (Peng et al., 2012), bDNA (Cui et al., 2012b)
CYP2A5 mRNA	Fetal development (1.7%)	20 days	Increased slowly	Adult age: 45 days (Cui et al., 2012b); 60 days (Peng et al., 2012); 90 days (Selwyn et al., 2015). Strain: C57BL/6 (mixed). Methods: RT-PCR (Cui et al., 2012b; Selwyn et al., 2015), RNA-seq (Peng et al., 2012), bDNA (Cui et al., 2012b)
CYP2B10 mRNA	0 days (20%)	5 days	Increased slowly	Adult age: 45 days (Cui et al., 2012b); 60 days (Peng et al., 2012; Li et al., 2016); Piekos et al., 2018), 90 days (Selwyn et al., 2015). Strain: C57BL/6 (mixed). Methods: RT-PCR (Cui et al., 2012b; Selwyn et al., 2015; Li et al., 2016; Piekos et al., 2018), RNA-seq (Peng et al., 2012), bDNA (Cui et al., 2012b)
CYP2B13 mRNA	M: 0 day (100%); F: NR (10 days: 17%)	M: 0 days; F: 45 days	M: inconsistent pattern; F: increased slowly	Adult age: 45 days (Cui et al., 2012b); 60 days (Peng et al., 2012). Strain: C57BL/6 (mixed). Methods: RT-PCR (Cui et al., 2012b), RNA-seq (Peng et al., 2012), bDNA (Cui et al., 2012b)
CYP2B23 mRNA	M: 0 days (100%); F: NR (10 days: 400%)	M: 30 days; F: 45 days	M: inconsistent pattern, decreased rapidly; F: Inconsistent pattern, decreased rapidly	Adult age: 45 days (Cui et al., 2012b); 60 days (Peng et al., 2012). Strain: C57BL/6 (mixed). Methods: RT-PCR (Cui et al., 2012b), RNA-seq (Peng et al., 2012), bDNA (Cui et al., 2012b)
CYP2B9 mRNA	M: 0 days (33%); F: NR (10 days: 85%)	M: 3 days; F: 10 days	M: inconsistent pattern; F: no changes	Adult age: 45 days (Cui et al., 2012b); 60 days (Peng et al., 2012); 90 days (Selwyn et al., 2015). Strain: C57BL/6 (mixed). Methods: RT-PCR (Cui et al., 2012b; Selwyn et al., 2015), RNA-seq (Peng et al., 2012), bDNA (Cui et al., 2012b)
CYP2C29 mRNA	M: fetal development (4%); F: NR (10 days: 4%)	20 days	Increased rapidly	Adult age: 45 days (Cui et al., 2012b); 60 days (Peng et al., 2012). Strain: C57BL/6 (mixed). Methods: RT-PCR (Cui et al., 2012b), RNA-seq (Peng et al., 2012), bDNA (Cui et al., 2012b)
CYP2C37 mRNA	Fetal development (20%)	15 days	Inconsistent pattern	Adult age: 45 days (Cui et al., 2012b); 60 days (Peng et al., 2012). Strain: C57BL/6 (mixed). Methods: RT-PCR (Cui et al., 2012b), RNA-seq (Peng et al., 2012), bDNA (Cui et al., 2012b)
CYP2C40 mRNA	M: NR (1 day: 23%); F: NR (10 days: 9%)	M: 30 days; F: 45 days	Increased slowly	Adult age: 45 days (Cui et al., 2012b); 90 days (Selwyn et al., 2015). Strain: C57BL/6 (mixed). Methods: RT-PCR (Cui et al., 2012b; Selwyn et al., 2015), bDNA (Cui et al., 2012b)
CYP2C44 mRNA	M: fetal development (15%); F: NR (15 days: 59%)	M: 45 days; F: 30 days	Increased slowly	Adult age: 45 days (Cui et al., 2012b); 60 days (Peng et al., 2012). Strain: C57BL/6 (mixed). Methods: RT-PCR (Cui et al., 2012b), RNA-seq (Peng et al., 2012), bDNA (Cui et al., 2012b)

(continued)

Ontogeny of Hepatic Transport and Drug Metabolism

TABLE 9—Continued

	Onset of Expression/ Activity	Adult Levels Reached	Age-Related Changes (% of Adult) in Expression/Activity after Birth	Comments	References
CYP2C50 mRNA	M: fetal development (1%–3%); F: NR (10 days: 23%)	M: 20 days; F: 20 days	Increased rapidly	Adult age: 45 days (Cui et al., 2012b), 60 days (Peng et al., 2012), 90 days (Selwyn et al., 2015). Strain: C57BL/6 (mixed). Methods: RT-PCR (Cui et al., 2012b; Selwyn et al., 2015), RNA-seq (Peng et al., 2012), bDNA (Cui et al., 2012b)	Cui et al. (2012b); Selwyn et al. (2015)
CYP2C54 mRNA	M: fetal development (2%); F: NR (20 days: 12%)	M: 45 days; F: 45 days	Increased rapidly	Adult age: 45 days (Cui et al., 2012b), 90 days (Selwyn et al., 2015). Strain: C57BL/6 (mixed). Methods: RT-PCR (Cui et al., 2012b; Selwyn et al., 2015), bDNA (Cui et al., 2012b)	Cui et al. (2012b); Selwyn et al. (2015)
CYP2C67 mRNA	NR (mixed 0 days: 33%)	M: 30 days; F: 45 days	Increased slowly	Adult age: 45 days (Cui et al., 2012b), 90 days (Selwyn et al., 2015). Strain: C57BL/6 (mixed). Methods: RT-PCR (Cui et al., 2012b; Selwyn et al., 2015), bDNA (Cui et al., 2012b)	Cui et al. (2012b); Selwyn et al. (2015)
CYP2C69 mRNA	M: fetal development (18%); F: NR (mixed 0 days: 61%)	M: 15 days; F: 45 days	Increased slowly	Adult age: 45 days (Cui et al., 2012b), 60 days (Peng et al., 2012), 90 days (Selwyn et al., 2015). Strain: C57BL/6 (mixed). Methods: RT-PCR (Cui et al., 2012b; Selwyn et al., 2015), RNA-seq (Peng et al., 2012), bDNA (Cui et al., 2012b)	Cui et al. (2012b); Peng et al. (2012); Selwyn et al. (2015)
CYP2C70 mRNA	Fetal development (7%)	15 days	Increased rapidly	Adult age: 45 days (Cui et al., 2012b), 60 days (Peng et al., 2012). Strain: C57BL/6 (mixed). Methods: RT-PCR (Cui et al., 2012b), RNA-seq (Peng et al., 2012), bDNA (Cui et al., 2012b)	Cui et al. (2012b); Peng et al. (2012)
CYP2D10 mRNA	M: fetal development (2%); F: NR (mixed 0 days: 8%)	45 days	Increased slowly	Adult age: 45 days (Cui et al., 2012b), 60 days (Peng et al., 2012). Strain: C57BL/6 (mixed). Methods: RT-PCR (Cui et al., 2012b), RNA-seq (Peng et al., 2012), bDNA (Cui et al., 2012b)	Cui et al. (2012b); Peng et al. (2012)
CYP2D22 mRNA	Fetal development (10%–15%)	20 days	Increased slowly	Adult age: 45 days (Cui et al., 2012b), 60 days (Peng et al., 2012). Strain: C57BL/6 (mixed). Methods: RT-PCR (Cui et al., 2012b), RNA-seq (Peng et al., 2012), bDNA (Cui et al., 2012b)	Cui et al. (2012b); Peng et al. (2012)
CYP2D26 mRNA	Fetal development (33%)	0 days	Increased rapidly	Adult age: 45 days (Cui et al., 2012b), 60 days (Peng et al., 2012). Strain: C57BL/6 (mixed). Methods: RT-PCR (Cui et al., 2012b), RNA-seq (Peng et al., 2012), bDNA (Cui et al., 2012b)	Cui et al. (2012b); Peng et al. (2012)
CYP2D9 mRNA	M: fetal development (2%); F: NR (mixed 0 days: 8.5%)	30 days	Increased slowly	Adult age: 45 days (Cui et al., 2012b), 60 days (Peng et al., 2012). Strain: C57BL/6 (mixed). Methods: RT-PCR (Cui et al., 2012b), RNA-seq (Peng et al., 2012), bDNA (Cui et al., 2012b)	Cui et al. (2012b); Peng et al. (2012)
CYP2E1 mRNA	M: fetal development (1%); F: NR (mixed 0 days: 33%)	5 days	Increased rapidly	Adult age: 45 days (Selwyn et al., 2015). Strain: C57BL/6 (mixed). Methods: RT-PCR (Cui et al., 2012b; Selwyn et al., 2015), RNA-seq (Peng et al., 2012), bDNA (Cui et al., 2012b)	Cui et al. (2012b); Peng et al. (2015); Selwyn et al. (2015)
CYP2F2 mRNA	0 days (3.5%–5%)	30 days	Increased slowly	Adult age: 45 days (Cui et al., 2012b), 60 days (Peng et al., 2012). Strain: C57BL/6 (mixed). Methods: RT-PCR (Cui et al., 2012b), RNA-seq (Peng et al., 2012), bDNA (Cui et al., 2012b)	Cui et al. (2012b); Peng et al. (2012)
CYP3A11 mRNA	Fetal development (2%)	20 days	Increased rapidly	Adult age: 45 days (Li et al., 2009; Cui et al., 2012b), 60 days (Peng et al., 2012; Li et al., 2016; Piekos et al., 2012b), 90 days (Selwyn et al., 2015). Strain: C57BL/6 (mixed). Methods: RT-PCR (Li et al., 2009; Cui et al., 2012b; Selwyn et al., 2015; Li et al., 2016; Piekos et al., 2018), RNA-seq (Peng et al., 2012; bDNA (Li et al., 2009; Cui et al., 2012b))	Li et al. (2009); Cui et al. (2012b); Peng et al. (2012); Selwyn et al. (2015); Li et al. (2016); Piekos et al. (2018)
CYP3A13					(continued)

TABLE 9—Continued

	Onset of Expression/ Activity	Adult Levels Reached	Age-Related Changes (% of Adult) in Expression/Activity after Birth	Comments	References
mRNA	Fetal development (10%–75%)	0 days	Nonlinear pattern		Cui et al. (2012b); Peng et al. (2012)
CYP3A25 mRNA	Fetal development (0%–30%)	20 days	Increased slowly	Adult age: 45 days (Cui et al., 2012b), 60 days (Peng et al., 2012). Strain: C57BL/6 (mixed). Methods: RT-PCR (Cui et al., 2012b), RNA-seq (Peng et al., 2012), bDNA (Cui et al., 2012b)	Li et al. (2009); Cui et al. (2012b); Peng et al. (2012); Selwyn et al. (2015)
CYP3A16 mRNA	Fetal development (75%–775%)	25 days	Decreased slowly	Adult age: 45 days (Li et al., 2009; Cui et al., 2012b), 60 days (Peng et al., 2012), 90 days (Selwyn et al., 2015). Strain: C57BL/6 (mixed). Methods: RT-PCR (Cui et al., 2012b; Selwyn et al., 2015), RNA-seq (Peng et al., 2012), bDNA (Li et al., 2009; Cui et al., 2012b)	Li et al. (2009); Cui et al. (2012b); Peng et al. (2012); Pieklos et al. (2018)
CYP3A41A mRNA	Fetal development (75%)	25 days	Increased slowly	Adult age: 45 days (Li et al., 2009; Cui et al., 2012b), 60 days (Peng et al., 2012). Strain: C57BL/6 (mixed). Methods: RT-PCR (Li et al., 2016; Pieklos et al., 2018), RNA-seq (Peng et al., 2012), bDNA (Li et al., 2009; Cui et al., 2012b)	Li et al. (2009); Cui et al. (2012b); Peng et al. (2012)
CYP3A41B mRNA	M: fetal development (15%–700%)	25 days	Inconsistent pattern	Adult age: 45 days (Cui et al., 2012b), 60 days (Peng et al., 2012). Strain: C57BL/6 (mixed). Methods: RT-PCR (Cui et al., 2012b), RNA-seq (Peng et al., 2012), bDNA (Cui et al., 2012b)	Cui et al. (2012b); Peng et al. (2012)
CYP3A44 mRNA	M: fetal development (75%); F: NR (mixed: 5%)	10 days	Increased rapidly	Adult age: 45 days (Cui et al., 2012b) (Li et al., 2009), 60 days (Peng et al., 2012). Strain: C57BL/6 (mixed). Methods: RT-PCR (Li et al., 2012b), RNA-seq (Peng et al., 2012), bDNA (Li et al., 2009; Cui et al., 2012b)	Li et al. (2009); Cui et al. (2012b); Peng et al. (2012)
CYP3A59 mRNA	M: fetal development (75%); F: NR (10 days: 100%)	45 days	Inconsistent pattern	Adult age: 45 days (Cui et al., 2012b), 60 days (Peng et al., 2012). Strain: C57BL/6 (mixed). Methods: RT-PCR (Cui et al., 2012b), RNA-seq (Peng et al., 2012), bDNA (Cui et al., 2012b)	Cui et al. (2012b); Peng et al. (2012)
CYP4A10 mRNA	M: 0 days (656%); F: NR (10 days: 69%)	Fluctuating around adult values	Inconsistent pattern	Adult age: 45 days (Cui et al., 2012b), 60 days (Peng et al., 2012). Strain: C57BL/6 (mixed). Methods: RT-PCR (Cui et al., 2012b; Selwyn et al., 2015), RNA-seq (Peng et al., 2012), bDNA (Cui et al., 2012b)	Cui et al. (2012b); Peng et al. (2012); Selwyn et al. (2015)
CYP4A14 mRNA	M: fetal development (7%); F: NR (10 days: 13%)	Fluctuating around adult values	Inconsistent pattern	Adult age: 45 days (Cui et al., 2012b), 60 days (Peng et al., 2012). Strain: C57BL/6 (mixed). Methods: RT-PCR (Cui et al., 2012b; Selwyn et al., 2015), RNA-seq (Peng et al., 2012), bDNA (Cui et al., 2012b)	Cui et al. (2012b); Peng et al. (2012); Selwyn et al. (2015)
CYP4A31 mRNA	NR (0 days: 30%)	Fluctuating around adult values	Inconsistent pattern	Adult age: 45 days (Cui et al., 2012b), 90 days (Selwyn et al., 2015). Strain: C57BL/6 (mixed). Methods: RT-PCR (Cui et al., 2012b; Selwyn et al., 2015), bDNA (Cui et al., 2012b)	Cui et al. (2012b); Selwyn et al. (2015)
CYP4A32 mRNA	M: 0 days (313%); F: NR (10 days: 43%)	Fluctuating around adult values	Inconsistent pattern	Adult age: 45 days (Cui et al., 2012b), 60 days (Peng et al., 2012). Strain: C57BL/6 (mixed). Methods: RT-PCR (Cui et al., 2012b; Selwyn et al., 2015), RNA-seq (Peng et al., 2012), bDNA (Cui et al., 2012b)	Cui et al. (2012b); Peng et al. (2012); Selwyn et al. (2015)
CYP4F13 mRNA	M: fetal development (16%); F: NR (10 days: 61%)	45 days	Increased slowly	Adult age: 45 days (Cui et al., 2012b), 60 days (Peng et al., 2012). Strain: C57BL/6 (mixed). Methods: RT-PCR (Cui et al., 2012b), RNA-seq (Peng et al., 2012), bDNA (Cui et al., 2012b)	Cui et al. (2012b); Peng et al. (2012)
CYP4F14					

(continued)

TABLE 9—Continued

	Onset of Expression/ Activity	Adult Levels Reached	Age-Related Changes (% of Adult) in Expression/Activity after Birth	Comments	References
mRNA	M: fetal development (4%); F: NR (10 days; 12%)	M: 20 days; F: 30 days	Increased rapidly	Adult age: 45 days (Cui et al., 2012b), 60 days (Peng et al., 2012). Strain: C57BL/6 (mixed). Methods: RT-PCR (Cui et al., 2012b), RNA-seq (Peng et al., 2012); bDNA (Cui et al., 2012b)	Cui et al. (2012b); Peng et al. (2012)
CYP4F15 mRNA	Fetal development (10%)	10 days	Increased slowly	Adult age: 45 days (Cui et al., 2012b), 60 days (Peng et al., 2012). Strain: C57BL/6 (mixed). Methods: RT-PCR (Cui et al., 2012b), RNA-seq (Peng et al., 2012); bDNA (Cui et al., 2012b)	Cui et al. (2012b); Peng et al. (2012)
CYP4F16 mRNA	Fetal development (100%)	25 days	Inconsistent pattern	Adult age: 45 days (Cui et al., 2012b), 60 days (Peng et al., 2012). Strain: C57BL/6 (mixed). Methods: RT-PCR (Cui et al., 2012b), RNA-seq (Peng et al., 2012); bDNA (Cui et al., 2012b)	Cui et al. (2012b); Peng et al. (2012)
FMO1 mRNA	Fetal development (40%)	30 days	Inconsistent pattern	Adult age: 56 days (Jammohamed et al., 2004), 60 days (Peng et al., 2013), 90 days (Selwyn et al., 2015). Strain: C57BL/6 (M) (Peng et al., 2013; Selwyn et al., 2015) and I29/SV (mixed) (Jammohamed et al., 2004). Methods: RT-PCR (Selwyn et al., 2015), RNA-seq (Peng et al., 2013), RNase protection assay (Jammohamed et al., 2004)	Jammohamed et al. (2004); Peng et al. (2013); Selwyn et al. (2015)
FMO2 mRNA	0 days (6%)	45 days	Inconsistent pattern	Adult age: 56 days (Jammohamed et al., 2004), 60 days (Peng et al., 2013), 90 days (Selwyn et al., 2015). Strain: C57BL/6 (M) (Peng et al., 2013; Selwyn et al., 2015) and I29/SV (mixed) (Jammohamed et al., 2004). Methods: RT-PCR (Selwyn et al., 2015), RNA-seq (Peng et al., 2013), RNase protection assay (Jammohamed et al., 2004)	Jammohamed et al. (2004); Peng et al. (2013); Selwyn et al. (2015)
FMO3 mRNA	Fetal development (132%)	60 days	Inconsistent pattern	Adult age: 56 days (Jammohamed et al., 2004), 60 days (Peng et al., 2013). Strain: C57BL/6 (M) (Peng et al., 2013; Selwyn et al., 2015) and I29/SV (mixed) (Jammohamed et al., 2004). Methods: RNA-seq (Peng et al., 2013), RNase protection assay (Jammohamed et al., 2004)	Jammohamed et al. (2004); Peng et al. (2013)
FMO4 mRNA	Fetal development (81%)	60 days	Inconsistent pattern	Adult age: 56 days (Jammohamed et al., 2004), 60 days (Peng et al., 2013). Strain: C57BL/6 (M) (Peng et al., 2013; Selwyn et al., 2015) and I29/SV (mixed) (Jammohamed et al., 2004). Methods: RNA-seq (Peng et al., 2013), RNase protection assay (Jammohamed et al., 2004)	Jammohamed et al. (2004); Peng et al. (2013)
FMO5 mRNA	Fetal development (2%)	45 days	Increased slowly	Adult age: 56 days (Jammohamed et al., 2004), 60 days (Peng et al., 2013), 90 days (Selwyn et al., 2015). Strain: C57BL/6 (M) (Peng et al., 2013; Selwyn et al., 2015) and I29/SV (mixed) (Jammohamed et al., 2004). Methods: RT-PCR (Selwyn et al., 2015), RNA-seq (Peng et al., 2013), RNase protection assay (Jammohamed et al., 2004)	Jammohamed et al. (2004); Peng et al. (2013); Selwyn et al. (2015)
NQO1 mRNA	Fetal development (255%)	Fluctuating around adult values	Inconsistent pattern	Adult age: 60 days (Peng et al., 2013), 90 days (Selwyn et al., 2015). Strain: C57BL/6 (M). Methods: RT-PCR (Selwyn et al., 2015), RNA-seq (Peng et al., 2013)	Peng et al. (2013); Selwyn et al. (2015)
POR mRNA	Fetal development (41%)	30 days	Inconsistent pattern	Adult age: 60 days (Peng et al., 2013), 90 days (Selwyn et al., 2015). Strain: C57BL/6 (M). Methods: RT-PCR (Selwyn et al., 2015), RNA-seq (Peng et al., 2013)	Peng et al. (2013); Selwyn et al. (2015)
ADH1 mRNA	Fetal development (3%–41%)	20 days	Increased rapidly	Adult age: 60 days. Strain: C57BL/6 (M). Methods: RNA-seq	Peng et al. (2013)
ALDH1A1 mRNA	Fetal development (6%)	45 days	Increased slowly	Adult age: 45 days (Alnouti and Klaassen, 2008) and 60 days (Li et al., 2016). Strain: C57BL/6 (mixed). Methods: RT-PCR (Li et al., 2016) and bDNA (Alnouti and Klaassen, 2008)	Alnouti and Klaassen (2008); Li et al., 2016

(continued)

TABLE 9—Continued

	Onset of Expression/ Activity	Adult Levels Reached	Age-Related Changes (% of Adult) in Expression/Activity after Birth	Comments	References
ALDH1B1 mRNA	Fetal development (35%)	15 days	Increased slowly	Adult age: 45 days (Ahouti and Klaassen, 2008) and 90 days (Selwyn et al., 2015). Strain: C57BL/6 (mixed). Methods: RT-PCR (Selwyn et al., 2015) and bDNA (Ahouti and Klaassen, 2008)	
ALDH3A2 mRNA	Fetal development (M: 32%, F: 16%)	M: 10 days; F: 45 days	Increased rapidly	Adult age: 45 days (Ahouti and Klaassen, 2008) and 90 days (Selwyn et al., 2015). Strain: C57BL/6 (mixed). Methods: RT-PCR (Selwyn et al., 2015) and bDNA (Ahouti and Klaassen, 2008)	
ALDH1A7 mRNA	Fetal development (2%–3%)	25 days	Increased slowly	Adult age: 45 days (Ahouti and Klaassen, 2008) and 60 days (Li et al., 2016). Strain: C57BL/6 (mixed). Methods: RT-PCR (Li et al., 2016) and bDNA (Ahouti and Klaassen, 2008)	
ALDH7A1 mRNA	Fetal development (25%)	22 days	Increased slowly	Adult age: 45 days (Ahouti and Klaassen, 2008) and 60 days (Li et al., 2016). Strain: C57BL/6 (mixed). Methods: RT-PCR (Li et al., 2016) and bDNA (Ahouti and Klaassen, 2008)	
AOX mRNA	1 day (2%)	60 days	Increased slowly	Adult age: 60 days (Peng et al., 2013), 90 days (Selwyn et al., 2015). Strain: C57BL/6 (M)	Peng et al. (2013); Selwyn et al. (2015)
CES2A mRNA	Fetal development (3%)	60 days	Increased slowly	Adult age: 60 days (Peng et al., 2013), 90 days (Selwyn et al., 2015). Strain: C57BL/6 (M)	Peng et al. (2013); Selwyn et al. (2015)

AOX, alternative oxidase; bDNA, branched DNA signal amplification assay; F, female; M, male; NQO1, NAD(P)H:quinone acceptor oxidoreductase-1; NR, not reported; POR, NADPH-cytochrome P450 reductase; RNA-seq, RNA sequencing; RT-PCR, reverse-transcriptase polymerase chain reaction.

3. Mouse. The results are depicted in Fig. 18, Supplemental Fig. 21, and Table 16 and are further explained below.

a. Age-related increase in activity/expression. The majority of phase II enzymes showed a lower expression in younger age groups than in older age groups. For some enzymes, the expression increased progressively directly after birth, often reaching adult values before or at 22 days postnatal age (PNA) [CES1B, CES1C, CES1G, CES2E, CES5, GSTA3, GSTA4, GSTK1, GSTM1, GSTM2, GSTM3, GSTM4, GSTT1, GSTT3, GSTZ1, NAT1, NAT12, SULT1B1, and UGT1A1 (male)], whereas for other enzymes the expression increased more gradually, reaching adult levels after 22 days PNA [CES1A, CES1D, CES1E, CES1F, CES2A, CES3B, CES6, CES7, microsomal glutathione S-transferase (MGST1), NAT2, NAT6, SULT3A1 (female), UGT1A1 (female), and UGT3A2].

b. Age-related decrease in expression. Only a few phase II enzymes showed a consistently higher expression at younger age groups than in older age groups (GSTCD, GSTM5, MGST2, NAT5, NAT11, NAT13).

c. No age-related changes in expression. There are few phase II enzymes that show no age-related changes in expression [CES2G, GSTT2, GSTM7, and SULT3A1 (male)].

d. Complex and/or inconsistent ontogeny patterns in expression. A number of phase II enzymes showed particular patterns of age-related changes in expression. These enzymes showed low expression at fetal age, which was followed by a peak in expression generally between 5 and 30 days PNA, after which expression dropped back to adult levels (SULT1A1, SULT1C2, SULT1D1, SULT2A1, SULT2A12, SULT2A2, SULT2A3, SULT2A4, SULT2A5, SULT2A6, and UGT1A9).

A few enzymes showed peaks and troughs in expression between younger and older age groups with an overall increasing trend (CES2B, CES2C, CES2D, CESPS, CES2F, CES3A, CES4A, GSTA1, GSTA2, GSTP1, GSTP2, NAT8, SULT2A7, SULT5A1, UGT1A2, UGT1A5, UGT1A6A, UGT1A6B, UGT1A10, UGT2B1, UGT2B5, UGT2B34, UGT2B35, UGT2B36, UGT2B37, UGT2B38, UGT3A1, and UGT3A2), whereas others did so with a general decreasing trend (SULT1E1, SULT2B1, and UGT1A7C).

Some enzymes showed contradictory (i.e., study-dependent) age-related expression profiles (MGST3, SULT1C1, GSTO1, and NAT10).

4. Nonrodents. The results are depicted in Fig. 19 and Table 17. For Göttingen minipig only, general UGT activity was studied. UGT activity gradually increased as a function of age, although only three data points are reported in literature.

IV. Discussion

A. Key Findings

- Multilevel and cross-species compilation of published ontogeny profiles of hepatic DTs and DMEs

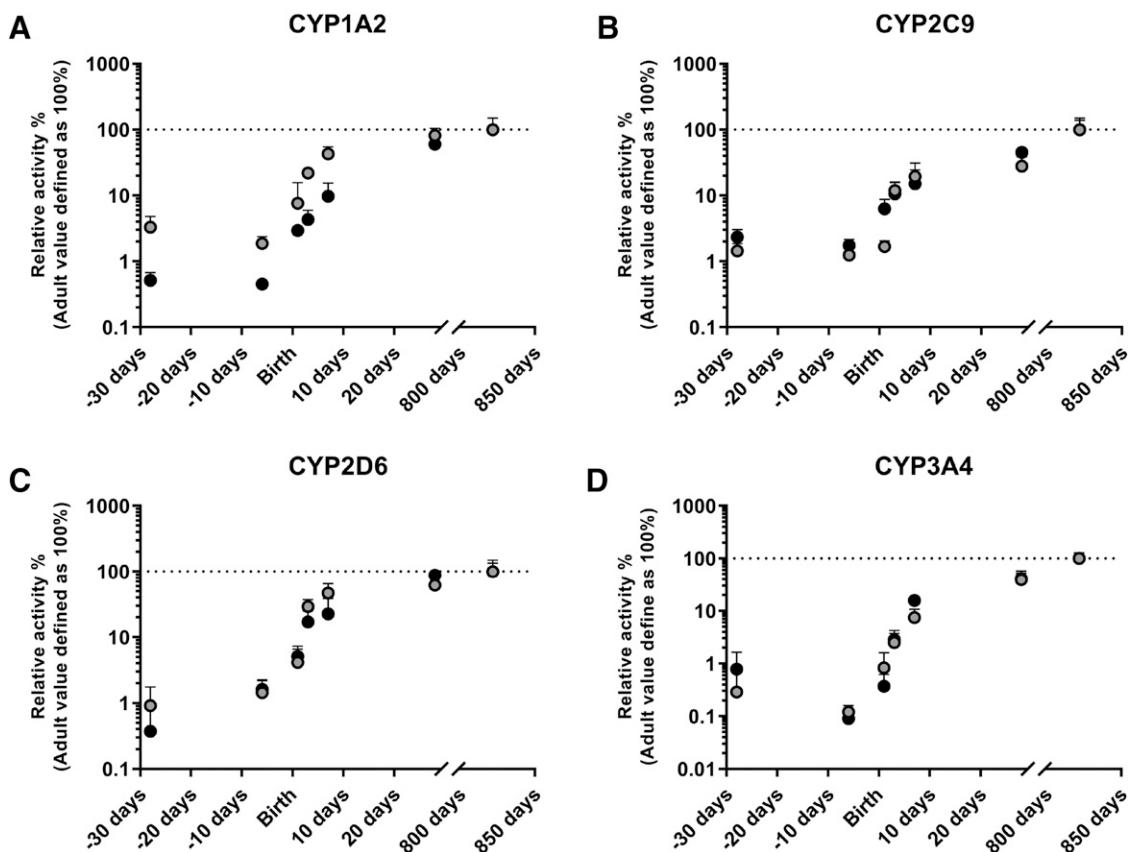


Fig. 12. Pooled literature data on the ontogeny of metabolic activity of hepatic phase I enzymes in Göttingen minipig: CYP1A2 (A), CYP2C9 (B), CYP2D6 (C), and CYP3A4 (D). The symbols represent the relative activity in each age group, and the dotted line indicates the adult value defined as 100%. If multiple values were obtained for the same age group, the symbols represent the average relative activity, and the error bars show the S.D. See Table 10 for explanation on the ontogeny profiles and literature references.

was used to obtain a quantitative understanding of the developmental biology of drug metabolism and transport.

- Most DTs and DMEs undergo substantial maturation after birth, typically showing developmental patterns with either significant increase or decrease in expression or activity levels with progressing age.
- The limitations associated with interpretation and application of ontogeny profiles across multiple studies should be acknowledged: technical, methodological, and analytical sources of variability confound accurate registration of interindividual variability in activity or expression levels. Examples of study-specific sources of variability include postmortem versus surgical biopsies, lack of subject demographic information, Western blotting versus LC-MS/MS-based proteomic quantitative methods, and different units of normalization or expression.
- Substantial knowledge gaps regarding the ontogeny of hepatic DTs/DMEs remain to be addressed. A notable example is the clear lack of information regarding ontogeny of DTs and DMEs in monkeys despite their frequent use in ontogeny-related studies. Also, no sex- or ethnicity-related differences

in ontogeny profile of human hepatic DTs and DMEs could be identified based on the currently available data.

B. Discussion

The overarching goal of this review was to compile multilevel (i.e., at mRNA expression, protein expression, and activity levels) ontogeny profiles of hepatic DTs and DMEs in humans and in nonclinical species. For many isoforms, data from multiple studies were combined, thus frequently yielding high-resolution ontogeny profiles sometimes at the protein activity level as well as the mRNA/protein expression levels. Subsequent interpretation of these profiles allowed obtaining more complete quantitative insight into the developmental changes in expression and activity levels of these pivotal hepatic DTs and DMEs.

Various developmental patterns emerged, which were classified as follows: 1) age-associated increase in activity/expression; 2) age-associated decrease in activity/expression; 3) no obvious associations of age with activity/expression; and 4) complex and/or inconsistent ontogeny profile(s). For those that showed an age-related change in activity/expression, developmental patterns were particularly isoform-dependent and

TABLE 10
Ontogeny profile of hepatic CYP enzymes in Göttingen minipig based on metabolic activity, protein expression, and mRNA expression levels

Percentages represent expression/activity relative to adult levels.

	Onset of Activity and/or Expression	Adult Levels Reached	Age-Related Changes in Activity/Expression	Comments	References
CYP1A2 Catalytic activity	M: -28 days (0.5%); F: -28 days (3%)	M: NR (60% at 28 days); F: NR (81% at 28 days)	Increased rapidly	Single study, not clear whether substrate is specific in Göttingen minipigs. Adult age: 822 days. Substrate: phenacetin O-deethylation. Methods: PLM. Strain: Göttingen minipig (M/F)	Van Peer et al. (2017)
CYP2C9 Catalytic activity	M: -28 days (2%); F: -28 days (1.5%)	M: NR (45% at 28 days); F: NR (28% at 28 days)	Increased rapidly	Single study, not clear whether substrate is specific in Göttingen minipigs. Adult age: 822 days. Substrate: 4-hydroxylation of tolbutamide. Methods: PLM. Strain: Göttingen minipig (M/F)	Van Peer et al. (2017)
CYP2D6 Catalytic activity	M: -28 days (0.4%); F: -28 days (1%)	M: 28 days; F: NR (61% at 28 days)	Increased rapidly	Single study, not clear whether substrate is specific in Göttingen minipigs. Adult age: 822 days. Substrate: dextromethorphan O-demethylation. Methods: PLM. Strain: Göttingen minipig (M/F)	Van Peer et al. (2017)
CYP3A Catalytic activity	M: -28 days (0.8%); F: -28 days (0.3%)	M: NR (39% at 28 days); F: NR (41% at 28 days)	Increased rapidly	Single study, not clear whether substrate is specific in Göttingen minipigs. Adult age: 822 days. Substrate: Luciferin-IPA, Midazolam. Methods: PLM. Strain: Göttingen minipig (M/F)	Van Peer et al., (2014, 2015, 2017)
Protein expression	M/F: -30 days (31%)	150 days (85%)	Increased rapidly	Single study, conflicting data between papers. Adult age: 822 days. Substrate: rabbit polyclonal anti-human anti-CYP3A4 antibody/CYP3A22, CYP3A29, CYP3A39, CYP3A46. Methods: PLM. Strain: Göttingen minipig (M/F)	

F, female; IPA, isopropyl acetate; M, male; NR, not reported; PLM, pig liver microsome.

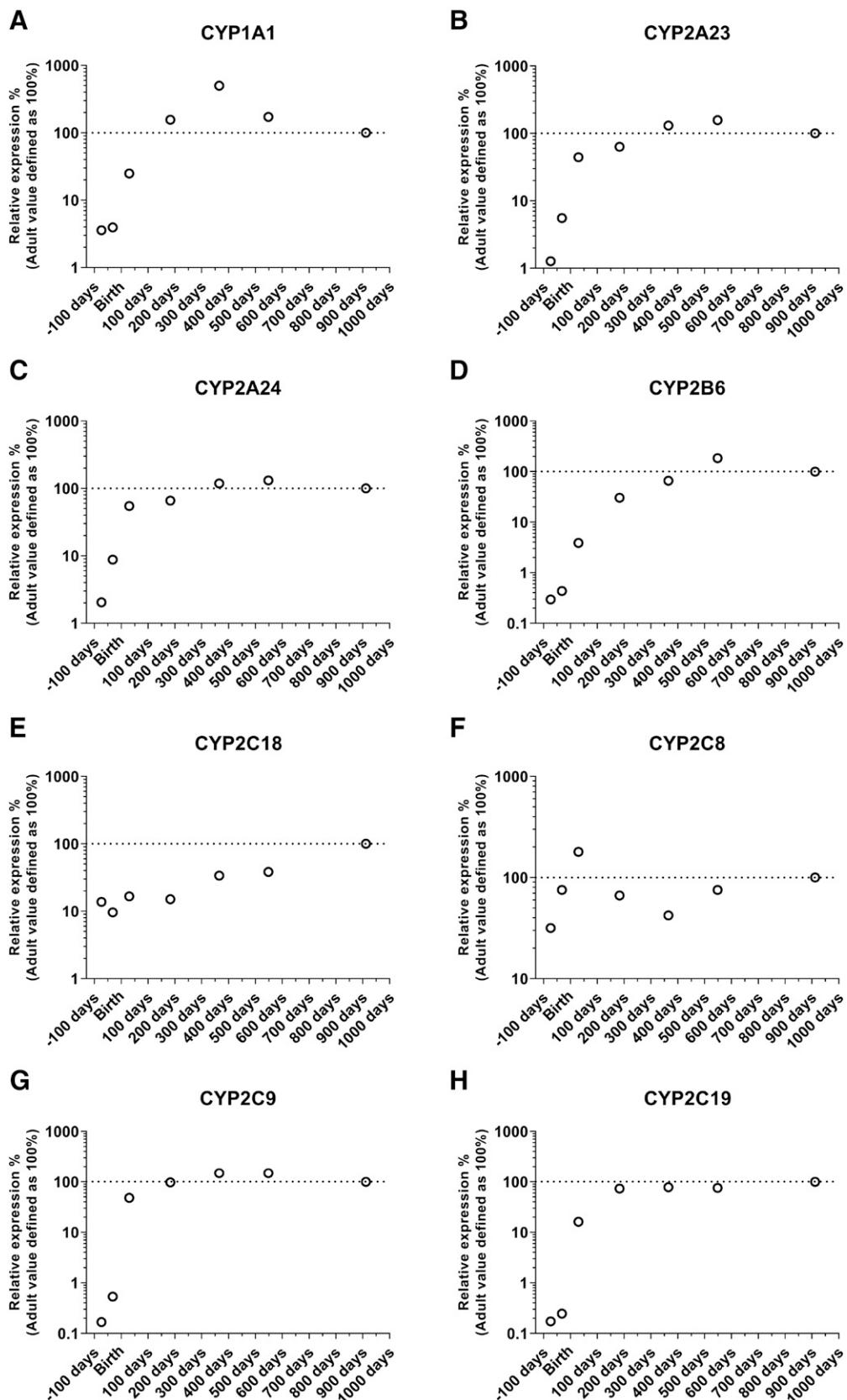


Fig. 13. Pooled literature data on the ontogeny of hepatic mRNA expression of hepatic phase I enzymes in cynomolgus monkey: CYP1A1 (A), CYP2A23 (B), CYP2A24 (C), CYP2B6 (D), CYP2C18 (E), CYP2C8 (F), CYP2C9 (G), CYP2C19 (H), CYP2C76 (I), CYP2D17 (J), CYP2E1 (K), CYP2J2 (L), CYP3A4 (M), CYP3A5 (N), CYP3A43 (O), CYP4A11 (P), CYP4F3 (Q), CYP4F11 (R), CYP4F12 (S), and CYP4F2 (T). The symbols represent the relative mRNA expression in each age group, and the dotted line indicates the adult value defined as 100%. See Table 11 for explanation on the ontogeny profiles and literature references.

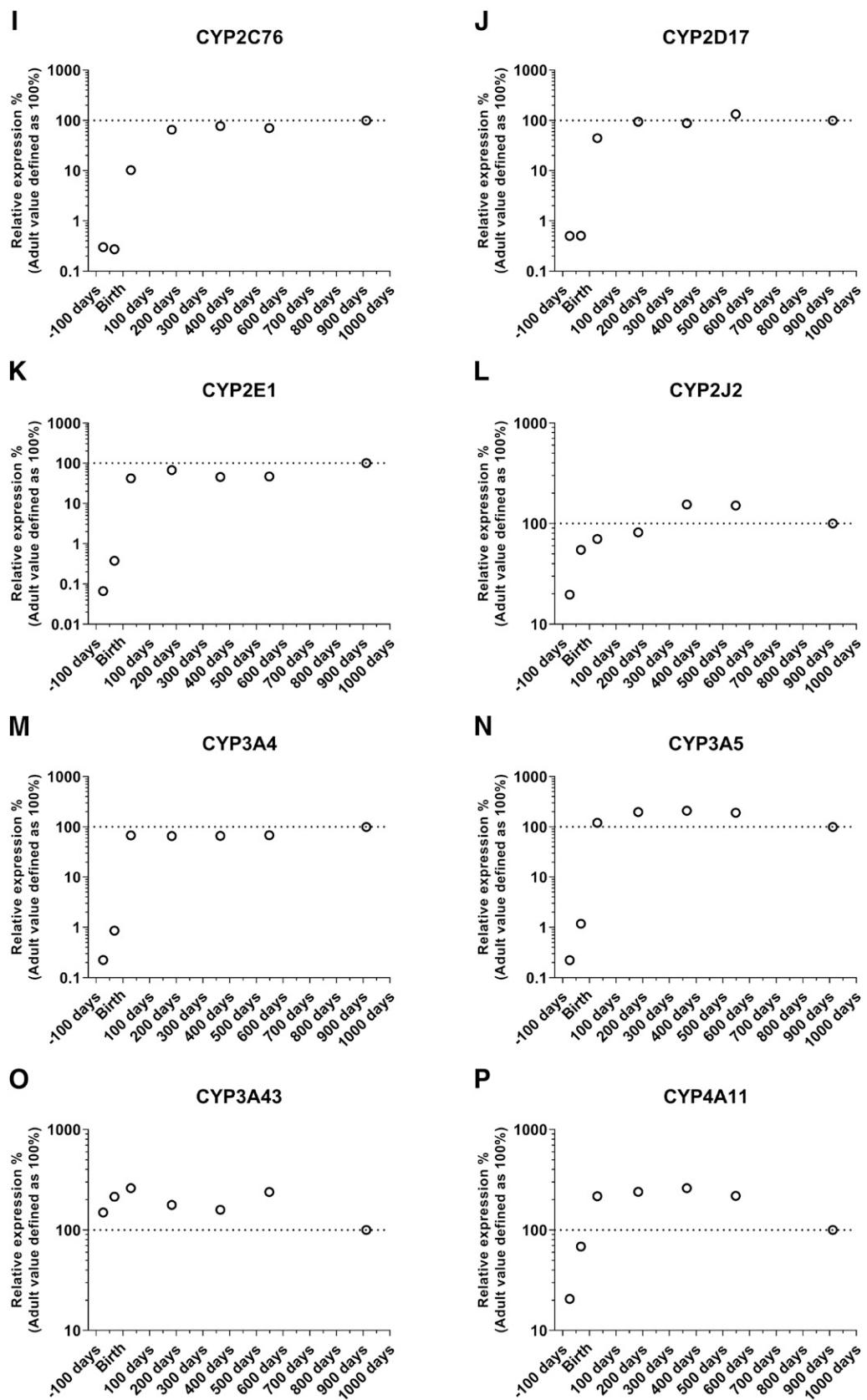


Fig. 13. Continued.

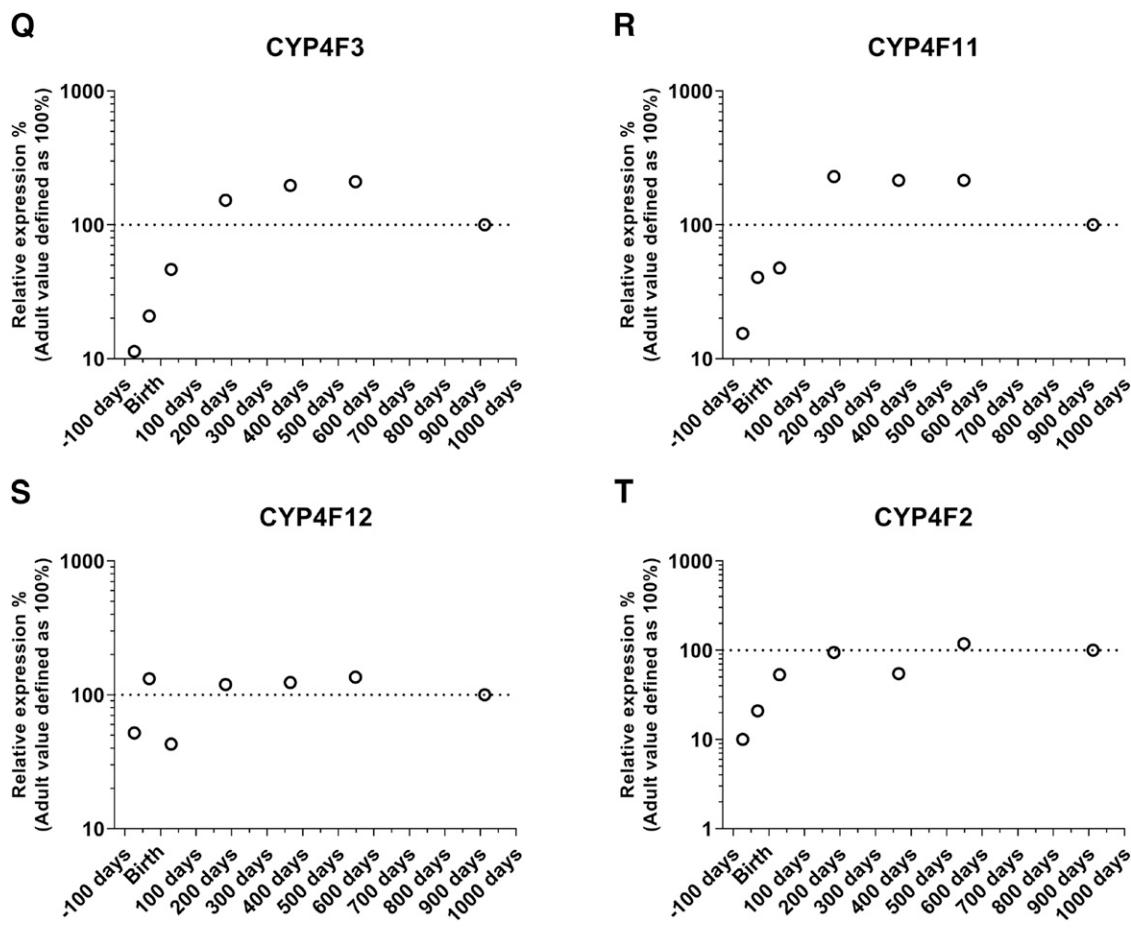


Fig. 13. Continued.

emerged at various rates. This resulted in identification of DTs and DMEs that reached adult levels shortly after birth [e.g., mRNA expression of MRP6 in mice (Table 6) or CYP27 in rats (Table 8) both increased rapidly], whereas others showed a more gradual increase/decrease [e.g., mRNA expression of CYP2A5 in mice increased slowly from fetal age until adult values were reached at 20 days of age (Table 9)].

Despite the increase in literature data, important knowledge gaps were identified while compiling these ontogeny data. One of the reasons is the scarcity of pediatric liver tissue, especially neonates (Brouwer et al., 2015). Human protein expression data remain limited for CYP2A6, CYP2C8, CYP2C9, CYP2C19, GSTP1, and SULT1A1, as data on older age groups were missing. The same holds true for mRNA expression of CYP3A7 and SULT2A1. In mice and cynomolgus monkey, all ontogeny profiles on CYP enzymes were derived from mRNA data only, and several CYP enzymes lack activity data in all other nonclinical species. In mice, most ontogeny profiles of phase II enzymes were derived from mRNA expression, whereas no phase II ontogeny data were available in nonrodent species. Finally, the mechanisms underlying the ontogeny of DTs and DMEs are largely unknown. A potential

mechanism is that nuclear transcription factor activity/expression involved in the expression of DTs and DMEs is also subject to age-related changes. For example, the mRNA expression of the nuclear transcription factor pregnane X receptor (PXR) was correlated with age in human livers (0–25 yr of age) (Neumann et al., 2016). In mouse livers the induction potential using substrates for aryl hydrocarbon receptor, PXR, and constitutive androstane receptor was age-specific (Li et al., 2016). The same was seen in rat livers for aryl hydrocarbon receptor, PXR, and constitutive androstane receptor mRNA expression (Xu et al., 2019). However, how these findings relate to DT and DME ontogeny should be further studied. The same considerations can be made for cofactors involved in metabolism, such as uridine diphosphate glucuronic acid, which supports compound glucuronidation. However, to the best of our knowledge, no literature is available on age-related changes in these cofactors levels.

Interestingly, in the case of DTs, data on mRNA expression were more limited than data on protein expression, whereas for DMEs, this was the other way around. A potential explanation is that the impact of DTs on drug disposition was recognized later than that of DMEs. In the earlier days, information on DMEs

TABLE 11
Ontogeny profile of hepatic phase I enzymes in cynomolgus monkey based on mRNA expression levels

Percentages represent expression/activity relative to adult levels.

	Onset of Activity and/or Expression	Adult Levels Reached	Age-Related Changes in Activity/Expression	Comments	References
CYP1A1 mRNA expression	F: -74 days (3.6%)	NR (156% at 183 days)	Inconsistent pattern/increased rapidly	Single study, only M at 6 mo and 2 to 3 yr, only F at -74 days. Adult age: 2 to 3 yr. Fetal development: -74 days only F. Methods: DNA microarray. Strain: cynomolgus macaque (M/F)	Ise et al. (2011)
CYP2A23 mRNA expression	F: -74 days (1.3%)	NR (130% at 365 days)	Increased rapidly	Single study, only M at 6 mo and 2 to 3 yr, only F at -74 days. Adult age: 2 to 3 yr. Fetal development: -74 days only F. Methods: DNA microarray. Strain: cynomolgus macaque (M/F)	Ise et al. (2011)
CYP2A24 mRNA expression	F: -74 days (2%)	NR (118% at 365 days)	Increased rapidly	Single study, only M at 6 mo and 2 to 3 yr, only F at -74 days. Adult age: 2 to 3 yr. Fetal development: -74 days only F. Methods: DNA microarray. Strain: cynomolgus macaque (M/F)	Ise et al. (2011)
CYP2B6 mRNA expression	F: -74 days (0.3%)	NR (183% at 365 days)	Increased slowly	Single study, only M at 6 mo and 2 to 3 yr, only F at -74 days. Adult age: 2 to 3 yr. Fetal development: -74 days only F. Methods: DNA microarray. Strain: cynomolgus macaque (M/F)	Ise et al. (2011)
CYP2C8 mRNA expression	F: -74 days (32%)	NR (75% at 365 days)	Inconsistent pattern	Single study, only M at 6 mo and 2 to 3 yr, only F at -74 days. Adult age: 2 to 3 yr. Fetal development: -74 days only F. Methods: DNA microarray. Strain: cynomolgus macaque (M/F)	Ise et al. (2011)
CYP2C9 mRNA expression	F: -74 days (0.2%)	183 days	Increased rapidly	Single study, only M at 6 mo and 2 to 3 yr, only F at -74 days. Adult age: 2 to 3 yr. Fetal development: -74 days only F. Methods: DNA microarray. Strain: cynomolgus macaque (M/F)	Ise et al. (2011)
CYP2C18 mRNA expression	F: -74 days (14%)	NR (38% at 365 days)	Increased slowly	Single study, only M at 6 mo and 2 to 3 yr, only F at -74 days. Adult age: 2 to 3 yr. Fetal development: -74 days only F. Methods: DNA microarray. Strain: cynomolgus macaque (M/F)	Ise et al. (2011)
CYP2C19 mRNA expression	F: -74 days (0.2%)	NR (75% at 548 days)	Increased rapidly/increased progressively	Single study, only M at 6 mo and 2 to 3 yr, only F at -74 days. Adult age: 2 to 3 yr. Fetal development: -74 days only F. Methods: DNA microarray. Strain: cynomolgus macaque (M/F)	Ise et al. (2011)
CYP2C76 mRNA expression	F: -74 days (0.3%)	NR (70% at 548 days)	Increased rapidly/increased progressively	Single study, only M at 6 mo and 2 to 3 yr, only F at -74 days. Adult age: 2 to 3 yr. Fetal development: -74 days only F. Methods: DNA microarray. Strain: cynomolgus macaque (M/F)	Ise et al. (2011)
CYP2D17 mRNA expression	F: -74 days (0.5%)	183 days	Increased rapidly	Single study, only M at 6 mo and 2 to 3 yr, only F at -74 days. Adult age: 2 to 3 yr. Fetal development: -74 days only F. Methods: DNA microarray. Strain: cynomolgus macaque (M/F)	Ise et al. (2011)
CYP2E1 mRNA expression	F: -74 days (0.07%)	NR (46% at 548 days)	Increased rapidly/increased progressively	Single study, only M at 6 mo and 2 to 3 yr, only F at -74 days. Adult age: 2 to 3 yr. Fetal development: -74 days only F. Methods: DNA microarray. Strain: cynomolgus macaque (M/F)	Ise et al. (2011)
CYP2B2 mRNA expression	F: -74 days (20%)	NR (81% at 183 days)	Nonlinear pattern/increased rapidly	Single study, only M at 6 mo and 2 to 3 yr, only F at -74 days. Adult age: 2 to 3 yr. Fetal development: -74 days only F. Methods: DNA microarray. Strain: cynomolgus macaque (M/F)	Ise et al. (2011)
CYP3A4 mRNA expression	F: -74 days (0.2%)	NR (68% at 30 days)	Increased rapidly	Single study, only M at 6 mo and 2 to 3 yr, only F at -74 days. Adult age: 2 to 3 yr. Fetal development: -74 days only F. Methods: DNA microarray. Strain: cynomolgus macaque (M/F)	Ise et al. (2011)
CYP3A5					

(continued)

TABLE 11—Continued

	Onset of Activity and/or Expression	Adult Levels Reached	Age-Related Changes in Activity/Expression	Comments	References
mRNA expression	F: -74 days (0.2%)	30 days	Inconsistent pattern	Single study, only M at 6 mo and 2 to 3 yr, only F at -74 days. Adult age: 2 to 3 yr. Fetal development: -74 days only F. Methods: DNA microarray. Strain: cynomolgus macaque (M/F)	Ise et al. (2011)
CYP3A43 mRNA expression	F: -74 days (149%)	-74 days	No changes/inconsistent pattern	Single study, only M at 6 mo and 2 to 3 yr, only F at -74 days. Adult age: 2 to 3 yr. Fetal development: -74 days only F. Methods: DNA microarray. Strain: cynomolgus macaque (M/F)	Ise et al. (2011)
CYP4A11 mRNA expression	F: -74 days (21%)	NR (216% at 30 days)	Inconsistent pattern/increased rapidly	Single study, only M at 6 mo and 2 to 3 yr, only F at -74 days. Adult age: 2 to 3 yr. Fetal development: -74 days only F. Methods: DNA microarray. Strain: cynomolgus macaque (M/F)	Ise et al. (2011)
CYP4F3 mRNA expression	F: -74 days (11%)	NR (152% at 183 days)	Inconsistent pattern/increased rapidly	Single study, only M at 6 mo and 2 to 3 yr, only F at -74 days. Adult age: 2 to 3 yr. Fetal development: -74 days only F. Methods: DNA microarray. Strain: cynomolgus macaque (M/F)	Ise et al. (2011)
CYP4F11 mRNA expression	F: -74 days (15%)	NR (230% at 183 days)	Inconsistent pattern/increased rapidly	Single study, only M at 6 mo and 2 to 3 yr, only F at -74 days. Adult age: 2 to 3 yr. Fetal development: -74 days only F. Methods: DNA microarray. Strain: cynomolgus macaque (M/F)	Ise et al. (2011)
CYP4F12 mRNA expression	F: -74 days (52%)	-74–30 days	No changes (variable)	Single study, only M at 6 mo and 2 to 3 yr, only F at -74 days. Adult age: 2 to 3 yr. Fetal development: -74 days only F. Methods: DNA microarray. Strain: cynomolgus macaque (M/F)	Ise et al. (2011)
CYP4F2 mRNA expression	F: -74 days (10%)	183 days	Increased rapidly	Single study, only M at 6 mo and 2 to 3 yr, only F at -74 days. Adult age: 2 to 3 yr. Fetal development: -74 days only F. Methods: DNA microarray. Strain: cynomolgus macaque (M/F)	Ise et al. (2011)

F, female; M, male; NR, not reported.

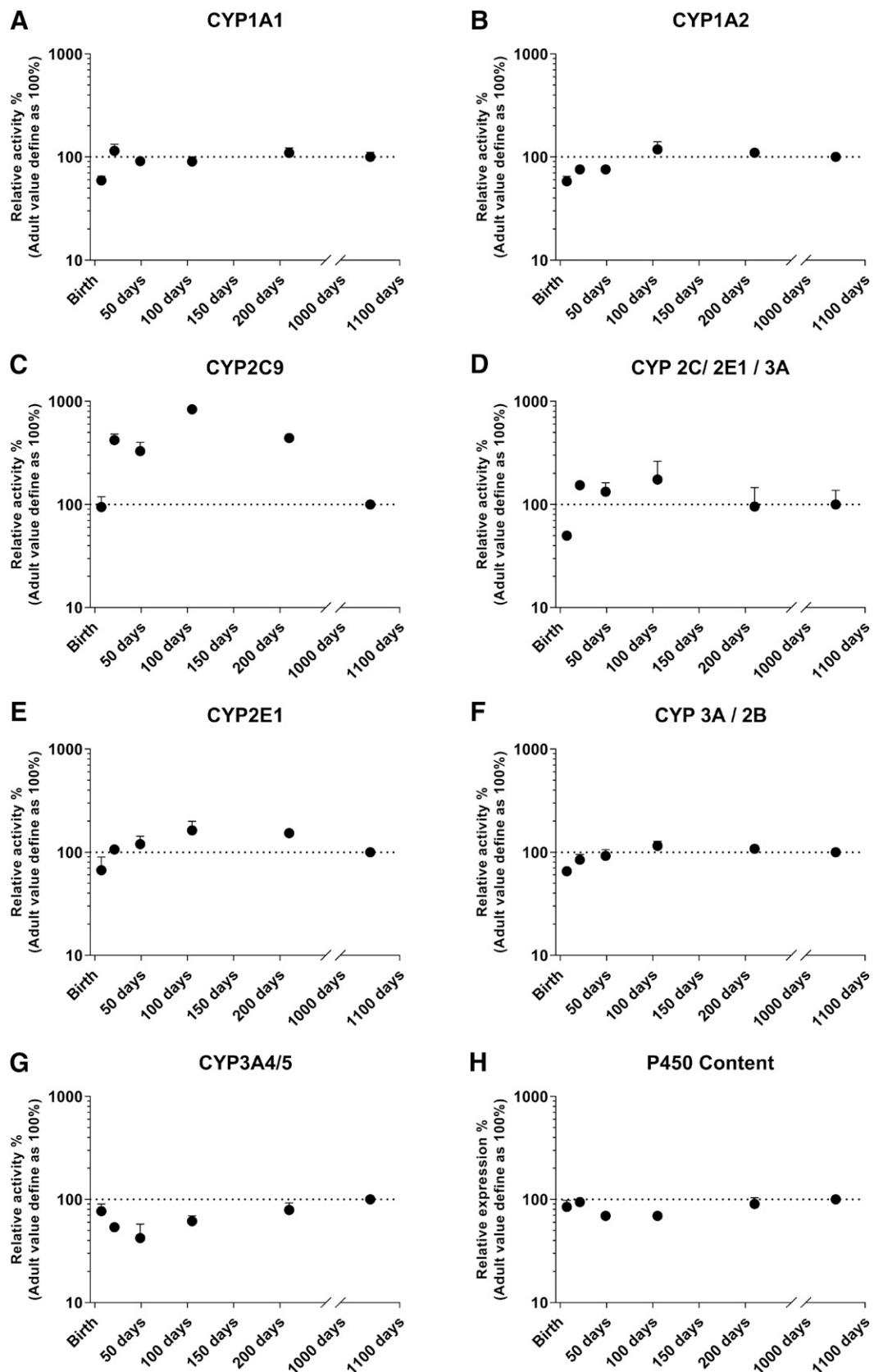


Fig. 14. Pooled literature data on the ontogeny of hepatic metabolic activity of phase I enzymes in Beagle dogs: CYP1A1 (A), CYP1A2 (B), CYP2C/2E1/3A (D), CYP2E1 (E), CYP3A/2B (F), CYP3A4/5 (G), and P450 content (H). The symbols represent the relative activity in each age group, and the dotted line indicates the adult value defined as 100%. If multiple values were obtained for the same age group, the symbols represent the average relative activity, and the error bars show the S.D. See Table 12 for explanation on the ontogeny profiles and literature references. P450, cytochrome P450.

TABLE 12
Ontogeny profile of hepatic CYP enzymes and hepatic CYP content in Beagle dog based on metabolic activity and protein expression levels

	Onset of Activity and/or Expression	Adult Levels Reached	Age-Related Changes in Activity/ Expression	Comments	References
CYP1A1 Catalytic activity	7 days (59%)	21 days	No changes	Single study, not clear whether substrate is specific in Beagle dogs. Fetal development: NR. Adult age: 1050 days. Substrate: p-nitroanisole O-demethylation. Methods: DLM. Strain: Beagle dog (M)	Tanaka et al. (1998)
CYP2A2 Catalytic activity	7 days (58%)	105 days	Increased rapidly/no changes	Single study, not clear whether substrate is specific in Beagle dogs. Fetal development: NR. Adult age: 1050 days. Substrate: caffeine N-demethylation. Methods: DLM. Strain: Beagle dog (M)	Tanaka et al. (1998)
CYP2C9 Catalytic activity	7 days (94%)	7 days	Increased rapidly/inconsistent pattern	Single study, not clear whether substrate is specific in Beagle dogs. Fetal development: NR. Adult age: 1050 days. Substrate: phenytoin hydroxylation. Methods: DLM. Strain: Beagle dog (M)	Tanaka et al. (1998)
CYP2E1 Catalytic activity	7 days (66%)	21 days	Increased rapidly/no changes	Single study, not clear whether substrate is specific in Beagle dogs. Fetal development: NR. Adult age: 1050 days. Substrate: aniline hydroxylation. Methods: DLM. Strain: Beagle dog (M)	Tanaka et al. (1998)
CYP3A4/5 Catalytic activity	7 days (77%)	NR (variable)	Inconsistent pattern	Single study, not clear whether substrate is specific in Beagle dogs. Fetal development: NR. Adult age: 1050 days. Substrate: erythromycin N-demethylation. Methods: DLM. Strain: Beagle dog (M)	Tanaka et al. (1998)
CYP3A2B Catalytic activity	7 days (65%)	49 days	Increased rapidly/no changes	Single study, not clear whether substrate is specific in Beagle dogs. Fetal development: NR. Adult age: 1050 days. Substrate: benzphetamine N-demethylation. Methods: DLM. Strain: Beagle dog (M)	Tanaka et al. (1998)
CYP2C/2E1/3A Catalytic activity	7 days (50%)	NR (154% at 21 days)	Increased rapidly/inconsistent pattern	Single study, not clear whether substrate is specific in Beagle dogs. Fetal development: NR. Adult age: 1050 days. Substrate: trimethadion N-demethylation. Methods: DLM. Strain: Beagle dog (M)	Tanaka et al. (1998)
Cytochrome P450 content Protein expression	NR	NR	Inconsistent pattern	Single study. Fetal development: NR. Adult age: 1050 days. Methods: Omura and Sato (DLM). Unit: nanomoles per milligram microsomal protein	Tanaka et al. (1998)

DLM, dog liver microsome; M, male; NR, not reported.

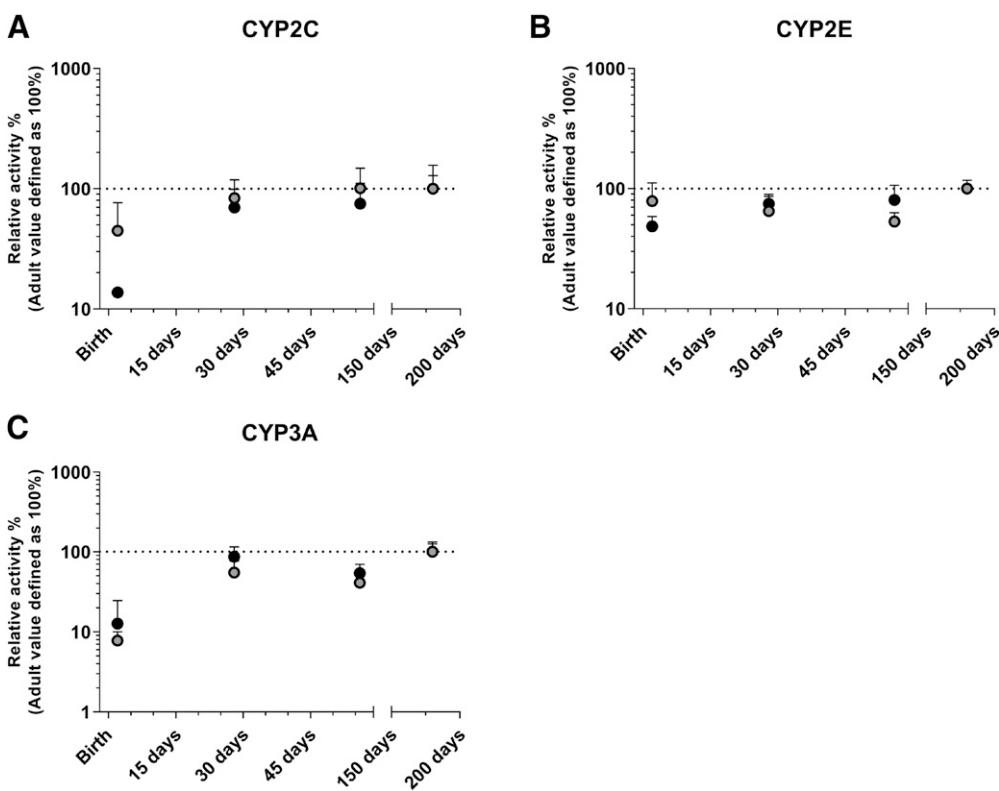


Fig. 15. Pooled literature data on the ontogeny of hepatic metabolic activity of phase I enzymes in the domestic pig: CYP2C (A), CYP2E (B), and CYP3A (C). The symbols represent the relative activity in each age group and the dotted line indicates the adult value defined as 100%. If multiple values were obtained for the same age group, the symbols represent the average relative activity and the error bars show the S.D. See Table 13 for explanation on the ontogeny profiles and literature references.

mostly came from mRNA expression data because analytical methods for protein quantification became more advanced just recently. There is still a major lack of knowledge on transporter ontogeny in all species. At the activity level, few studies were published on age-dependent activity of OATP, OCT1, and NTCP in suspended rat hepatocytes. This represents a critical gap because gene expression or protein abundance rarely correlates well with transporter activity. Hence, activity data are essential to accurately predict the impact of age-related changes on drug disposition (Brouwer et al., 2015). For several DMEs, validated *in vivo* markers are available, as exemplified by the drug midazolam that is often used for CYP3A activity (de Wildt et al., 2009) or dextromethorphan for CYP2D6 activity (Leeder et al., 2008). However, transporter substrates most often rely on multiple DTs; hence validated markers are lacking, which makes it challenging to fill this knowledge gap. Furthermore, the lack of data on hepatic DTs in nonclinical species precludes straightforward translation of *in vivo* data animal with a transporter substrate from nonclinical species to humans. Still, DTs and DMEs in animals that are orthologs of human isoforms do not always recognize the same substrates. This adds another layer of complexity to translating nonclinical animal data to humans.

Filling the knowledge gaps on ontogeny of human DT and DME activity remains challenging. Even if biologic material were available to perform *in vitro* experiments, the *in vitro*-to-*in vivo* extrapolation would not be straightforward. The isolation process of cells or enzymes from biologic material is known to impact enzyme and transporter activity. Even the isolation efficiency of enzymes from donor material from different age groups can be subject to age (unpublished observation). The importance of age-specific scaling is also reflected by the reported age dependency of hepatocellularity (i.e., the number of hepatocytes per gram liver in rat liver). Standard (i.e., age-independent) scaling factors to extrapolate catalytic activity from *in vitro* to *in vivo* could therefore introduce bias to the data and skew predictions of *in vivo* drug disposition. More studies should attempt to translate *in vitro* observations to the clinical setting to better understand how these ontogeny profiles, which are often determined *in vitro*, can aid in clinical dose predictions. In this context, it also needs to be emphasized that reliable prediction and understanding of the effect of age on the disposition of a given drug require consideration and integration of combined influences of ontogeny of all enzymes/transporters mediating the disposition of said drug. Consistently, developmental patterns based on *in vitro* activity measurements should also be validated

TABLE 13
Ontogeny profile of hepatic CYP enzymes in domestic pig based on metabolic activity, protein expression, and mRNA expression levels
Percentages represent expression/activity relative to adult levels.

	Onset of Activity and/or Expression	Adult Levels Reached	Age-Related Changes in Activity/Expression	Comments	References
CYP1A2 Protein expression	M: 2 days (5.4%); F: 2 days (2.2%)	NR (180 days)	M: increased rapidly; F: increased slowly	Single study, sparse data. Adult age: 180 days. Fetal development: NR. Methods: LC-MS/MS (PLM). Strain: Seghers hybrid (M/F)	Millecam et al. (2018)
CYP2A19 Protein expression	M: 2 days (11%); F: 2 days (2.3%)	NR (180 days)	Increased slowly	Single study, sparse data. Adult age: 180 days. Fetal development: NR. Methods: LC-MS/MS (PLM). Strain: Seghers hybrid (M/F)	Millecam et al. (2018)
CYP2B22 Protein expression	M: 2 days (8.6%); F: 2 days (10%)	180 days	Increased slowly	Single study, sparse data. Adult age: 180 days. Fetal development: NR. Methods: LC-MS/MS (PLM). Strain: Seghers hybrid (M/F)	Millecam et al. (2018)
CYP2C Catalytic activity	M: 2 days (14%); F: 2 days (45%)	M: NR; F: 56 days	M: increased progressively; F: increased rapidly	Single study, sparse data. Adult age: 180 days. Fetal development: NR. Substrate: Tolbutamide 4-hydroxylation. Methods: PLM. Strain: Seghers hybrid (M/F)	Millecam et al. (2018)
CYP2C33 Protein expression	M: 2 days (8.6%); F: 2 days (10%)	180 days	Increased progressively	Single study, sparse data. Adult age: 180 days. Fetal development: NR. Methods: LC-MS/MS (PLM). Strain: Seghers hybrid (M/F)	Millecam et al. (2018)
CYP2C34 Protein expression	M: 2 days (38%); F: 2 days (30%)	M: NR (210% at 28 days); F: NR (165% at 28 days)	Increased rapidly	Single study, sparse data. Adult age: 180 days. Fetal development: NR. Methods: LC-MS/MS (PLM). Strain: Seghers hybrid (M/F)	Millecam et al. (2018)
CYP2C35 Protein expression	M: 2 days (30%); F: 2 days (24%)	M: NR (155% at 28 days); F: NR (129% at 28 days)	Increased rapidly/inconsistent pattern	Single study, sparse data. Adult age: 180 days. Fetal development: NR. Methods: LC-MS/MS (PLM). Strain: Seghers hybrid (M/F)	Millecam et al. (2018)
CYP2C36 Protein expression	M: 2 days (9%); F: 2 days (58%)	M: NR (180 days); F: M: increased slowly; F: increased rapidly 28 days	M: increased slowly; F: increased rapidly	Single study, sparse data. Adult age: 180 days. Fetal development: NR. Methods: LC-MS/MS (PLM). Strain: Seghers hybrid (M/F)	Millecam et al. (2018)
CYP2C49 Protein expression	M: 2 days (12%); F: 2 days (10%)	28 days	Increased rapidly	Single study, sparse data. Adult age: 180 days. Fetal development: NR. Methods: LC-MS/MS (PLM). Strain: Seghers hybrid (M/F)	Millecam et al. (2018)
CYP2D6 Protein expression	M: 2 days (6%); F: 2 days (5%)	NR (180 days)	Increased rapidly/increased progressively	Single study, sparse data. Adult age: 180 days. Fetal development: NR. Methods: LC-MS/MS (PLM). Strain: Seghers hybrid (M/F)	Millecam et al. (2018)
CYP2E Catalytic activity	M: 2 days (49%); F: 2 days (78%)	NR (180 days)	Increased slowly/no changes	Single study, sparse data. Adult age: 180 days. Fetal development: NR. Substrate: Chlorzoxazone 6-hydroxylation. Methods: PLM. Strain: Seghers hybrid (M/F)	Millecam et al. (2018)
CYP2E1 Protein expression	M: 2 days (108%); F: 2 days (31%)	M: 2 days; F: NR (180 days)	M: no changes; F: increased slowly	Single study, sparse data. Adult age: 180 days. Fetal development: NR. Methods: LC-MS/MS (PLM). Strain: Seghers hybrid (M/F)	Millecam et al. (2018)
CYP3A Catalytic activity	M: 2 days (13%); F: 2 days (8%)	NR (180 days)	Increased slowly	Single study, sparse data. Adult age: 180 days. Fetal development: NR. Substrate: Midazolam 1-hydroxylation. Methods: PLM. Strain: Seghers hybrid (M/F)	Millecam et al. (2018)
CYP3A22					(continued)

TABLE 13—Continued

	Onset of Activity and/or Expression	Adult Levels Reached	Age-Related Changes in Activity/Expression	Comments	References
Protein expression	M: 2 days (35%); F: 2 days (20%)	NR (180 days)	Increased slowly	Single study, sparse data. Adult age: 180 days. Fetal development: NR. Methods: LC-MS/MS (PLM). Strain: Seghers hybrid (M/F)	Millecam et al. (2018)
CYP3A46 Protein expression	M: 2 days (2.3%); F: 2 days (2.9%)	NR (180 days)	Increased slowly	Single study, sparse data. Adult age: 180 days. Fetal development: NR. Methods: LC-MS/MS (PLM). Strain: Seghers hybrid (M/F)	Millecam et al. (2018)
CYP4A21 Protein expression	M: 2 days (16%); F: 2 days (17%)	NR (180 days)	Increased slowly	Single study, sparse data. Adult age: 180 days. Fetal development: NR. Methods: LC-MS/MS (PLM). Strain: Seghers hybrid (M/F)	Millecam et al. (2018)
CYP4A24 Protein expression	M: 2 days (44%); F: 2 days (60%)	NR (180 days)	Increased slowly	Single study, sparse data. Adult age: 180 days. Fetal development: NR. Methods: LC-MS/MS (PLM). Strain: Seghers hybrid (M/F)	Millecam et al. (2018)
CYP20A1 Protein expression	M: 2 days (60%); F: 2 days (45%)	NR (180 days)	Increased slowly/no changes	Single study, sparse data. Adult age: 180 days. Fetal development: NR. Methods: LC-MS/MS (PLM). Strain: Seghers hybrid (M/F)	Millecam et al. (2018)
CYP51A1 Protein expression	M: 2 days (26%); F: 2 days (17%)	1 NR (180 days)	Increased slowly	Single study, sparse data. Adult age: 180 days. Fetal development: NR. Methods: LC-MS/MS (PLM). Strain: Seghers hybrid (M/F)	Millecam et al. (2018)

F, female; M, male; NR, not reported; PLM, pig liver microsome.

because, for example, the isolation process of cells or subcellular fractions from biologic material could impact protein activity. Moreover, since substrates differ in their affinity for DT and/or DME isoforms, the results from one substrate may not be translated directly to another drug substrate. Understanding the disposition kinetics of the substrate itself is therefore a crucial step in the interpretation of age-dependent activities. Exemplar of this is the interplay between different individual DTs and/or, subsequently, with DMEs, which hampers reliable deconvolution and extraction of individual DT and DME ontogeny profiles from population PK data (Nigam, 2015). However, PBPK models allow incorporation of multiple ontogeny profiles and can be used for hypothesis-driven exploration and ultimately verification of developmental patterns against pediatric PK data (Emoto et al., 2018; Zhou et al., 2018; Cheung et al., 2019). Also, PBPK models are increasingly used for pediatric dose finding—for example, for oseltamivir for pediatric clinical trials (Parrott et al., 2011).

Currently, an opportunity lies in the fact that endogenous substrates/metabolites have been identified as potential markers to phenotype the activity of DTs and DMEs *in vivo*. Examples include the use of the urinary 6-β-hydroxycortisol/cortisol ratio for CYP3A activity, thiamine for OCT1 activity, and dehydroepiandrosterone sulfate (DHEAS) for OATP1B1/3 activity (Muller et al., 2018). Recently, testosterone glucuronide normalized by andosterone glucuronide was shown to be promising as a urinary biomarker to phenotype UGT2B17 activity in children 7–18 years (Zhang et al., 2020). Using endogenous substrates to phenotype DTs and DMEs does not require administration of an exogenous probe, thereby overcoming one of the challenges in pediatric research. However, pediatric homeostatic levels may differ from those in adults, and reference values of endogenous substrate levels in children are lacking. For example, DHEAS levels at birth are high and decrease drastically over the first month of life, which is then followed by a more progressive decrease until reaching 6 months of age (de Peretti and Forest, 1978). Hence, specific reference values for these endogenous substrates/metabolites in various age groups should be obtained in the near future.

Our data compilation effort also revealed notable inconsistencies between results obtained in different studies on the same protein isoforms. Clearly, understanding differential contributions of technical, methodological, analytical, and interindividual variability in activity or expression levels remains challenging. First, several studies that were included in this review used human pediatric liver tissue—mostly postmortem tissue—obtained from biobanks. Not all biobanks collect data on the primary diagnosis, age, and sex. Moreover, it is challenging to determine the severity of disease, comedication, ethnicity, feeding status, smoking, drug

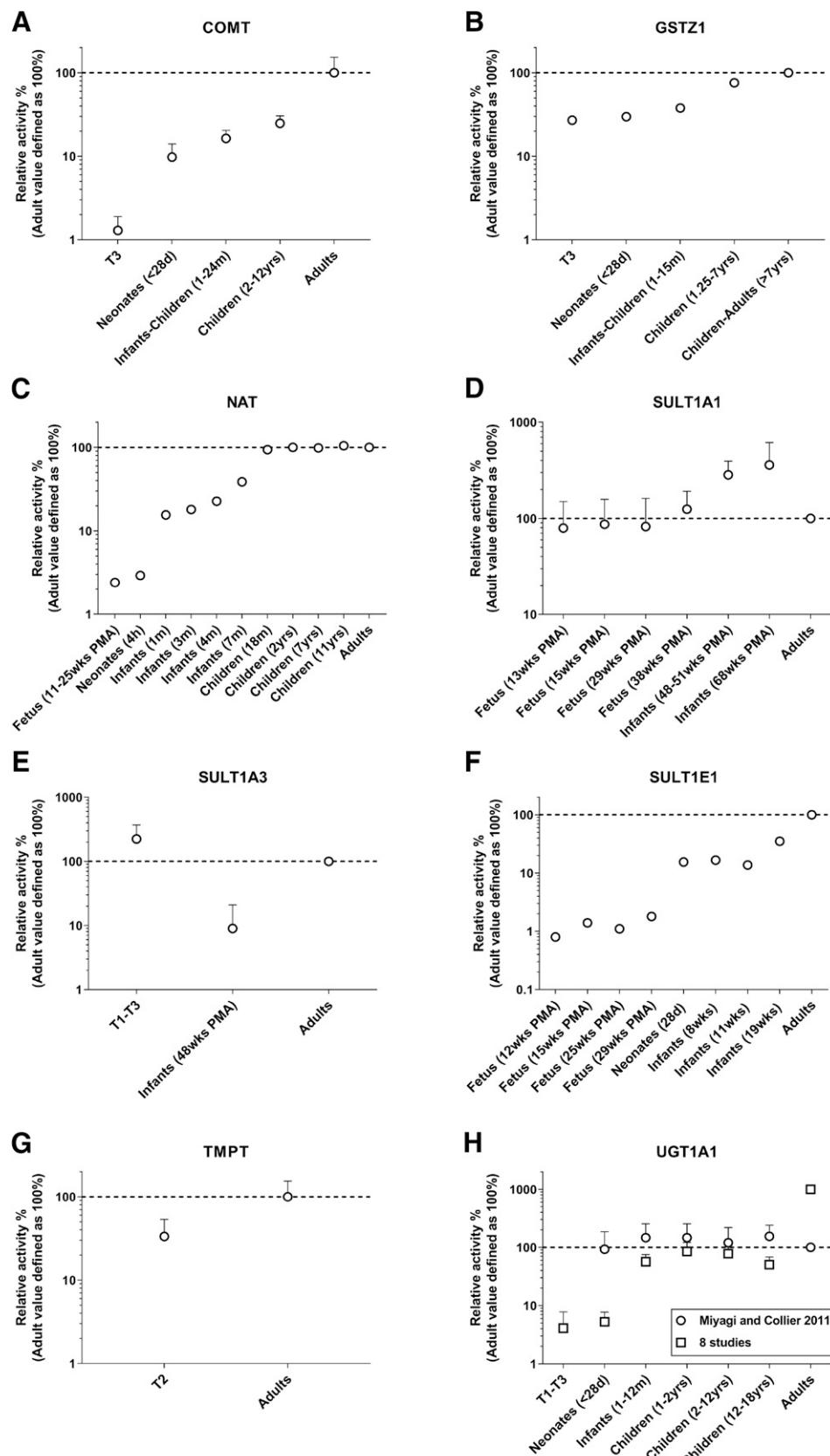


Fig. 16. Pooled literature data on the ontogeny of metabolic activity of hepatic phase II enzymes in humans: COMT (A), GSTZ1 (B), NAT (C), SULT1A1 (D), SULT1A3 (E), SULT1E1 (F), TMPT (G), UGT1A1 (H), UGT1A4 (I), UGT1A6 (J), UGT1A9 (K), UGT2B7 (L), UGT2B15 (M), and UGT2B17 (N). The symbols represent the relative activity in each age group, and the dotted line indicates the adult value defined as 100%. If multiple values were obtained for the same age group, the symbols represent the average relative activity, and the error bars show the S.D. See Table 14 for explanation on the ontogeny profiles and literature references. TMPT, thiopurine-methyltransferase.

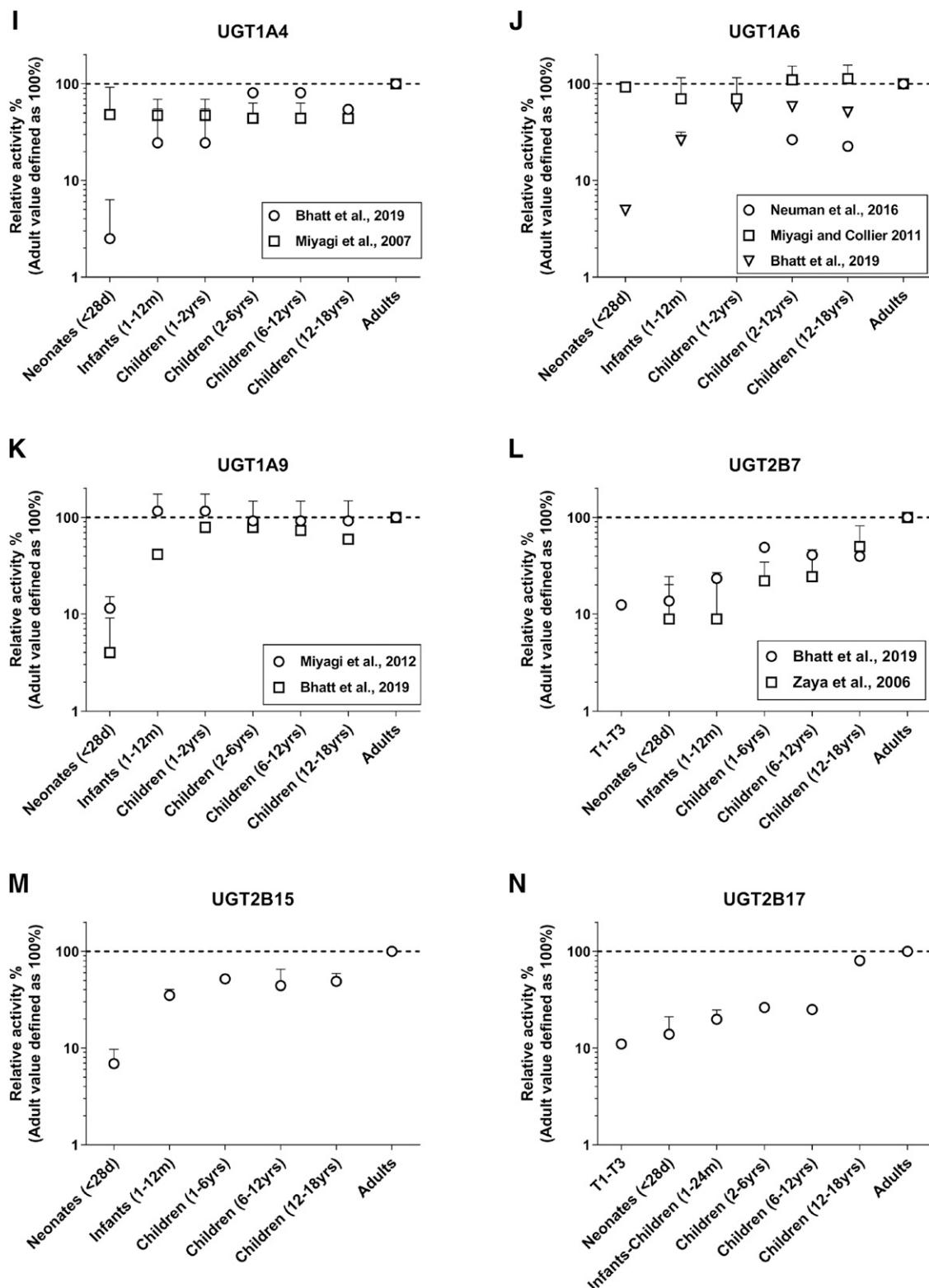


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history, or alcohol intake and the impact of these factors. For data obtained in tissues from nonclinical animals, strain differences may have an especially significant impact apart from housing conditions and diet. Taken together, this implies that data compiled in

this review were obtained in various subpopulations. Second, the exact experimental conditions and specific analytical methods used to quantify mRNA expression, protein abundance, or activity may differ between studies and may thus complicate the meta-analysis of

TABLE 14
Ontogeny profile of hepatic phase II enzymes in humans based on metabolic activity, protein expression, and mRNA expression levels

	Onset of Activity and/or Expression	Adult Levels Reached	Age-Related Changes in Activity and/or Expression	C	Comments	References
COMT Catalytic activity	Birth (T3: <2%)	NR (12 yr: 25%)	Increased slowly then decreased in elderly	12–18 yr: NR. Substrate: methyepinephrine. Matrix: liver cytosol. Methods: HLC, spectrophotometry		Agathopoulos et al. (1971)
GSTA1 Protein expression	Fetal development (T1–T3: 70%)	Neonates–1 yr	Increased rapidly	1–18 yr: ND. Methods: electrophoresis and radioimmunoassay		Strange et al. (1989); McCarter and Hines (2002)
GSTA2 Protein expression	Fetal development (T1–T3: 25%)	>1 yr (60%)	Increased progressively	1–18 yr: ND. Methods: electrophoresis and radioimmunoassay		Strange et al. (1989); McCarter and Hines (2002)
GSTM Protein expression	Fetal development (T1–T3: 20%)	Neonates–1 yr	Increased rapidly	1–18 yr: ND. Methods: electrophoresis and radioimmunoassay		Strange et al. (1989); McCarter and Hines (2002)
GSTP1 Protein expression	Fetal development (T1–T3: >1000%)	Fetal development	Inconsistent pattern	1–18 yr: ND. Methods: electrophoresis and radioimmunoassay		Strange et al. (1989); McCarter and Hines (2002)
GSTZ1 Catalytic activity	Fetal development (T1–T3: <30%)	>7 yr	Increased slowly (7 yr: 75%)	7–18 yr: NR. Substrate: dichloroacetic acid. Matrix: liver cytosol. Methods: HPLC coupled with radioactivity flow detector		Li et al. (2012)
Protein expression	Birth (2.5 wk: <10%)	>7 yr	Increased slowly (7 yr: 75%)	7–18 yr: NR. Methods: Western blotting		Li et al. (2012)
NAT Catalytic activity	Birth (T1–T3: <3%)	1.5 yr	Increased progressively	11–18 yr: NR. Substrates: glycine, benzoic acid. Matrix: liver homogenates. Methods: radioactivity, spectrophotometry		Pacifici et al. (1991a); Mawal et al. (1997)
SULT1A1 Catalytic activity	Fetal development (T1–T3: >80%)	Fetal development	Peaked at 6 mo (3-fold)	6 mo to 18 yr: NR. Substrates: 1-naphthol, 2-naphthol, 4-nitrophenol, N-hydroxy-4-acetylaminobiphenyl, N-hydroxy-4-acetylaminobiphenyl. Matrix: liver cytosol. Methods: radioactivity HPLC, chromatography		Pacifici et al. (1988); Cappiello et al. (1991); Gilissen et al. (1994); Richard et al. (2001)
Protein expression	Fetal development (T1–T3: 93%)	Fetal development	No changes	Methods: electrophoresis and radioimmunoassay		McCarter and Hines (2002); Duanmu et al. (2006)
SULT1A3 Catalytic activity	Fetal development (T1–T3: >200%)	Fetal development	Decreased in neonates (2.5-fold)	Neonates–18 yr: NR. Substrate: dopamine. Matrix: liver cytosol. Methods: radioactivity, chromatography		Powis et al. (1988); Cappiello et al. (1991); Pacifici et al. (1993); Richard et al. (2001)
SULT1E1 Catalytic activity	Birth (T1–T3: <2%)	19 wk: 35%	Increased progressively			Barker et al. (1994)
Protein expression	Fetal development (T1–T3: >100% or 20%)	Fetal development or 19 wk (92%)	Decreased in infants (2-fold) or increased rapidly (3 wk: 69%)	19–18 yr: NR. Methods: immunoblotting, Western blotting		Barker et al. (1994); Duanmu et al. (2006)
SULT2A1 Protein expression	Fetal development (T1–T3: <10%)	Neonates–1 yr (160%)	Increased rapidly	Methods: electrophoresis and radioimmunoassay		McCarter and Hines (2002); Duanmu et al. (2006)
mRNA expression	Fetal development (T1: 30%)	NR	NR	T2–30 yr: NR. Methods: qRT-PCR		Ekstrom and Rane (2015)
TMPT Catalytic activity	Fetal development (T2: 30%)	NR	NR	Birth–18 yr: NR. Substrate: 6-mercaptopurine. Matrix: liver cytosol. Methods: radioactivity		Pacifici et al. (1991b)
UGT1A1						

(continued)

TABLE 14—Continued

	Onset of Activity and/or Expression	Adult Levels Reached	Age-Related Changes in Activity and/or Expression	Comments	References
Catalytic activity	Birth (T1-T3: <10%)	Neonates or 1–2 yr (90%)	Increased rapidly or progressively	Conflicting results. Substrates: bilirubin, 2-aminopheno β -estradiol*, Matrix: liver microsomes, liver homogenates. Methods: high-pressure LC, spectrophotometry, HPLC (-MS/MS)	Onishi et al. (1979); Kawade and Onishi (1981); Leakey et al. (1987); Coughtrie et al. (1988); Burchell et al. (1989); Miyagi and Collier (2011); Nie et al. (2017); Bhatt et al. (2019)
Protein expression mRNA expression	Fetal development (T1-T3: 10%) Birth (T1-T3: 0.5%)	1–2 yr (90%) 6 mo	Increased progressively Increased progressively	Methods: Western blotting, proteomics-HPLC	Miyagi and Collier (2011); Nie et al. (2017); Bhatt et al. (2019)
UGT1A3 Catalytic activity	Fetal development (T2: 30%)	NR	NR	Birth–6 mo: NR. 2–18 yr: NR. Methods: qRT-PCR	Strassburg et al. (2002); Nie et al. (2017)
UGT1A4 Catalytic activity	Birth (neonates: 50% or 10%)	NR (12–18 yr: 44%) or 2–6 yr (80%)	Increased slowly or progressively	T1: UD. Substrate: NA. Matrix: NA de Wildt et al., (1999); McCarver and Hines (2002)	
Protein expression UGT1A6 Catalytic activity	Birth (neonates: <2%)	6–12 yr (70%)	Increased slowly	Fetal development: NR. Substrate: trifluoperazine. Conflicting results. Matrix: liver microsomes. Methods: fluorometry, HPLC-MS/MS.	Miyagi and Collier (2007); Bhatt et al. (2019)
Protein expression UGT1A9 Catalytic activity	Birth (neonates: 10% or 100%)	NR (12–18 yr: 50%) or neonates	Increased progressively or no changes	Fetal development: NR. Substrates: serotonin, 4-hydroxyindole. Conflicting results. Matrix: liver microsomes. Methods: proteomics-HPLC (-MS/MS)	Miyagi and Collier (2007); Neumann et al. (2016); Bhatt et al. (2019)
Protein expression mRNA expression	Birth (neonates: <5%) Birth (neonates: <20%)	NR (12–18 yr: 41%) or 2–6 yr 1 mo to 1 yr or 1 to 2 yr (80%)	Increased slowly or progressively No changes or increased progressively	Fetal development: NR. Conflicting results. Matrix: ELISA assay, HPLC	Miyagi and Collier (2007); Neumann et al. (2016); Bhatt et al. (2019)
UGT2B4 mRNA expression	Birth (T2: UD)	NR (12–18 yr: 37%) or 1 mo to 1 yr: 85% >2 yr (2 yr: 68%)	Increased progressively or decreased at 2 yr (50%) Increased slowly	Fetal development: NR. Conflicting results. Matrix: proteomics-HPLC, Western blotting	Miyagi and Collier (2007); Bhatt et al. (2019)
UGT2B7 Catalytic activity	Birth (T2: UD)	>2 yr (2 yr: <40%)	NR	Methods: qRT-PCR	Strassburg et al. (2002)
Protein expression mRNA expression	Fetal development (T1-T3: 13%)	NR (12–18 yr: 40%–50%)	Increased slowly	Conflicting results. Substrates: epirubicine, morphine, naloxone. Matrix: liver microsomes. Methods: HPLC-MS/MS, HPLC coupled with fluorescence	Pacifci et al. (1982, 1989); Zaya et al. (2006); Bhatt et al. (2019)
UGT2B15 Catalytic activity	Birth (T2: UD)	6 mo to 1 yr	Increased rapidly	Fetal development: NR. Conflicting results. Matrix: electronhoresis and immunoblotting, proteomics-HPLC	Zaya et al. (2006); Bhatt et al. (2019)
Protein expression UGT2B17	Birth (neonates: <13%)	NR (12–18 yr: 70%)	Increased slowly	Fetal development: NR. Conflicting results. Matrix: liver microsomes. Methods: HPLC-MS/MS	Strassburg et al. (2002); Neumann et al. (2016)
Protein expression mRNA expression	Birth (T2: UD)	NR (12–18 yr: 50%)	Increased slowly	Fetal development: NR. Methods: qRT-PCR, qPCR	Bhatt et al., (2019)
Protein expression UGT2B17	Fetal development (T3: <20%)	12–18 yr: (80%)	Increased slowly	Methods: immunoquantification, proteomics-HPLC	Divakaran et al. (2014); Bhatt et al. (2019)

(continued)

TABLE 14—Continued

	Onset of Activity and/or Expression	Adult Levels Reached	Age-Related Changes in Activity and/or Expression	Comments	References
Catalytic activity	Fetal development (T1-T3: 10%)	12–18 yr: (80%)	Increased slowly	Substrate: testosterone. Matrix: liver microsomes. Methods: High-pressure LC, HPLC (MS/MS)	Leakey et al. (1987); Coughtrie et al. (1988); Neumann et al. (2016); Bhatt et al. (2018)
Protein expression	Birth (neonates: <5%)	NR (12–18 yr: 60%)	Increased slowly	Fetal development: NR. Methods: proteomics-HPLC	Bhatt et al. (2018)

HPLC, high performance liquid chromatography; HPLC, high-performance LC; NA, not available; ND, not detectable; NR, not reported; qPCR, quantitative polymerase chain reaction; qRT-PCR, quantitative reverse-transcriptase polymerase chain reaction; TMPT, thiopurine-methyltransferase; T1, trimester 1 of fetal life; T2, trimester 2 of fetal life; T3, trimester 3 of fetal life; UD, undetected.
^aβ-estradiol-3-glucuronide formation, **4-MU coincubated with niflumic acid.

ontogeny profiles. This is most obvious for protein abundance, wherein Western blot and LC-MS/MS do not necessarily give the same results (Aebersold et al., 2013; Achour et al., 2017). Third, for LC-MS/MS-based quantification, there is a significant interlaboratory variability in absolute protein levels (Wegler et al., 2017; Prasad et al., 2019). Sources of variability include type of membrane fractionation, tissue homogenization, degree of protein solubility, digestion conditions of proteins (e.g., type of digestion enzyme), digestion time, and temperature and amount of enzymes per total protein, quantification method, and choice of specific peptides and probe substrate (Badée et al., 2019). In addition, the units to express protein abundance are of importance when comparing between studies and when extrapolating normalized values for protein abundance to, for instance, the organ level. One example is that Prasad et al. (2016) used protein abundance in relation to “microgram membrane protein,” whereas van Groen et al. (2018) used protein abundance in relation to “gram liver tissue.” The reason for the latter is that membrane protein yield per gram tissue showed an age-related pattern and needed a correction factor to scale up to total organ expression (van Groen et al., 2018). Consistently, the number of isolated liver cells per gram liver (i.e., hepatocellularity) was also shown to be age-dependent in rats, necessitating the use of age-specific values for hepatocellularity, such as, for instance, when extrapolating in vitro hepatocytic uptake data to the in vivo level (Fattah et al., 2016). Despite the fact that we have normalized the results presented in this review to adult levels in an attempt to correct for interstudy variability, readers should consider the implications of these differences in data generation when using the data (e.g., for PBPK modeling). Moreover, because consensus is lacking on standard practice for performing LC-MS/MS-based protein quantitation studies (Prasad et al., 2019), the scientific community should harmonize guidelines in these areas to further improve use of the quantitative data presented in this review.

Not surprisingly, we identified several asynchronous ontogeny profiles of DTs and DMEs when comparing mRNA expression, protein expression, and activity levels. This was, for instance, the case for protein and mRNA expression levels of CNT2 in rats that increased with age, reaching maximal levels at either 21 or 45 days, respectively (Supplemental Fig. 3), whereas maximal uptake activity levels of uridine mediated by CNT1/2 were achieved during fetal development and then rapidly decreased in neonatal animals (Fig. 4C) (del Santo et al., 2001). In addition, UGT2B7 mRNA expression levels decreased with increasing age in humans, showing maximal levels during fetal life and reaching adult levels in older children of 12 years of age (Supplemental Fig. 18B), but this expression profile was inconsistent with the respective protein (Supplemental Fig. 17B) and activity (Fig. 16L) levels. Taken together,

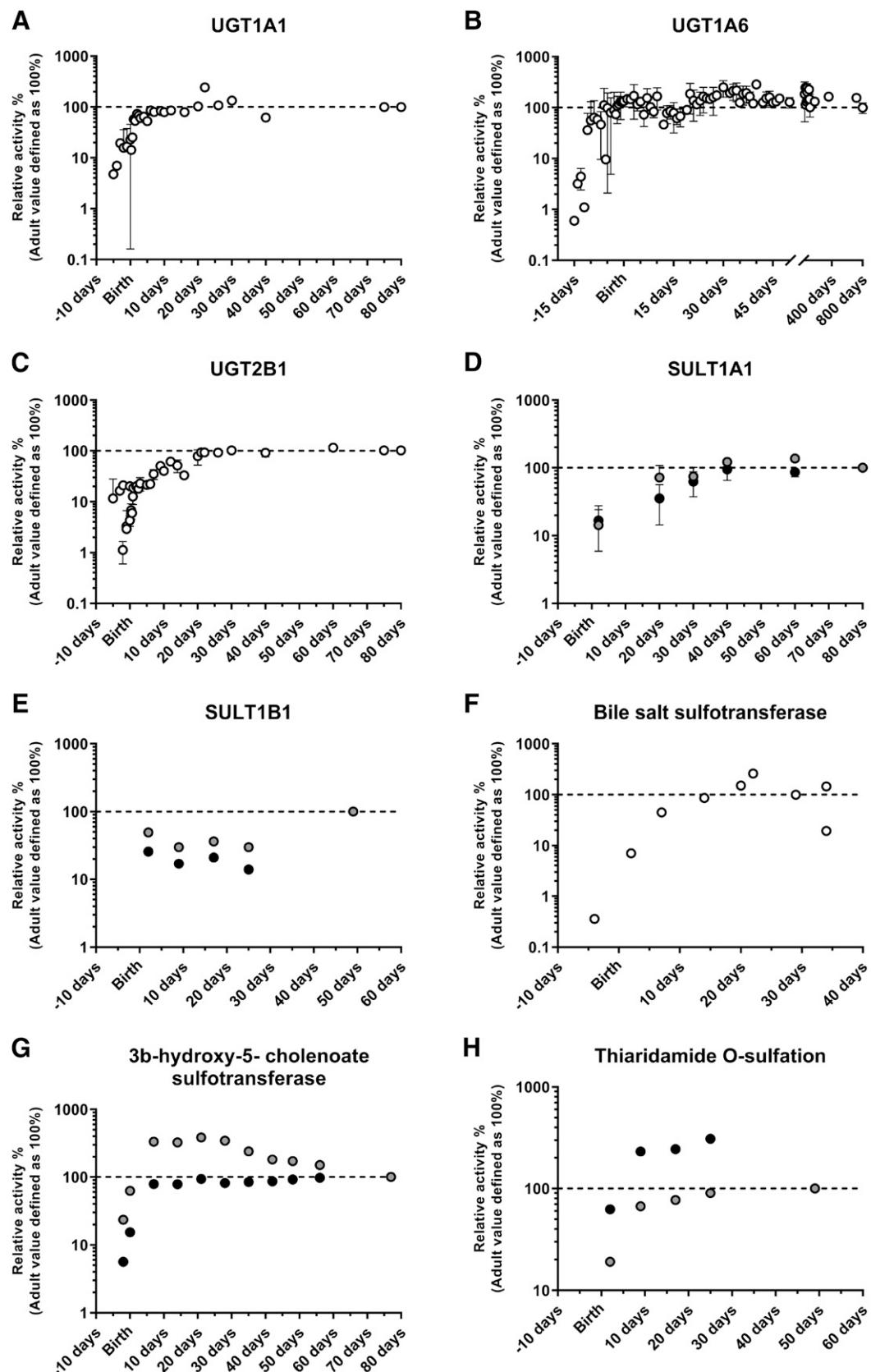


Fig. 17. Pooled literature data on the ontogeny of metabolic activity of hepatic phase II enzymes in rats: UGT1A1 (A), UGT1A6 (B), UGT2B1 (C), SULT1A1 (D), SULT1B1 (E), Bile salt sulfotransferase (F), 3b-hydroxy-5-cholenoate sulfotransferase (G), Thiaridamide O-sulfation (H), Piperazine derivate (DETR) N-sulfation (I), Aniline N-sulfation (J), Piperazine derivate (PTHP) N-sulfation (K), Desipramine N-sulfation (SULT2A1) (L), Androsterone O-sulfation (low activity) (M), Androsterone O-sulfation (high activity) (N), GST (O), NAT1 (P), GSH reductase (Q), and GSH peroxidase (R). The symbols represent the relative activity in each age group, and the dotted line indicates the adult value defined as 100%. If multiple values were obtained for the same age group, the symbols represent the average relative activity, and the error bars show the S.D. See Table 15 for explanation on the ontogeny profiles and literature references. DETR, Piperazine derivate; PTHP, Piperidine derivative.

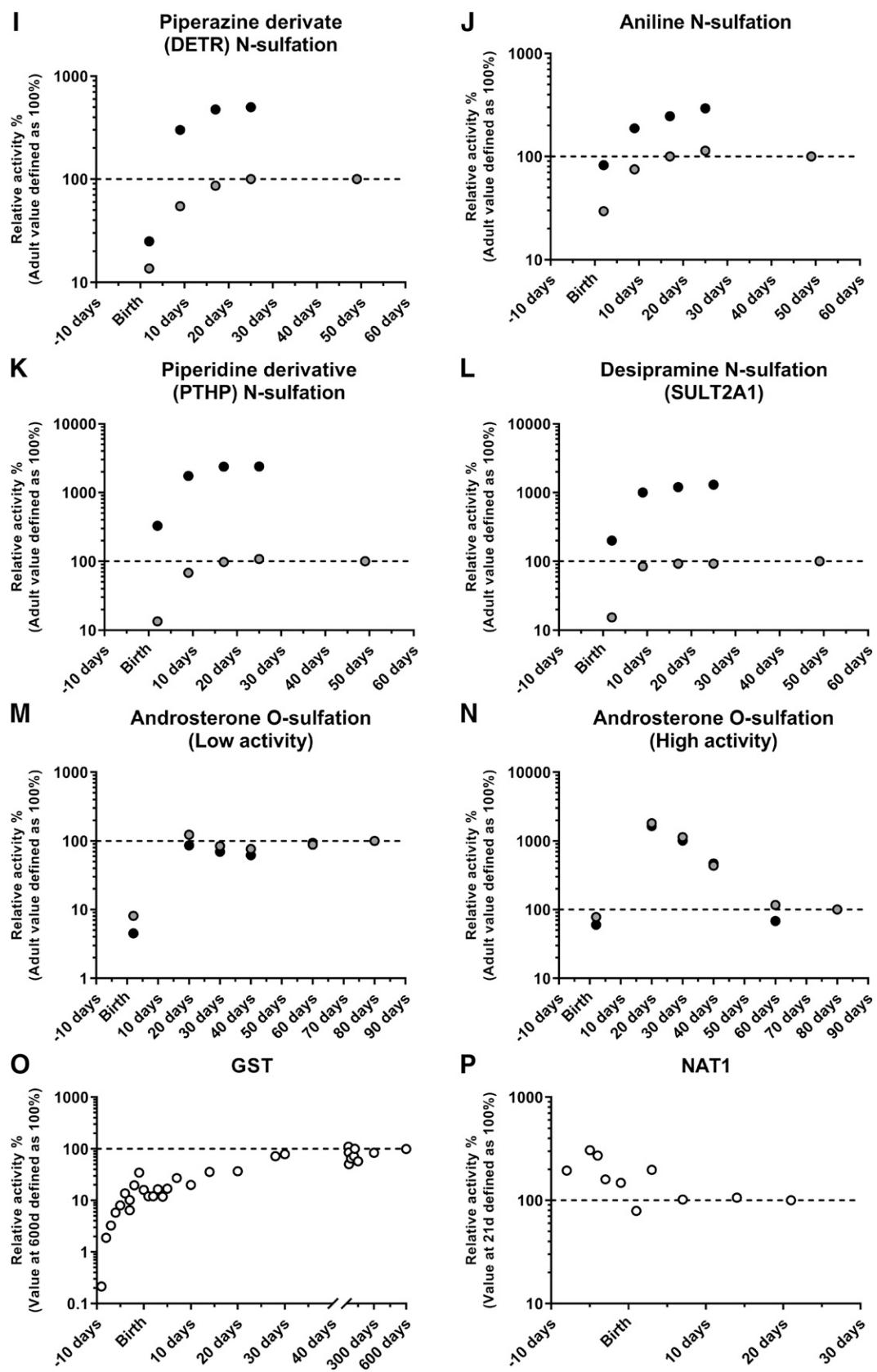


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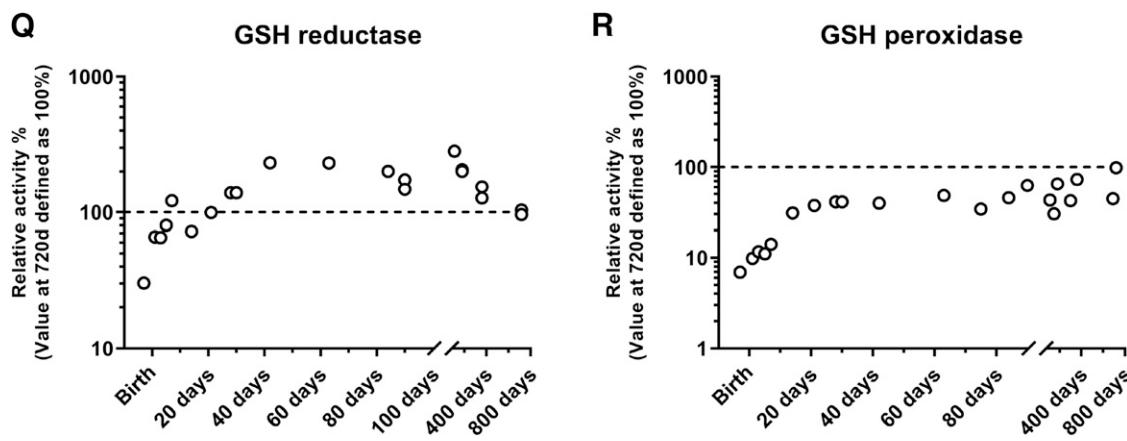


Fig. 17. Continued.

these examples of asynchronous maturation in expression and activity further demonstrate the need for cross-validation of DT/DME ontogeny profiles for individual isoforms. Importantly, for several transporter isoforms, the prior identification and characterization of isoform-selective drug/probe substrates will be required to support the generation of reliable activity data. The current review may show that mRNA expression levels could correlate quantitatively and qualitatively with

protein expression levels for some DMEs and DTs (van Groen et al., 2018). However, caution is warranted for deriving quantitative maturation functions solely from mRNA expression levels. Any correlation between mRNA and protein expression levels that may come up from the current literature review could apply solely to the patient population that was studied in the original research papers. Demographic and environmental factors may affect transcription factors and

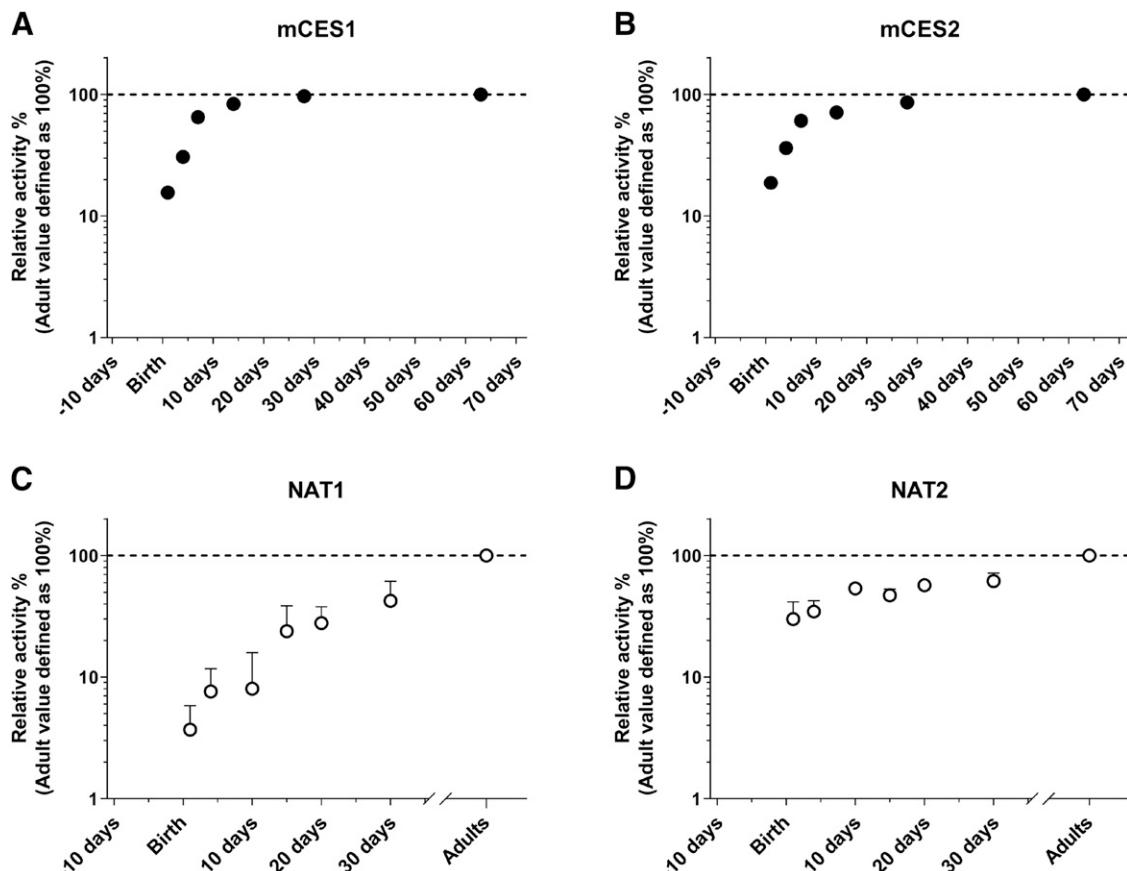


Fig. 18. Pooled literature data on the ontogeny of metabolic activity of hepatic esterases and phase II enzymes in mice: mCES1 (A), mCES2 (B), NAT1 (C), and NAT2 (D). The symbols represent the relative activity in each age group, and the dotted line indicates the adult value defined as 100%. See Table 16 for explanation on the ontogeny profiles and literature references. mCES, mice carboxylesterase.

TABLE 15
Ontogeny profile of hepatic phase II enzymes in rats based on metabolic activity, protein expression, and mRNA expression levels
Percentages represent expression/activity relative to adult levels.

	Onset of Activity and/or Expression	Adult Levels Reached	Age-Related Changes in Activity/Expression	Comments	References
UGT1A1 Catalytic activity	-5 days (5%)	20 days	Increased rapidly	Ontogeny profile established. Adult age: 80 days. Substrates: bilirubin, furosemide. Methods: RLM, RLH. Strain: Wistar (mixed sex). WAG (M)	Wishart (1978); Campbell and Wishart (1980); Rachmel and Hazelton (1986); Coughtrie et al. (1988)
mRNA expression	-1 day (3%)	>30 days	Inconsistent pattern/increased progressively	Single study. Peak (200%) after 1 day. Adult age: 56 days. Methods: qPCR. Strain: Wistar (M)	Kishi et al. (2008)
UGT1A5 mRNA expression	-5 days (3%)	0-56 days	Inconsistent pattern/increased rapidly	Single study, peak (300%) after 7 days. Adult age: 56 days. Methods: qPCR. Strain: Wistar (M)	Kishi et al. (2008)
UGT1A6 Catalytic activity	-2 wk (3%)	0 days	Inconsistent pattern/increased rapidly	Single study, high resolution, large variability. Adult age: 800 days. Substrates: 2-aminophenol, 1-nitrophenol, 4-nitrophenol. Methods: RLH, RLH, liver snips. Strain: Wistar (mixed), Sprague-Dawley (mixed). WAG (M)	Wishart (1978); Matsui and Watanabe (1982); Seragg et al. (1983); Rachmel and Hazelton (1986); Santa Maria and Machado (1988); Matsumoto et al. (2002); Kishi et al. (2008)
mRNA expression	-5 days (29%)	-0.5-28 days	Inconsistent pattern/increased rapidly	Single study, low resolution. Adult age: 56 days. Methods: qPCR. Strain: Wistar (M)	Kishi et al. (2008)
UGT2B1 Catalytic activity	-5 days (23%)	3 wk	Increased progressively	High resolution, nine compounds over 17 data sets. Adult age: 80 days. Substrates: testosterone, androsterone, bisphenol A, nonylphenol, octylphenol, diethylstilbestrol, 4-hydroxybiphenyl, estrone, morphine. Methods: RLM, RLH. Strain: Wistar (mixed), Sprague-Dawley (mixed), WAG (M)	Campbell and Wishart (1980); Matsui and Watanabe (1982); Rachmel and Hazelton (1986); Coughtrie et al. (1988); Matsumoto et al. (2002)
UGT2B2 mRNA expression	-2 days (3%)	14 days	Increased rapidly	Single study, semiquantitative, no data after 21 days. Adult age: 21 days. Methods: Northern blot. Strain: Wistar (mixed)	Kishi et al. (2008)
SULT1A1 Catalytic activity	2 days (14%)	40 days	Increased rapidly	Single study, data for high and low sulfation activity were pooled (S.D. provided in graphs). Adult age: 80 days. Fetal development: NR.	Matsui and Watanabe (1982)
mRNA expression	M: 1.5 days (28%); F: 1.5 days (75%)	M: 15 days; F: 7 days	Inconsistent pattern/increased rapidly	Substrate: 4-nitrophenol O-sulfation. Methods: RLC. Strain: Wistar (M/F)	Klaassen et al. (1998)
SULT1B1 Catalytic activity	M: 2 days (26%); F: 2 days (49%)	49 days (100%)	Inconsistent pattern/increased slowly	Single study. Adult age: 49 days. Fetal development: NR. Substrate: naphthol O-sulfation. Methods: RLC. Strain: not defined (M/F)	Iwasaki et al. (1994)

(continued)

TABLE 15—Continued

	Onset of Activity and/or Expression	Adult Levels Reached	Age-Related Changes in Activity/Expression	Comments	References
SULT1C1 mRNA expression	M: 1.5 days (5%); F: 1.5 days (46%)	M: 45 days; F: 15 days	M: increased slowly; F: increased rapidly	Single study. Adult age: 90 days. Fetal development: NR. Methods: qPCR. Strain: NR (M/F)	Klaassen et al. (1998)
SULT1E1 mRNA expression	M: 1.5 days (2.5%); F: 1.5 days (35%)	M: 60 days; F: 90 days	M: inconsistent pattern/increase slowly; F: increased rapidly	Single study. Adult age: 90 days. Fetal development: NR. Methods: qPCR. Strain: NR (M/F)	Klaassen et al. (1998)
SULT2A1/sult-20/21 Catalytic activity	M: (25%–300%); F: 2 days (13%–30%)	M: 25–49 days; F: 17 days	M: inconsistent pattern/increased rapidly; F: increased progressively	All substrate data reported by Iwasaki and coworkers (1994) correlate with desipramine sulfation [T]Iamamide O-sulfation, Piperazine derivative (DETR), N-sulfation, Aniline N-sulfation, Piperidine derivative (PTHP), N-sulfation, Desipramine N-sulfation). Desipramine sulfation has been associated with SULT2A1. Androsterone sulfation showed a similar variable profile (distinction between high and low activity). Adult age: 49/80 days. Fetal development: NR. Methods: RLC. Strain: Wistar (M/F) and unknown (M/F)	Iwasaki et al. (1994); Matsui and Watanabe (1982); Iwasaki et al. (1994)
mRNA expression (SULT-20/21)	M: 1.5 days (25%); F: 1.5 days (8%)	M: 60 days; F: 60 days	Increased slowly	Single study. Adult age: 30 days. Substrate: bile salt mixture. Substrate: unknown. Methods: RLC. Strain: Sprague-Dawley (M/F)	Chen (1982)
Bile-salt sulfotransferase/sult2a1 Catalytic activity	2 days (7%)	M: NR; F: NR	Inconsistent pattern	Single study, isoform unknown. Adult age: 77 days. Fetal development: NR (F). Substrate: 3 β -hydroxy-5-cholenate. Methods: RLC. Strain: Sprague-Dawley (M/F)	Kane and Chen (1991)
3 β -hydroxy-5-cholenate sulfotransferase (not defined) Catalytic activity	M: –2 days (5%); F: –2 days (24%)	M: 21 days; F: 0/77 days	M: increased rapidly; F: inconsistent pattern/increased rapidly	Single study, isoform unknown. Adult age: 77 days. Fetal development: NR (F). Substrate: 3 β -hydroxy-5-cholenate. Methods: RLC. Strain: Sprague-Dawley (M/F)	Kane and Chen (1991)
SULT-40/41/hydroxysteroid sulfotransferase/sult2a6 mRNA expression	M: NR; F: NR	M: NR; F: NR	M: inconsistent pattern; F: inconsistent pattern	Single study. Adult age: 90 days. Fetal development: NR. Methods: qPCR. Strain: NR (M/F)	Klaassen et al. (1998)
SULT-60/hydroxysteroid sulfotransferase/sult2a2 mRNA expression	M: 1.5 days (50%); F: 1.5 days (1.5%)	M: 0 days; F: 45 days	M: inconsistent pattern/no changes; F: increased slowly	Single study. Adult age: 90 days. Fetal development: NR. Methods: qPCR. Strain: NR (M/F)	Klaassen et al. (1998)
NAT1 Catalytic activity	NR	NR (195% at –8 days)	No changes	Single study. Adult age: 21 days. Fetal development: NR (–8 days). Substrate: p-amino benzoic acid. Methods: RLC. Strain: CD (Charles River) (M/F)	Lucier et al. (1975)
mRNA expression	1 day (3%)	182 days	Increased slowly	Single study. Adult age: 365 days. Fetal development: NR. Methods: qPCR. Strain: F344 (M) and Sprague-Dawley (M)	Hein et al. (2008)

(continued)

TABLE 15—Continued

	Onset of Activity and/or Expression	Adult Levels Reached	Age-Related Changes in Activity/Expression	Comments	References
NAT2 mRNA expression	1 day (58%)	Variable	No changes	Single study. Adult age: 365 days. Fetal development: NR. Methods: qPCR. Strain: F344 (M) and Sprague-Dawley (M)	Hein et al. (2008)
GST Catalytic activity	-8 (2%)	30 days	Increased rapidly	Adult age: 600 days. Substrates: 1-chloro-2,4-dinitrobenzene, 1,2-epoxy-(3-nitrophenoxy)propane, 1-chloro-3,4-dinitrobenzene, 3,4-dichloronitrobenzene, bromosulfophthalain, ethacrynic acid, trans-4-phenyl-3-butene-2-one.	Gregus et al. (1985); Tee et al. (1992); Jang et al. (1998); Elbarbary and Alcorn (2009)
GSH peroxidase Catalytic activity	-3 days (7%)	NR (720 days)	Increased slowly	Methods: RLC. Strain: Wistar (M/F) and Sprague-Dawley (M)	Santa Maria and Machado (1988); Jang et al. (1998); Elbarbary and Alcorn (2009)
GSH reductase Catalytic activity	-3 days (30%)	21 days	Inconsistent pattern	Adult age: 720 days. Substrate: GSSG reduction coupled to NADPH oxidation; Glutathione peroxidase kit. Methods: RLC, RLH. Strain: Wistar (M/F) and Sprague-Dawley (M)	Santa Maria and Machado (1988); Elbarbary and Alcorn (2009)

F, female; GSSG, glutathione disulfide; M, male; NR, not reported; qPCR, quantitative polymerase chain reaction; RLC, rat liver cytosol; RLH, rat liver homogenate; RLM, rat liver microsome.

TABLE 16
Ontogeny profile of hepatic phase II enzymes in mice based on metabolic activity, protein expression, and mRNA expression levels

Percentages represent expression/activity relative to adult levels.

	Onset of Activity and/or Expression	Adult Levels Reached	Age-Related Changes in Activity/Expression	Comments	References
mCES1 Protein activity	Before or at 1 day (30%)	14 days	Increased rapidly	Substrate: MPH. Adult age: 9 wk. Methods: hydrolysis reaction study. Strain: FVB mice (M)	Zhu et al. (2009)
mRNA expression	Before or at 1 day (30%)	28 days	Increased progressively	Adult age: 67 days. Methods: RNA-seq. Strain: FVB mice (M)	Lee et al. (2011)
mCES2 Protein activity	During or before 1 day (20%)	14 days	Increased rapidly	Substrate: irinotecan. Adult age: 9 wk. Methods: hydrolysis reaction study. Strain: FVB mice (M)	Zhu et al. (2009)
mRNA expression	Before or at 1 day (20%)	14 days	Increased rapidly	Adult age: 67 days. Methods: RNA-seq. Strain: FVB mice (M)	Lee et al. (2011)
CES1A mRNA expression	3 days (14%)	60 days	Increased slowly	The amount detected was very low throughout the entire age range. Adult age: 60 days. Methods: RNA-seq. Strain: C57BL/6 (M)	Peng et al. (2013)
CES1B mRNA expression	Fetal development (0.036%)	20 days	Increased rapidly	Adult age: 60 days. Methods: RNA-seq. Strain: C57BL/6 (M)	Peng et al. (2013)
CES1C mRNA expression	Fetal development (0.19%)	15 days	Increased rapidly	Most abundant of the Ces1 family. Adult age: 60 days. Methods: RNA-seq. Strain: C57BL/6 (M)	Peng et al. (2013)
CES1D mRNA expression	Fetal development (2%)	30 days	Increased rapidly	Adult age: 60 days. Methods: RNA-seq. Strain: C57BL/6 (M)	Peng et al. (2013)
CES1E mRNA expression	Fetal development (2%)	60 days	Increased slowly	Adult age: 60 days. Methods: RNA-seq. Strain: C57BL/6 (M)	Peng et al. (2013)
CES1F mRNA expression	Fetal development (0.146%)	60 days	Increased slowly	Adult age: 60 days. Methods: RNA-seq. Strain: C57BL/6 (M)	Peng et al. (2013)
CES1G mRNA expression	Fetal development (3%)	20 days	Increased rapidly	Adult age: 60 days. Methods: RNA-seq. Strain: C57BL/6 (M)	Peng et al. (2013)
CES2A mRNA expression	Fetal development (1%)	45 days	Increased rapidly	Adult age: 60 days. Methods: RNA-seq. Strain: C57BL/6 (M)	Peng et al. (2013)
CES2B mRNA expression	Fetal development (20%)	30 days	Inconsistent pattern	Adult age: 60 days. Methods: RNA-seq. Strain: C57BL/6 (M)	Peng et al. (2013)
CES2C mRNA expression	Fetal development (0.86%)	45 days	Inconsistent pattern	Adult age: 60 days. Methods: RNA-seq. Strain: C57BL/6 (M)	Peng et al. (2013)
CES2D-PS mRNA expression	Fetal development (0.80%)	30 days	Inconsistent pattern	Adult age: 60 days. Methods: RNA-seq. Strain: C57BL/6 (M)	Peng et al. (2013)
CES2E mRNA expression	Fetal development (0.67%)	25 days	Increased progressively	Adult age: 60 days. Methods: RNA-seq. Strain: C57BL/6 (M)	Peng et al. (2013)
CES2F mRNA expression	Fetal development (4%)	45 days	Inconsistent pattern	Adult age: 60 days. Methods: RNA-seq. Strain: C57BL/6 (M)	Peng et al. (2013)
CES2G					

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TABLE 16—Continued

	Onset of Activity and/or Expression	Adult Levels Reached	Age-Related Changes in Activity/Expression	Comments	References
mRNA expression CESB mRNA expression CES3A mRNA expression CES3B mRNA expression CES4A mRNA expression	Fetal development 0 days (76%) Fetal development 7 days (1.4%) Fetal development 30 days (0.04%) Fetal development 30 days (0.01%) 0 days (3%)	Hovering around adult value Increased progressively Increased moderately Inconsistent pattern Increased rapidly		Adult age: 60 days. Methods: RNA-seq. Strain: C57BL/6 (M) Adult age: 12 mo. Methods: RT-PCR. Strain: FVB mice (M) Adult age: 60 days. Methods: RNA-seq. Strain: C57BL/6 (M) Adult age: 60 days. Methods: RNA-seq. Strain: C57BL/6 (M)	Peng et al. (2013) Lee et al. (2011) Peng et al. (2013) Peng et al. (2013)
GSTA1 mRNA expression GSTA1/2 mRNA expression	Fetal development 60 days (2%) M: fetal development 30 days (12%; F: fetal development (6%); M: 7 days (0.04%))	Inconsistent pattern M: increased rapidly; F: inconsistent pattern; M: increased progressively		The amount detected was very low throughout the entire age range. Adult age: 60 days. Methods: RNA-seq. Strain: C57BL/6 (M)	Peng et al. (2013)
GSTA2 mRNA expression	Fetal development 30 days (0.15%) (Lu et al., 2013) 7 days (0.02%) (Lee et al., 2011)	Increased rapidly		Adult age: 60 days at the prenatal stage is difficult, so pooling of sexes might have occurred at these timepoints. Adult age: 45 days (Cui et al., 2010), 12 mo (Lee et al., 2011). Methods: ChIP-Seq analysis (Cui et al., 2010), RT-PCR (Lee et al., 2011). Strain: C57BL/6 (M & F) (Cui et al., 2010), FVB Mice (M) (Lee et al., 2011)	Cui et al. (2010); Lee et al. (2011)
GSTA3 mRNA expression	Fetal development 30 days (16%)	Increased rapidly		Adult age: 45 days (Cui et al., 2010), 12 mo (Lee et al., 2011), 60 days (Lu et al., 2013). Methods: ChIP-Seq analysis (Cui et al., 2010), RT-PCR (Lee et al., 2011). Strain: C57BL/6 (M & F) (Cui et al., 2010) (Lu et al., 2013), FVB mice (M) (Lee et al., 2011)	Cui et al. (2010); Lee et al. (2011); Lu et al. (2013)
GSTA4 mRNA expression	Fetal development 10 days (2%-50%)	Increased rapidly		Adult age: 45 days (Cui et al., 2010), 12 mo (Lee et al., 2011), 60 days (Lu et al., 2013). Methods: ChIP-Seq analysis (Cui et al., 2010), RT-PCR (Lee et al., 2011). Strain: C57BL/6 (M & F) (Cui et al., 2010; Lu et al., 2013), FVB mice (M) (Lee et al., 2011)	Cui et al. (2010); Lee et al. (2011); Lu et al. (2013)
GSTCD mRNA expression GSTK1	Fetal development 7 days (560%)	Decreased rapidly		Adult age: 12 mo. Methods: RT-PCR. Strain: FVB mice (M)	Lee et al. (2011)

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TABLE 16—Continued

	Onset of Activity and/or Expression	Adult Levels Reached	Age-Related Changes in Activity/Expression	Comments	References
mRNA expression	Fetal development (5%)	15 days	Increased rapidly	Adult age: 45 days (Cui et al., 2010), 12 mo (Lee et al., 2011), 60 days (Lu et al., 2013). Methods: ChIP-Seq analysis (Cui et al., 2010), RT-PCR (Lee et al., 2011). RNA-seq (Lu et al., 2013). Strain: C57BL/6 (M & F) (Cui et al., 2010; Lu et al., 2013), FVB mice (M) (Lee et al., 2011)	Cui et al. (2010); Lee et al. (2011); Lu et al. (2013)
GSTM1 mRNA expression	Fetal development (10%) (M & F)	22 days	Increased rapidly	Adult age: 45 days (Cui et al., 2010), 12 mo (Lee et al., 2011), 60 days (Lu et al., 2013). Methods: ChIP-Seq analysis (Cui et al., 2010), RT-PCR (Lee et al., 2011), RNA-seq (Lu et al., 2013). Strain: C57BL/6 (M & F) (Cui et al., 2010; Lu et al., 2013), FVB mice (M) (Lee et al., 2011)	Cui et al. (2010); Lee et al. (2011); Lu et al. (2013)
GSTM2 mRNA expression	Fetal development (6%)	10 days (Cui et al., 2010); 32 days (Lee et al., 2011)	Inconsistent pattern (Lu et al., 2010); increased progressively (Lee et al., 2011)	Adult age: 45 days (Cui et al., 2010), 12 mo (Lee et al., 2011), 60 days (Lu et al., 2013). Methods: ChIP-Seq analysis (Cui et al., 2010), RT-PCR (Lee et al., 2011), RNA-seq (Lu et al., 2013). Strain: C57BL/6 (M & F) (Cui et al., 2010; Lu et al., 2013), FVB mice (M) (Lee et al., 2011)	Cui et al. (2010); Lee et al. (2011); Lu et al. (2013)
GSTM3 mRNA expression	Fetal development (0.02%–11%)	15 days (Lu et al., 2013; Cui et al., 2010); 32 days (Lee et al., 2011)	Increased moderately (Lu et al., 2013) (Cui et al., 2010); increased progressively (Lee et al., 2011)	Adult age: 45 days (Cui et al., 2010), 12 mo (Lee et al., 2011), 60 days (Lu et al., 2013). Methods: ChIP-Seq analysis (Cui et al., 2010), RT-PCR (Lee et al., 2011), RNA-seq (Lu et al., 2013). Strain: C57BL/6 (M & F) (Cui et al., 2010; Lu et al., 2013), FVB mice (M) (Lee et al., 2011)	Cui et al. (2010); Lee et al. (2011); Lu et al. (2013)
GSTM4 mRNA expression	Fetal development (10%)	22 days	Increased rapidly	Adult age: 45 days (Cui et al., 2010), 12 mo (Lee et al., 2011), 60 days (Lu et al., 2013). Methods: ChIP-Seq analysis (Cui et al., 2010), RT-PCR (Lee et al., 2011), RNA-seq (Lu et al., 2013). Strain: C57BL/6 (M & F) (Cui et al., 2010; Lu et al., 2013), FVB mice (M) (Lee et al., 2011)	Cui et al. (2010); Lee et al. (2011); Lu et al. (2013)
GSTM5 mRNA expression	Fetal development (3922%) (M & F)	15 days	Decreased rapidly	Adult age: 45 days (Cui et al., 2010), 12 mo (Lee et al., 2011), 60 days (Lu et al., 2013). Methods: ChIP-Seq analysis (Cui et al., 2010), RT-PCR (Lee et al., 2011), RNA-seq (Lu et al., 2013). Strain: C57BL/6 (M & F) (Cui et al., 2010; Lu et al., 2013), FVB mice (M) (Lee et al., 2011)	Cui et al. (2010); Lee et al. (2011); Lu et al. (2013)
GSTM6 mRNA expression	Fetal development (20%) (Cui et al., 2010; Lu et al., 2013) 7 days (Lee et al., 2011)	22 days (Cui et al., 2010; Lu et al., 2013); 32 days (Lee et al., 2011)	Increased moderately (Cui et al., 2010; Lu et al., 2013); increased progressively (Lee et al., 2011)	Adult age: 45 days (Cui et al., 2010), 12 mo (Lee et al., 2011), 60 days (Lu et al., 2013). Methods: ChIP-Seq analysis (Cui et al., 2010), RT-PCR (Lee et al., 2011), RNA-seq (Lu et al., 2013). Strain: C57BL/6 (M & F) (Cui et al., 2010; Lu et al., 2013), FVB mice (M) (Lee et al., 2011)	Cui et al. (2010); Lee et al. (2011); Lu et al. (2013)
GSTM7 mRNA expression	Fetal development (100%) (Lu et al., 2013); fetal development (100%) (Lee et al., 2011)	No change (Lu et al., 2013); 32 days (Lee et al., 2011)	No change (Lu et al., 2013); increased moderately (Lee et al., 2011)	Adult age: 60 days (Lu et al., 2013), 12 mo (Lee et al., 2011). Methods: RNA-seq (Lu et al., 2013); RT-PCR (Lee et al., 2011). Strain: C57BL/6 (M) (Lu et al., 2013), FVB mice (M) (Lee et al., 2011)	Lee et al. (2011); Lu et al. (2013)

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TABLE 16—Continued

	Onset of Activity and/or Expression	Adult Levels Reached	Age-Related Changes in Activity/Expression	Comments	References
GSTO1 mRNA expression	Fetal development (64%)(M); fetal development (31% or 235%)	M: 0 day (22 days); F: NR	Inconsistent pattern (Cui et al., 2010; Lu et al., 2013); decreased or increased slowly (Lee et al., 2011)	Adult age: 45 days (Cui et al., 2010), 12 mo (Lee et al., 2011), 60 days (Lu et al., 2013). Methods: ChIP-Seq analysis (Cui et al., 2010), RT-PCR (Lee et al., 2011), RNA-seq (Lu et al., 2013). Strain: C57BL/6 (M & F) (Cui et al., 2010; Lu et al., 2013), FVB mice (M) (Lee et al., 2011)	Cui et al. (2010); Lee et al. (2011); Lu et al. (2013))
GSTP1 mRNA expression	Fetal development (13%)	30 days	Nonlinear pattern	Adult age: 60 days (Lu et al., 2013), 12 mo (Lee et al., 2011). Methods: RNA-seq (Lu et al., 2013), RT-PCR (Lee et al., 2011). Strain: C57BL/6 (M) (Lu et al., 2013), FVB mice (M) (Lee et al., 2011)	Lee et al. (2011); Lu et al. (2013)
GSTP1/2 mRNA expression	M: fetal development (16%); F: no change	M: 45 days; F: No change	M: increased rapidly (Cui et al., 2010; Peng et al., 2013); F: no change (Cui et al., 2010)	Adult age: 45 days (Cui et al., 2010), 60 days (Peng et al., 2013). Methods: ChIP-Seq (Cui et al., 2010), RNA-seq (Peng et al., 2010), C57BL/6 (M & F) (Cui et al., 2010), C57BL/6 (M) (Peng et al., 2013)	Cui et al. (2010); Peng et al. (2013)
GSTP2 mRNA expression	Fetal development (11%)(M)	25 days	Increased rapidly	Adult age: 60 days. Methods: RNA-Seq, Strain: C57BL/6 (M)	Lu et al. (2013)
GSTT1 mRNA expression	Fetal development (15%–81%)	M: 10 days; F: 30 days	Increased rapidly	Adult age: 45 days (Cui et al., 2010), 12 mo (Lee et al., 2011), 60 days (Lu et al., 2013). Methods: ChIP-Seq analysis (Cui et al., 2010), RT-PCR (Lee et al., 2011), RNA-seq (Lu et al., 2013). Strain: C57BL/6 (M & F) (Cui et al., 2010; Lu et al., 2013), FVB mice (M) (Lee et al., 2011)	Cui et al. (2010); Lee et al. (2011); Lu et al. (2013)
GSTT2 mRNA expression	No changes	No changes	No changes	Adult age: 45 days (Cui et al., 2010), 12 mo (Lee et al., 2011), 60 days (Lu et al., 2013). Methods: ChIP-Seq analysis (Cui et al., 2010), RT-PCR (Lee et al., 2011), RNA-seq (Lu et al., 2013). Strain: C57BL/6 (M & F) (Cui et al., 2010; Lu et al., 2013), FVB mice (M) (Lee et al., 2011)	Cui et al. (2010); Lee et al. (2011); Lu et al. (2013)
GSTT3 mRNA expression	Fetal development (7%–55%)	Between 0 and 32 days	Increased rapidly	Adult age: 45 days (Cui et al., 2010), 12 mo (Lee et al., 2011), 60 days (Lu et al., 2013). Methods: ChIP-Seq analysis (Cui et al., 2010), RT-PCR (Lee et al., 2011), RNA-seq (Lu et al., 2013). Strain: C57BL/6 (M & F) (Cui et al., 2010; Lu et al., 2013), FVB mice (M) (Lee et al., 2011)	Cui et al. (2010); Lee et al. (2011); Lu et al. (2013)
GSTZ1 mRNA expression	Fetal development (29%)	3 days	Increased progressively	Adult age: 45 days (Cui et al., 2010), 12 mo (Lee et al., 2011), 60 days (Lu et al., 2013). Methods: ChIP-Seq analysis (Cui et al., 2010), RT-PCR (Lee et al., 2011), RNA-seq (Lu et al., 2013). Strain: C57BL/6 (M & F) (Cui et al., 2010; Lu et al., 2013), FVB mice (M) (Lee et al., 2011)	Cui et al. (2010); Lee et al. (2011); Lu et al. (2013)
MGST1					(continued)

TABLE 16—Continued

	Onset of Activity and/or Expression	Adult Levels Reached	Age-Related Changes in Activity/Expression	Comments	References
mRNA expression	Fetal development (7%)	30 days	Increased rapidly	Female expression was generally lower. Adult age: 45 days (Cui et al., 2010), 60 days (Lu et al., 2013). Methods: ChIP-Seq (Cui et al., 2010), RNA-seq (Lu et al., 2013). Strain: C57BL/6 (M & F) (Cui et al., 2010), C57BL/6 (M) (Lu et al., 2013)	Cui et al. (2010); Lu et al. (2013)
MGST2 mRNA expression	NR	15 days	Decreased rapidly	Adult age: 45 days. Methods: ChIP-Seq. Strain: C57BL/6 (M & F)	Cui et al. (2010)
MGST3 mRNA expression	Fetal development (50%); fetal development (23.09%)	0 days (Cui et al., 2010); 5 days (Lu et al., 2013)	Increased progressively (Cui et al., 2010); decreased progressively (Lu et al., 2013)	Adult age: 45 days (Cui et al., 2010), 60 days (Lu et al., 2013). Methods: ChIP-Seq (Cui et al., 2010), RNA-seq (Lu et al., 2013). Strain: C57BL/6 (M & F) (Cui et al., 2010), C57BL/6 (M) (Lu et al., 2013)	Cui et al. (2010); Lu et al. (2013)
NAT1 Protein activity	Before or at 4 days (1%)	NR	Increased rapidly	Substrates were isoniazid (INH), p-aminobenzoic acid (PABA), 4ABP, and 2AF. Adult age: not defined. Methods: RNA assay. Strain: C57BL/6 (M & F)	McQueen and Chau (2003)
mRNA expression	Before or at 4 days (7%)	25 days	Increased progressively	Adult age: 60 days (Lu et al., 2013), 12 mo (Lee et al., 2011). Methods: RNA-seq (Lu et al., 2013), RT-PCR (Lee et al., 2011). Strain: C57BL/6 (M) (Lu et al., 2013), FVB mice (M) (Lee et al., 2011)	Lee et al. (2011); Lu et al. (2013)
NAT2 Protein activity	Before or at 4 days (3%)	NR	Increased rapidly	Substrates were isoniazid (INH), p-aminobenzoic acid (PABA), 4ABP, and 2AF. Adult age: not defined. Methods: RNA assay. Strain: C57BL/6 (M & F)	McQueen and Chau (2003)
mRNA expression	Hovering around adult value	—	Inconsistent pattern	Adult age: 60 days (Lu et al., 2013), 12 mo (Lee et al., 2011). Methods: RNA-seq (Lu et al., 2013), RT-PCR (Lee et al., 2011). Strain: C57BL/6 (M) (Lu et al., 2013), FVB mice (M) (Lee et al., 2011)	Lee et al. (2011); Lu et al. (2013)
NAT5 mRNA expression	Fetal development (14.7%)	32 days	Decreased rapidly	Adult age: 12 mo. Methods: RT-PCR. Strain: FVB mice (M)	Lee et al. (2011)
NAT6 mRNA expression	Fetal development (37%)	32 days	Increased rapidly	Adult age: 12 mo. Methods: RT-PCR. Strain: FVB mice (M)	Lee et al. (2011)
NAT8 mRNA expression	0 days (1%)	0 days	Inconsistent pattern	Adult age: 60 days. Methods: RNA assay. Strain: C57BL/6 (M)	Lee et al. (2011)
NAT10 mRNA expression	Fetal development (34%–1425%)	32 days	Inconsistent pattern	The article reported both an increasing and decreasing pattern. Adult age: 12 mo. Methods: RT-PCR. Strain: FVB mice (M)	Lee et al. (2011)
NAT11 mRNA expression	Fetal development (643%)	32 days	Decreased rapidly	Adult age: 12 mo. Methods: RT-PCR. Strain: FVB mice (M)	Lee et al. (2011)
NAT12 mRNA expression	Fetal development (43%)	7 days	Increased rapidly	Adult age: 12 mo. Methods: RT-PCR. Strain: FVB mice (M)	Lee et al. (2011)
NAT13					

(continued)

TABLE 16—Continued

	Onset of Activity and/or Expression	Adult Levels Reached	Age-Related Changes in Activity/Expression	Comments	References
mRNA expression	Fetal development (200%)	32 days	Decreased slowly	Adult age: 12 mo. Methods: RT-PCR. Strain: FVB mice (M)	Lee et al. (2011)
SULT1A1 mRNA expression	Fetal development (20%–100%)	1 days	Increased progressively	Adult age: 45 days (Ahouti and Klaassen, 2006), 60 days (Lu et al., 2013; Peng et al., 2013), 12 mo (Lee et al., 2011). Methods: bDNA signal amplification assay (Ahouti and Klaassen, 2006), RNA assay (Lu et al., 2013), RNA-seq (Peng et al., 2013), RT-PCR (Lee et al., 2011). Strain: C57BL/6 (M) (Lu et al., 2013; Peng et al., 2013), C57BL/6 (M & F) (Ahouti and Klaassen, 2006), FVB mice (M) (Lee et al., 2011)	Ahouti and Klaassen (2006); Lee et al. (2011); Lu et al. (2013); Peng et al. (2013)
SULT1B1 mRNA expression	Fetal development (10%)	5 days	Increased rapidly	Adult age: 60 days (Lu et al., 2013; Peng et al., 2013), 12 mo (Lee et al., 2011). Methods: RNA assay (Lu et al., 2013), RNA-seq (Peng et al., 2013), RT-PCR (Lee et al., 2011). Strain: C57BL/6 (M) (Lu et al., 2013; Peng et al., 2013), FVB mice (M) (Lee et al., 2011)	Lee et al. (2011); Lu et al. (2013); Peng et al. (2013)
SULT1C1 mRNA expression	Fetal development (29%) (Lu et al., 2013; fetal development (22220%))	5 days (Lu et al., 2013); 22 days (Ahouti and Klaassen, 2006)	Increased progressively (Lu et al., 2013); decreased rapidly	Adult age: 45 days (Ahouti and Klaassen, 2006), 60 days (Lu et al., 2013). Methods: bDNA amplification assay (Ahouti and Klaassen, 2006), RNA assay (Lu et al., 2013). Strain: C57BL/6 (M & F) (Ahouti and Klaassen, 2006), C57BL/6 (M) (Lu et al., 2013)	Ahouti and Klaassen (2006); Lu et al. (2013)
SULT1C2 mRNA expression	0 days (6%)	1 day	Increased progressively	Adult age: 45 days (Ahouti and Klaassen, 2006), 60 days (Lu et al., 2013; Peng et al., 2013), 12 mo (Lee et al., 2011). Methods: bDNA signal amplification assay (Ahouti and Klaassen, 2006), RNA assay (Lu et al., 2013), RNA-seq (Peng et al., 2013), RT-PCR (Lee et al., 2011). Strain: C57BL/6 (M) (Lu et al., 2013; Peng et al., 2013), C57BL/6 (M & F) (Ahouti and Klaassen, 2006), FVB mice (M) (Lee et al., 2011)	Ahouti and Klaassen (2006); Lu et al. (2013); Peng et al. (2013)
SULT1D1 mRNA expression	Fetal development (13%)	1 day	Inconsistent pattern	Adult age: 45 days (Ahouti and Klaassen, 2006), 60 days (Lu et al., 2013; Peng et al., 2013), 12 mo (Lee et al., 2011). Methods: bDNA signal amplification assay (Ahouti and Klaassen, 2006), RNA assay (Lu et al., 2013), RNA-seq (Peng et al., 2013), RT-PCR (Lee et al., 2011). Strain: C57BL/6 (M) (Lu et al., 2013; Peng et al., 2013), C57BL/6 (M & F) (Ahouti and Klaassen, 2006), FVB mice (M) (Lee et al., 2011)	Ahouti and Klaassen (2006); Lu et al. (2013); Peng et al. (2013)
SULT1E1 mRNA expression	Fetal development (207%)	10 days	Inconsistent pattern	Adult age: 60 days. Methods: RNA-seq. Strain: C57BL/6 (M)	Peng et al. (2013)
SULT2A1					(continued)

TABLE 16—Continued

	Onset of Activity and/or Expression	Adult Levels Reached	Age-Related Changes in Activity/Expression	Comments	References
mRNA expression	Fetal development (900%)	30 days	Inconsistent pattern	Adult age: 60 days (Lu et al., 2013; Peng et al., 2013). Methods: RNA-seq (Peng et al., 2013), RNA assay (Lu et al., 2013). Strain: C57BL/6 (M) (Lu et al., 2013; Peng et al., 2013)	Lu et al. (2013); Peng et al. (2013)
SULT2A12 mRNA expression	0 days (10%)	10 days	Inconsistent pattern	Adult age: 45 days. Methods: bDNA signal amplification assay. Strain: C57BL/6 (F)	Alnouti and Klaassen (2006)
SULT2A2 mRNA expression	Fetal development (600%)	30 days	Inconsistent pattern	Adult age: 60 days (Lu et al., 2013; Peng et al., 2013). Methods: RNA-seq (Peng et al., 2013), RNA assay (Lu et al., 2013). Strain: C57BL/6 (M) (Lu et al., 2013; Peng et al., 2013)	Lu et al. (2013); Peng et al. (2013)
SULT2A3 mRNA expression	Before or at 5 days (248%)	60 days	Inconsistent pattern	Adult age: 60 days. Methods: RNA-seq. Strain: C57BL/6 (M)	Peng et al. (2013)
SULT2A4 mRNA expression	Before or at 5 days (248%)	60 days	Inconsistent pattern	Adult age: 60 days. Methods: RNA-seq. Strain: C57BL/6 (M)	Peng et al. (2013)
SULT2A5 mRNA expression	Before or at 5 days (248%)	60 days	Inconsistent pattern	Adult age: 60 days. Methods: RNA-seq. Strain: C57BL/6 (M)	Peng et al. (2013)
SULT2A6 mRNA expression	Before or at 1 day (447%)	60 days	Inconsistent pattern	Adult age: 60 days. Methods: RNA-seq. Strain: C57BL/6 (M)	Peng et al. (2013)
SULT2A7 mRNA expression	15 days (200%)	30 days	Inconsistent pattern	Adult age: 60 days. Methods: RNA assay. Strain: C57BL/6 (M)	Peng et al. (2013)
SULT2B1 mRNA expression	Fetal development (300%)	15 days	Inconsistent pattern	Adult age: 60 days. Methods: RNA assay. Strain: C57BL/6 (M)	Peng et al. (2013)
SULT3A1 mRNA expression	M: fetal development (200%); F: fetal development (6%)	M: 15 days; F: 45 days	M: inconsistent pattern; F: increased slowly	Adult age: 45 days. Methods: bDNA signal amplification assay. Strain: C57BL/6 (M & F)	Alnouti and Klaassen (2006)
SULT3A1 mRNA expression	No data	7 days	Decreased progressively	Adult age: 12 mo. Methods: RT-PCR. Strain: FVB mice (M)	Lee et al. (2011)
SULT5A1 mRNA expression	Fetal development (2%)	15 days	Increases rapidly (Lu et al., 2013; Peng et al., 2013); inconsistent pattern (Lee et al., 2011)	Adult age: 60 days (Lu et al., 2013; Peng et al., 2013); 12 mo (Lee et al., 2011). Methods: RNA assay (Lu et al., 2013), RNA-seq (Peng et al., 2013), RT-PCR (Lee et al., 2011). Strain: C57BL/6 (M) (Lu et al., 2013; Peng et al., 2013), FVB mice (M) (Lee et al., 2011)	Lee et al. (2011); Lu et al. (2013); Peng et al. (2013)
UGT1A1 mRNA expression	M: fetal development (11%); F: fetal development (3%)	M: 5 days; F: 45 days	M: increased progressively; F: increases progressively until 5 days then rapidly	Primer: Mn0260337_m1 (Nakajima et al., 2012). Adult age: 60 days (Lu et al., 2013), >9 wk (Nakajima et al., 2012). Methods: real-time PCR (Nakajima et al., 2012), RNA assay (Lu et al., 2013). Strain: BALB/c (M & F) (Nakajima et al., 2012), C57BL/6 (M) (Lu et al., 2013)	Nakajima et al. (2012); Lu et al. (2013)
UGT1A2					(continued)

TABLE 16—Continued

	Onset of Activity and/or Expression	Adult Levels Reached	Age-Related Changes in Activity/Expression	Comments	References
mRNA expression	Fetal development 1 day (8%)	Inconsistent pattern		Adult age: 60 days. Methods: RNA assay. Strain: C57BL/6 (M)	Lu et al. (2013)
UGT1A5 mRNA expression	Fetal development 3 days (2%)	Inconsistent pattern		Adult age: 60 days. Methods: RNA assay. Strain: C57BL/6 (M)	Lu et al. (2013)
UGT1A6 mRNA expression	Fetal development 3 days (30%) (mixed)	Increased rapidly		Data are not normalized as the highest reported age is too low. Adult age: 21 days. Methods: RT-PCR. Strain: C57BL/6 (M & F)	Yabusaki et al. (2015)
UGT1A6A mRNA expression	Fetal development 60 days (10%)	Increased rapidly		Adult age: 60 days. Methods: RNA assay. Strain: C57BL/6 (M)	Lu et al. (2013)
UGT1A6B mRNA expression	Fetal development 10 days (9%)	Increased rapidly		Adult age: 60 days. Methods: RNA assay. Strain: C57BL/6 (M)	Lu et al. (2013)
UGT1A7C mRNA expression	Fetal development 5 days (380%)	Decreased progressively		Adult age: 60 days. Methods: RNA assay. Strain: C57BL/6 (M)	Lu et al. (2013)
UGT1A9 mRNA expression	1 day (1%)	60 days	Increased progressively until 5 days then rapidly until 30 days	Adult age: 60 days. Methods: RNA assay. Strain: C57BL/6 (M)	Lu et al. (2013)
UGT1A10 mRNA expression	Fetal development 1 day (133%)	Inconsistent pattern		Adult age: 60 days. Methods: RNA assay. Strain: C57BL/6 (M)	Lu et al. (2013)
UGT2A3 mRNA expression	1 day (1%)	60 days	Increased progressively until 5 days then rapidly until 30 days	Adult age: 60 days (Lu et al., 2013), 12 mo (Lee et al., 2011). Methods: RNA-seq (Lu et al., 2013), RT-PCR (Lee et al., 2011). Strain: C57BL/6 (M) (Lu et al., 2013), FVB mice (M) (Lee et al., 2011)	Lee et al. (2011); Lu et al. (2013)
UGT2B1 mRNA expression	Fetal development 25 days (0.5%)	Increased progressively until 5 days then rapidly until 30 days	Primer: Mn02603337_m1 (Nakajima et al., 2012). Adult age: 60 days (Lu et al., 2013), >9 wk (Nakajima et al., 2012), 12 mo (Lee et al., 2011). Methods: real-time PCR (Nakajima et al., 2012), RNA assay (Lu et al., 2013), RT-PCR (Lee et al., 2011). Strain: BALB/c (M & F) (Nakajima et al., 2012), C57BL/6 (M) (Lu et al., 2013), FVB mice (M) (Lee et al., 2011)	(Lee et al., 2011); Nakajima et al. (2012); Lu et al. (2013)	
UGT2B34 mRNA expression	Fetal development 60 days (23%)	Increased rapidly	Adult age: 60 days (Lu et al., 2013), 12 mo (Lee et al., 2011). Methods: RNA-seq (Lu et al., 2013), RT-PCR (Lee et al., 2011). Strain: C57BL/6 (M) (Lu et al., 2013), FVB mice (M) (Lee et al., 2011)	Lee et al. (2011); Lu et al. (2013)	
UGT2B35 mRNA expression	Fetal development 60 days (13%)	Increased rapidly	Adult age: 60 days (Lu et al., 2013), 12 mo (Lee et al., 2011). Methods: RNA-seq (Lu et al., 2013), RT-PCR (Lee et al., 2011). Strain: C57BL/6 (M) (Lu et al., 2013), FVB mice (M) (Lee et al., 2011)	Lee et al. (2011); Lu et al. (2013)	
UGT2B36					(continued)

TABLE 16—Continued

	Onset of Activity and/or Expression	Adult Levels Reached	Age-Related Changes in Activity/Expression	Comments	References
mRNA expression	Fetal development (5%)	25 days	Increased rapidly	Adult age: 60 days (Lu et al., 2013), 12 mo (Lee et al., 2011). Methods: RNA-seq (Lu et al., 2013), RT-PCR (Lee et al., 2011). Strain: C57BL/6 (M) (Lu et al., 2013), FVB mice (M) (Lee et al., 2011)	Lee et al. (2011); Lu et al. (2013)
UGT2B37 mRNA expression	Fetal development (3%)	25 days	Increased rapidly	Adult age: 60 days (Lu et al., 2013), 12 mo (Lee et al., 2011). Methods: RNA-seq (Lu et al., 2013), RT-PCR (Lee et al., 2011). Strain: C57BL/6 (M) (Lu et al., 2013), FVB mice (M) (Lee et al., 2011)	Lee et al. (2011); Lu et al. (2013)
UGT2B38 mRNA expression	Fetal development (2%)	60 days	Increased rapidly	Adult age: 60 days (Lu et al., 2013), 12 mo (Lee et al., 2011). Methods: RNA-seq (Lu et al., 2013), RT-PCR (Lee et al., 2011). Strain: C57BL/6 (M) (Lu et al., 2013), FVB mice (M) (Lee et al., 2011)	Lee et al. (2011); Lu et al. (2013)
UGT2B38 mRNA expression	No data	32 days	Inconsistent pattern	Adult age: 12 mo. Methods: RT-PCR. Strain: FVB mice (M)	Lee et al. (2011); Lu et al. (2011)
UGT2B5 mRNA expression	Fetal development (2%–18%)	32 days	Increased rapidly	Adult age: 60 days (Lu et al., 2013), 12 mo (Lee et al., 2011). Methods: RNA-seq (Lu et al., 2013), RT-PCR (Lee et al., 2011). Strain: C57BL/6 (M) (Lu et al., 2013), FVB mice (M) (Lee et al., 2011)	Lee et al. (2011); Lu et al. (2013)
UGT3A1 mRNA expression	Fetal development (2%)	30 days	Inconsistent pattern (Lu et al., 2013); increases progressively (Lee et al., 2011)	Adult age: 60 days (Lu et al., 2013), 12 mo (Lee et al., 2011). Methods: RNA-seq (Lu et al., 2013), RT-PCR (Lee et al., 2011). Strain: C57BL/6 (M) (Lu et al., 2013), FVB mice (M) (Lee et al., 2011)	Lee et al. (2011); Lu et al. (2013)
UGT3A2 mRNA expression	Fetal development (0.5%)	30 days	Increased progressively until 5 days then rapidly until 30 days	Adult age: 60 days (Lu et al., 2013), 12 mo (Lee et al., 2011). Methods: RNA-seq (Lu et al., 2013), RT-PCR (Lee et al., 2011). Strain: C57BL/6 (M) (Lu et al., 2013), FVB mice (M) (Lee et al., 2011)	Lee et al. (2011); Lu et al. (2013)

⁴AFP, 4-aminobiphenyl; 2AF, 2-aminofluorene; bDNA, branched DNA signal amplification assay; ChIP-Seq, chromatin immunoprecipitation sequencing; F, female; M, male; mCES, mouse CES; NR, not reported; PCR, polymerase chain reaction; RNA-seq, RNA-sequencing; RT-PCR, reverse-transcriptase PCR.

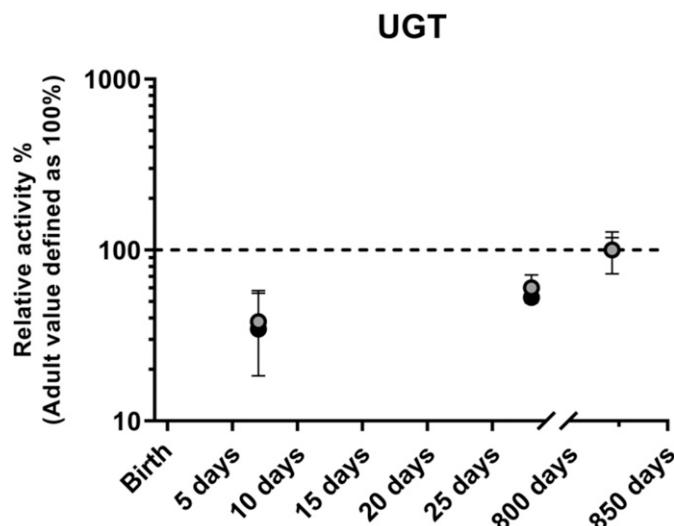


Fig. 19. Pooled literature data on the ontogeny of metabolic activity of hepatic UGT activity in Göttingen minipig. The symbols represent the relative activity in each age group, and the dotted line indicates the adult value defined as 100%. If multiple values were obtained for the same age group, the symbols represent the average relative activity, and the error bars show the S.D. See Table 17 for explanation on the ontogeny profiles and literature references.

post-translational modifications, which could remove the correlation in the study populations of future research. This could lead to false conclusions. We therefore encourage future researchers to first establish the correlation between mRNA expression, protein expression, and, if possible, even protein activity for their study population interest before relying entirely on mRNA expression levels as a surrogate for protein expression or activity.

Numerous other covariates apart from age, such as sex or genotype, will also influence expression and activity of DTs and DMEs. However, because of a lack of available data and/or a limited number of individual measurements, no sex-related differences in ontogeny profile of human hepatic DTs and DMEs have been reported. In nonclinical species, including rats and mice, distinct profiles were identified between male and female animals. For instance, CYP2B13 in male mice was at adult values at birth, but in female mice adult values were only reached at 45 days (see Table 9), whereas SULT1A1 mRNA expression in 1.5-day-old rats was 28% and 75% of adults values for males and females, respectively (see Table 15).

In this review, no genetic differences in ontogeny profiles were explored. Nevertheless, for the polymorphic DME CYP2D6, for instance, both age and genotype are known to contribute to interindividual variability in CYP2D6 metabolism during human development (Stevens et al., 2008). For DTs (e.g., OATP1B1), it has been shown that some polymorphisms can influence mRNA and/or protein expression levels. Yet there are conflicting reports, as one study did not find a genotype–protein expression relationship for OATP1B1 in human liver tissue of fetuses and children <3 months old (van Groen et al., 2018). Another group reported that OATP1B1 expression was associated with genotype in children >1 year of age (Prasad et al., 2016). Such apparent discrepancies can be explained by the interplay between genotype and ontogeny, in which a lower expression at young age may obscure an effect of genotype. Also for in vivo data, this was shown for the CYP3A5 substrate tacrolimus, in which younger age and CYP3A5 expresser genotype were independently associated with higher dosing requirements and lower tacrolimus concentration/dose ratios (Gijssen et al., 2011). Further studying the interplay between age and genotype would be of help to improve prediction of

TABLE 17
Ontogeny profile of hepatic phase II enzymes in Göttingen minipig based on metabolic activity, protein expression, and mRNA expression levels
Percentages represent expression/activity relative to adult levels.

Onset of Activity and/or Expression	Adult Levels Reached	Age-Related Changes in Activity/Expression	Comments	References	
UGT Catalytic activity	M: 7 days (35%); F: 7 days (38%)	M: NR (52% at 28 days); F: NR (60% at 28 days)	Increased rapidly	Single study, sparse data. Adult age: 822 days. Substrate: UGT-Glo. Methods: PLM. Strain: Göttingen minipig (M/F)	Van Peer et al. (2017)

F, female; M, male; NR, not reported; PLM, pig liver microsome.

drug PK mainly when polymorphic proteins, such as CYP2D6, CYP2C19, or UGT2B10, are involved.

Several suggestions can be made for further research. Because the strength of this review lies in the fact that all raw data from available literature were extracted, normalized, and pooled, we believe that this should become standard of practice. As such, to accelerate data availability, we encourage publishing the data set as a supplementary file along with the publication and initiatives for data-sharing platforms or repositories in which all raw data of published articles are available—the added value of this was recently shown by Ladumor et al. (2019). Scientists are encouraged to follow guidelines from initiatives like the go-FAIR initiative to make data “Findable, Accessible, Interoperable, and Reusable (FAIR)” (Wilkinson et al., 2016). Also, to further accelerate data generation, international databases with information on which samples are stored in various biobanks would have added value to overcome the scarcity of pediatric tissue. Lastly, current developments may make it possible to fill the knowledge gaps that were identified in this review. These include the use of organoids (Nantasanti et al., 2016) and exosomes (Rodrigues and Rowland, 2019) to study DT and DME activity and the interplay between DTs and DMEs. However, these techniques are currently hampered by the lack of knowledge regarding whether organoids and exosomes retain age-specific properties, which is a prerequisite for studying age-related changes in expression/activity of DTs and DMEs. Moreover, although this review is limited to ontogeny in the liver, DTs and/or DMEs are also abundant in other major organs, such as the kidney and gastrointestinal tract, and sanctuary sites, including the central nervous system (DeGorter et al., 2012). Developmental patterns of isoforms appear to be organ-dependent (Drozdzik et al., 2018; Li et al., 2019). However, for other organs, a quantitative approach as presented in this comprehensive review is not available but is highly needed. Once this is available, integration of ontogeny profiles in multiple tissues via a PBPK framework could provide a more holistic systems approach on the development of an entire organism (Smits et al., 2013).

In conclusion, we anticipate that our expedition to compile these hepatic ontogeny data across human and various nonclinical species will help us understand the developmental patterns of DTs and DMEs in human and nonclinical species and provide an excellent framework to support and trigger improvement in predicting drug disposition in pediatric and juvenile populations.

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