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Expression of CYP3A4 and CYP3A7 in Human Foetal Tissues and its Correlation with Nuclear Receptors

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Abstract: Previous reports have suggested that the nuclear receptors vitamin D receptor (VDR), peroxisome proliferator-activated receptor α (PPARα), pregnane X receptor (PXR) and constitutive androstane receptor (CAR) are involved in the regulation of the drug-metabolizing enzyme cytochrome P450 (CYP) 3A4 expression in adults. The aim of this study was to investigate the gene expression of CYP3A4 and the foetal CYP3A7 in human foetal tissues and their relation to gene expression and genetic variations in the nuclear receptors VDR, PPARα, PXR and CAR. We determined the relative expression of CYP3A4 and CYP3A7 and these nuclear receptors in foetal livers, intestines and adrenals, using quantitative PCR. In addition, the expression of these enzymes was also analysed in adult liver. There was a high interindividual variability in CYP3A4 and CYP3A7, 49 times and 326 times, respectively. Both CYP3A4 and CYP3A7 had the highest expression in the liver. There were significant correlations ($p < 0.001$) between the nuclear receptors studied and the expression of CYP3A4 and CYP3A7 in foetal liver, as well as the expression of CYP3A4 in foetal intestine. Polymorphisms in the VDR gene, rs1544410 and rs1523130 (*TaqI*), in the PXR gene, rs1523130, and in the PPARα gene, rs4253728, were not correlated with CYP3A4 or CYP3A7 expression. However, C-homozygous individuals of the *TaqI* VDR polymorphism had 60% lower VDR gene expression ($p < 0.05$), than individuals carrying one or two T alleles. In conclusion, differences in the expression of nuclear receptors might determine the variability in CYP3A4 and CYP3A7 expression observed in foetal liver.

The cytochrome P450 (CYP) 3A enzymes are considered to be the most important human drug-metabolizing enzymes with regard to number of different drug substrates [1]. The activities of these enzymes are known to show a significant variability not only between different individuals but also within the same individual at different time-points. CYP3A4 accounts for the main CYP3A enzyme in adults, whereas CYP3A7 is the main enzyme in foetuses [2–4].

CYP3A4 protein expression is low during the first trimester and rises during the second and third trimester of pregnancy [2]. Adult levels are reached at 2–3 years of age. CYP3A7 is the main CYP3A enzyme in foetuses. It decreases after birth but remains the main CYP3A enzyme during the first year after birth [2,3] and is still expressed in adult liver and intestine but at much lower levels [5]. Determining dosage of drugs in patients during neonatal period, infancy and childhood is complicated as there is no correlation between liver size and enzyme activity, and body-weight may not reflect age-related differences in the ability to metabolize drugs [6].

Although some medications may be suspended during pregnancy or replaced by safer alternatives, other drugs are required to prevent maternal complications. It has been shown that 40% of women received drug prescriptions during pregnancy [7] and the use of over-the-counter drugs is common [8]. This high prevalence of drug use by pregnant women underscores the importance of understanding drug metabolism in the foetus. Foetal metabolism is considered to be a significant contributor to non-placental foetal clearance [9]. However, studies of CYP enzymes in the foetus are scarce.

Nuclear receptors are transcription factors that interact with small lipophilic ligands, such as steroid hormones, thyroid hormones, leukotrienes, fatty acids and vitamin D. They bind to specific sequences in the DNA called hormone response elements and thereby change gene expression of their target genes. Polymorphisms in nuclear receptors have been shown to affect the expression of CYP3A4 in the intestine and liver [10–12]. Pregnane X receptor (PXR) is a nuclear receptor mainly expressed in the liver and intestines that induce CYP3A4 in response to pregnanes and several clinically used drugs [10]. PXR, constitutive androstane receptor (CAR) and vitamin D receptor (VDR) form heterodimers with retinoid X receptor (RXR). CAR is found in the cytoplasm and translocates to the nucleus upon activation.

1,25dihydroxyvitamin D_3 is the most active metabolite of vitamin D_3 and can induce CYP3A4 by binding to the VDR according to *in vitro* experiments [13]. In addition, previous findings from clinical samples have also suggested a possible role for vitamin D in CYP3A activity [14]. The VDR gene is polymorphic, and previous reports have shown that the SNP rs1544410 is important for the interindividual variability in CYP3A activity in the adult intestine [12]. The VDR SNP

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rs731236, also referred to as *TaqI*, has been shown to be associated with altered VDR function [15,16].

Peroxisome proliferator-activated receptor α (PPARα), a nuclear receptor involved in regulation of lipid metabolism, has also been linked to expression of CYP3A4 [17–19]. PPARα is also polymorphic, and the SNP rs4253728 has previously been shown to correlate with CYP3A activity [17]. Several SNPs in PXR have been studied in relation to CYP3A4 activity, of which a C>T promoter polymorphism (rs1523130) was consistently associated with CYP3A4 activity and expression [11].

The aim of this study was to investigate the expression of CYP3A4 and CYP3A7 in different human foetal tissues and study a possible correlation with expression patterns of the nuclear receptors VDR, CAR, PPARα and PXR.

Material and Methods

Study population. Foetal samples, intestines (n = 13), liver (n = 60) and adrenals (n = 46), were obtained from a biobank of foetal tissue from legal abortions performed for socio-medical reasons at Karolinska University Hospital [20]. The gestational ages were determined by crown-rump length and ranged from 5 to 12 weeks (median = 10.2). None of the women reported any chronic or acute diseases, drug abuse or regular drug use.

Adult liver samples (n = 21) were collected from patients between 30 and 75 years of age. The material was obtained from the liver bank at the Division of Clinical Pharmacology as previously described elsewhere [21]. Material consisted of total RNA, cDNA and genomic DNA from foetuses and adults.

The studies were approved by the National Board of Health and Welfare (51-9171/96) and the Ethics Review Board in Stockholm (foetus: DNR964/23, adult: DNR429/01).

RNA extraction and cDNA synthesis. Total RNA from 5 to 30 mg of foetal tissue samples and 200 mg adult liver tissue was prepared using AllPrep DNA/RNA Mini Kit and RNeasy kit (Qiagen, Hilden, Germany), respectively, according to the manufacturer's protocols. Reverse transcription was performed on 0.3–0.5 µg RNA samples. Master mix with RNase inhibitor was prepared according to High Capacity cDNA Reverse Transcription Kits Protocol (Applied Biosystems, Foster City, CA, USA) and was performed using PCR System 2700 (Applied Biosystems) under conditions 25°C for 10 min., 37°C for 120 min., 85°C for 5 min. and 4°C overnight.

Q-PCR gene expression. PCR Master mix was prepared: Taq Man 2XPCR mix, 20× gene assay and 1 µL cDNA in a final volume of 15 µL. TaqMan® Gene Expression Assays (Applied Biosystems) used were CYP3A4 (Hs00604506_m1), CYP3A7 (Hs00426361_m1), VDR (Hs00172113_m1), PPARα (Hs00947536_m1), PXR (Hs01114267_m1), CAR (Hs00901571_m1) and 18S (4310893E-1105050). The samples were analysed in duplicates or triplicates using QuantStudio™ 12K Flex Real-Time PCR system software version 1.1.2. or 2.0.6 (Applied Biosystems). Each PCR included activation (95°C for 10 min.) followed by 40 cycles of denaturation (95°C for 15 sec.) and annealing/elongation (60°C for 1 min.).

Relative expression was calculated according to the $2^{-\Delta\Delta Ct}$ formula [22] using housekeeping gene 18S as an internal control. As the PCR efficacy when using foetal cDNA was between 98 and 100% and the slope was ≤0.1 between 18S and the genes of interest, this formula could be employed. Comparing adults and foetuses, ΔΔCt was calculated using an adult sample as calibrator, and when comparing tissue-specific expression, a sample from foetal liver was chosen as calibrator. Comparing tissue-specific CYP3A7 and CYP3A4 expression in foetuses, a tissue-specific CYP3A7 value from a specific foetus was used as calibrator.

PCR genotyping. PCR Master mix was prepared containing Taq Man 2XPCR mix, 40× genotyping assay and 15–30 ng genomic DNA to a final volume of 10 µL. TaqMan® genotyping assays (Applied Biosystems) used were VDR rs1544410 (C__8716062_10), VDR rs731236 (C___2404008_10), PXR rs1523130 (C___9152783_20) and PPARα rs4253728 (C__31052401_10). Samples were analysed using QuantStudio™ 12K Flex Real-Time PCR system software version 1.1.2 (Applied Biosystems) using the PCR programme described above.

Statistical analysis. All statistical analyses were performed with Graph Pad Prism® (GraphPad Software, Inc., La Jolla, CA 92037, USA) version 4.03. Data could not be proven to follow Gaussian distribution; therefore, nonparametric tests were used. Spearman's rank correlation was used to investigate correlation between nuclear receptors and CYP3A4 and CYP3A7 and correlation of these genes with age. Mann–Whitney tests were used for determining differences in expression between adults and foetuses and tissue-specific CYP3A7 and CYP3A4 expression and when comparing expression of CYP3A4, CYP3A7 and VDR between different genotypes. For tissue-specific comparison, Kruskal–Wallis test was performed followed by Dunn's multiple comparisons post-test for the identification of which groups differed from the others.

Results

Hepatic gene expression of CYP3A4 and CYP3A7 in foetuses and adults.

Differences in expression between adults and foetuses were found in expression levels of CYP3A4, CYP3A7, VDR, PXR and CAR (fig. 1). Expression of CYP3A4, VDR, PXR and CAR was higher in adults than in foetuses. The expression level of PPARα was the same in adults and foetuses (fig. 1). Relative expression of CYP3A4 was 40,000 times higher in adults than in foetuses, and CYP3A7 was 5500 times higher in foetuses than in adults. The gene expression levels were not correlated with age or sex (adult samples) or gestation age (foetal samples).

Correlation between expression of CYP3A4 and CYP3A7 and nuclear receptors.

The correlation between VDR, PPARα, PXR, CAR and CYP3A4 was significant in foetal livers (table 1, fig. 2) and most prominent for CAR and PXR ($p < 0.001$). A correlation was also found between VDR, PPARα, PXR, CAR and CYP3A7 in foetal livers (table 1, fig. 2). In contrast, in adult liver tissue, no correlation could be established between VDR and CYP3A4 or CYP3A7. The correlation grade between PPARα, PXR, CAR and CYP3A4 in adult liver was the similar (rs 0.49–0.53; $p < 0.05$) (table 1).

It should also be noted that there are two outliers, one showing more than 10 times higher expression of CYP3A7 than the other samples and one showing very high VDR expression (fig. 2). However, when these two outliers were excluded from the analyses, the correlation coefficients were only marginally affected and did not change the overall results or the conclusions.

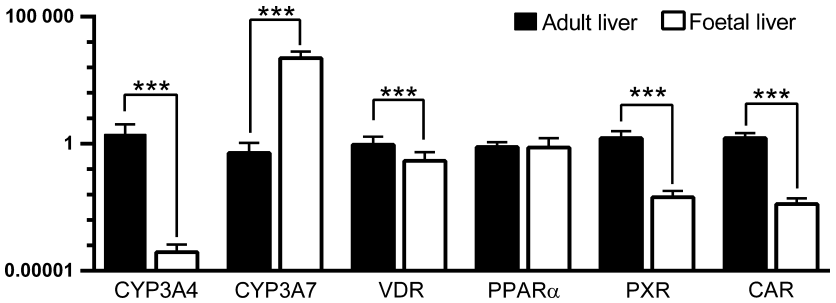

Fig. 1. Mean values of hepatic relative gene expression + S.D. of CYP3A4, CYP3A7, VDR, PPARα, PXR and CAR in adults (n = 21) and foetuses (n = 53). Relative expression is calculated using the same adult liver sample as calibrator for each gene. *p* values from Mann–Whitney tests are given. ***$p < 0.001$.

Table 1.
Correlation between CYP3A4 and CYP3A7 expression and nuclear receptors in foetal and adult tissue.

Gene	Tissue	VDR	PPARα	PXR	CAR
Foetal tissue					
CYP3A4	Liver	0.2796*	0.3916**	0.4993***	0.5900***
	Intestine	0.7348**	0.8674***	0.8122***	0.9002***
	Adrenals	−0.002276	0.232	−0.1304	0.1942
CYP3A7	Liver	0.4187**	0.6778***	0.6911***	0.7037***
	Intestine	0.3052	0.5421	0.1913	0.1868
	Adrenals	−0.03896	0.3824*	0.2271	0.4648**
Adult tissue					
CYP3A4	Liver	0.3143	0.5091*	0.4935*	0.5325*
CYP3A7	Liver	0.4000	0.6481**	0.5584**	0.6169**

Values of Spearman's rank correlation and *p* are given. *$p < 0.05$ **$p < 0.01$ ***$p < 0.001$.

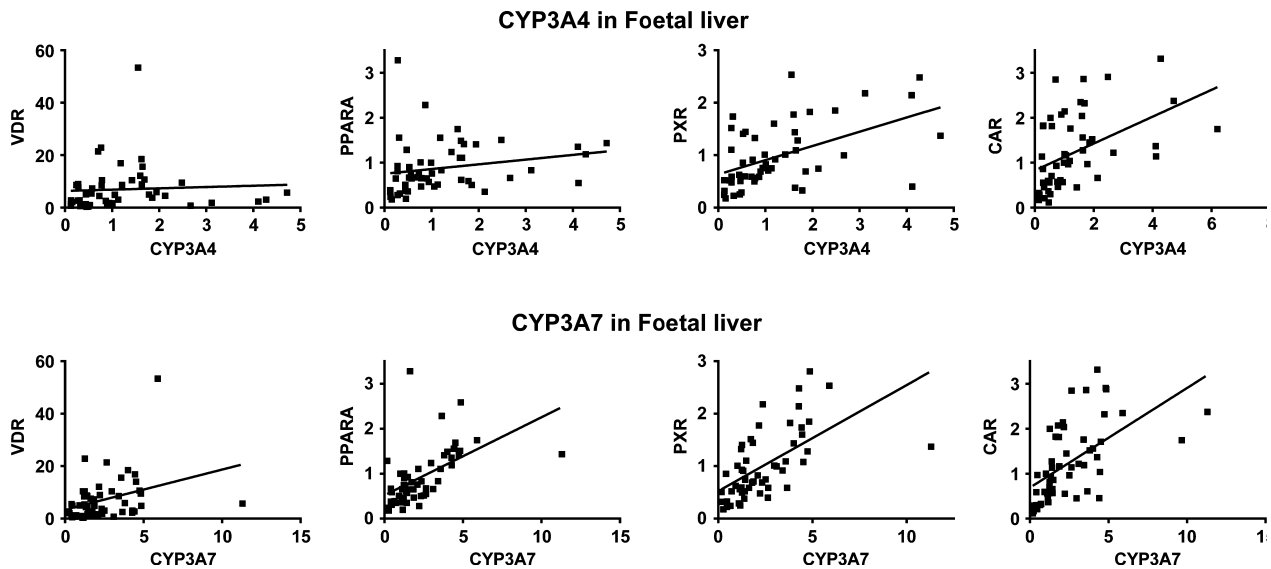

Fig. 2. Correlation between relative expression of CYP3A4 and CYP3A7 and nuclear receptors in foetal liver tissue. Values of Spearman's rank correlation and *p* values are given in table 1.

A pronounced correlation between intestinal CYP3A4 in foetus and VDR, PPARα, PXR and CAR was found. No such correlation was found in adrenals. CYP3A7 was correlated with PPARα and CAR in adrenals ($p < 0.05$ and $p < 0.01$, respectively). No correlation was found between CYP3A7 and any of the analysed nuclear receptors in intestines (table 1).

Extrahepatic gene expression and correlation with nuclear receptors in foetuses.

There was a higher expression of CYP3A7 than of CYP3A4 in foetal adrenals and livers ($p < 0.0001$). No significant difference in expression of CYP3A7 and CYP3A4 in foetal intestines could be detected (fig. 3).

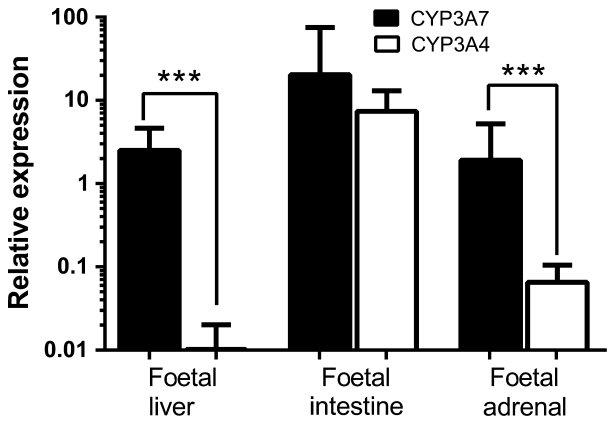

Fig. 3. Mean values of relative gene expression + S.D. of CYP3A7 and CYP3A4 in foetal livers (n = 60), intestines (n = 13) and adrenals (n = 46). Relative expression is calculated using a CYP3A7 value from each organ. *p* values from Mann–Whitney tests are given. ***$p < 0.001$.

CYP3A4 was detected in 90%, 77% and 77% of the foetal liver, intestine and adrenal samples, respectively. CYP3A4 expression was higher in liver than in adrenals ($p < 0.001$), but there was no significant difference in hepatic and intestinal CYP3A4 expression. CYP3A7 was detected in 95%, 77% and 96% of the foetal liver, intestine and adrenal samples, respectively. Hepatic CYP3A7 expression was higher than intestinal ($p < 0.001$) and adrenal expression ($p < 0.001$), while intestinal and adrenal expression did not differ significantly.

The mRNA expression of the nuclear factors VDR, PPARα, PXR and CAR was detected in >90% of the samples investigated, except for CAR mRNA which was only found in 39% of the adrenal samples. Expression of VDR was higher in adrenals than in livers and intestines ($p < 0.001$ and $p < 0.001$, respectively). Hepatic PPARα expression was higher than intestinal and adrenal expression ($p < 0.001$ and $p < 0.001$, respectively), while intestinal and adrenal expression did not differ significantly. Expression of PXR was higher in hepatic tissue and intestines than in adrenals ($p < 0.001$ and $p < 0.001$, respectively). There was no difference between expression of PXR in hepatic tissue and intestines. CAR exhibited a higher expression level in intestines than in livers and adrenals ($p < 0.01$ and $p < 0.001$, respectively), and hepatic expression was higher than adrenal expression ($p < 0.001$) (fig. 4).

Interindividual variability in gene expression.

A large interindividual variation of the gene expression of CYP3A enzymes and nuclear receptors was detected in foetal tissue (table 2). The highest interindividual variation was found for intestinal CYP3A7, a 326 times variation. Further, an interindividual variation found in expression of nuclear receptors ranged from 2 times to 178 times variation (table 2).

Genetic variation in VDR, PXR and PPARα in relation to CYP3A expression.

In this study, the VDR polymorphism, rs1544410 G>A, was not correlated with mRNA expression of CYP3A7, CYP3A4 or VDR in adult liver or foetal liver, intestine or adrenal (data not shown). Both alleles, G and A, had the same frequency (50%) (data not shown). The VDR polymorphism rs1523130 (*TaqI*) was not correlated with mRNA expression of CYP3A7 or CYP3A4. However, individuals homozygous for the C allele had 60% lower VDR expression compared to individuals carrying one or two T alleles ($p < 0.05$) (data not shown).

Likewise, the polymorphisms in PPARα (rs4253728 G>A) and PXR (rs1523130 C>T) were not correlated with relative gene expression of CYP3A4, CYP3A7 or PPARα/PXR in hepatic or extrahepatic tissue (data not shown). Minor allele frequency of PPARα (A) and PXR (T) was 25% and 39%, respectively.

Discussion

In this study, we have shown that there was a significant and strong correlation between the expression of the nuclear receptors PPARα, VDR, CAR and PXR and the expression of

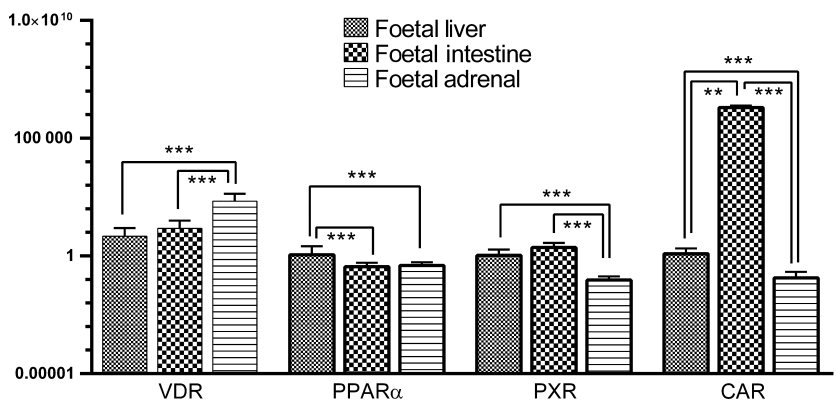

Fig. 4. Mean values + S.D. of relative expression of nuclear receptors in foetal livers (n = 57), intestines (n = 13) and adrenals (n = 44). Relative expression is calculated using the same foetal liver sample as calibrator for each gene. *p* values from Kruskal–Wallis tests are given. **$p < 0.01$ ***$p < 0.001$.

Table 2.
Interindividual fold variation in relative expression in different foetal tissues.

Gene/tissue	Liver	Intestine	Adrenals
CYP3A4	49	8	10
CYP3A7	64	326	214
VDR	178	165	81
PPARA	55	5	7
PXR	27	130	9
CAR	29	2	8

CYP3A4 and CYP3A7 in human foetal liver tissue. For CYP3A4, this correlation was also observed in the intestine. Notably, there was a high interindividual variation in mRNA expression of CYP3A4 and CYP3A7, in accordance with earlier reports [23,24].

In adults, the intestinal CYP3A enzymes play an important role in first-pass metabolism of xenobiotics, leading to reduced bioavailability of many orally administered drugs. In contrast, the intestinal CYP enzymes in foetuses probably play a minor role. Instead, the adrenal glands have been suggested to have a central role in the metabolism of xenobiotics during foetal development and, as a consequence, have been suggested to be involved in xenobiotic-induced foetal development toxicity [25]. In addition, the adrenal glands are one of the most important endocrine organs during foetal life and are also involved in the metabolism of hormones [26]. Interestingly, as opposed to foetal hepatic and intestinal CYP3A4 expression, adrenal gene expression of CYP enzymes was not correlated with expression levels of any nuclear receptor in this study, indicating a different transcriptional regulation in the adrenals. Possible tissue-specific regulators of CYP3A enzymes in extrahepatic tissues merit further investigation.

Previous studies of VDR in adults show that the expression is highest in intestines, followed by adrenals and liver [27]. Results from our study indicate a higher mRNA expression of VDR in adrenals than in livers and intestines during the first trimester. In adults, an association between the genetic polymorphism rs1544410 G>A in VDR and intestinal CYP3A4 expression and activity has been reported, while no association between hepatic CYP3A4 expression and activity has been found [12,28]. In this study, the polymorphism rs1544410 G>A in VDR was not significantly associated with relative expression of CYP3A4, CYP3A7 or VDR. The VDR SNP rs731236 (*TaqI)* has been shown to be associated with changed VDR function where individuals carrying the C allele have a beneficial VDR function and better outcome in infectious diseases [15,16]. Previous reports have suggested that individuals carrying the C allele have increased expression levels of VDR [16,29,30]. Interestingly, in this study, we show that individuals homozygous for the C allele have significantly lower VDR expression in foetal liver tissue, indicating that this polymorphism might have different effects on the expression pattern during foetal and adult life.

Consistent with results from previous studies in adults, our results show a higher relative expression of PPARα in livers than intestines and adrenals [27]. PPARα was found to be correlated with CYP3A4 in livers and intestines and with CYP3A7 in livers and adrenals. Genetic polymorphism in PPARα, rs4253728 G>A, has been shown to correlate with lower CYP3A4 expression and activity in the liver [17]. de Keyser *et al.*[17] linked the same polymorphism in PPARα to cholesterol-lowering effects of simvastatin, a statin drug mainly metabolized by CYP3A4. In the present study, the expression of CYP3A4 and CYP3A7 was not associated with the polymorphism rs4253728 G>A in PPARα in any of the tissues examined. Moreover, there was no association between the PXR promoter SNP investigated (rs1523130) and PXR, CYP3A4 and CYP3A7 mRNA levels in the foetuses. In adults, this polymorphism has been associated with CYP3A4 expression and activity in adult livers [11]. However, the small number of foetal intestinal samples limited genotyping correlations.

A previous study found correlations between PXR, CAR and CYP3A4 and between PXR, CAR and CYP3A7 [31]. Here, we found strong correlations between PXR, CAR and CYP3A4 and CYP3A7 in hepatic tissue. There were also strong correlations between PXR, CAR and CYP3A4 in intestines, whereas no correlation could be found with CYP3A7. Intestinal expression of CAR was higher than both hepatic and adrenal expression. In contrast, CAR expression is low in adult intestines [27], which may indicate that CAR is more important in the foetal intestine.

A limitation of this study is that we have examined gene expression of CYP3A enzymes and nuclear receptors and not activity or protein expression. Further studies in activity and protein expression of CYP3A enzymes and nuclear receptors of foetuses as well as neonates could further increase insight in the mechanisms of drug metabolism in foetuses and paediatric populations.

It should also be noted that there are two 'outliers' in the analyses, one with very high VDR expression and one with very high CYP3A7 expression. Given that these are post-mortem samples from electively terminated pregnancies and rely on self-reported drug use, one cannot assume that these samples necessarily represent constitutive levels of expression. Thus, these values should be interpreted with caution as actual interindividual variability in constitutive expression may be overestimated. However, the correlation coefficient was only marginally affected when the outliers were excluded from the analyses and did not affect the overall results or conclusions.

In conclusion, our findings support the hypothesis of an important role of PPARα, PXR, VDR and CAR in the regulation of CYP3A enzymes during foetal life. Consequently, the large interindividual variability in CYP3A4 and CYP3A7 expression in foetal livers might be explained by differences in the expression of these nuclear receptors. In contrast to previous reports, the interindividual variations in CYP3A enzymes could not be explained by genetic polymorphisms in VDR and PPARα.

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