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Nuclear Receptor Expression in Fetal and Pediatric Liver: Correlation with CYP3A Expression

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List of non-standard abbreviations:

NR, nuclear receptor; PXR, pregnane X receptor; CAR, constitutive androstane receptor;; RXR, retinoid X receptor; HNF4α; hepatocyte nuclear factor α; CYP3A, cytochrome P450 3A

Abstract

The mechanisms underlying interindividual variation and developmental changes in CYP3A expression and activity are not fully understood. Quantitative RT-PCR methods were used to detect, during human fetal and pediatric development, mRNA expression of nuclear receptors involved in the regulation of CYP3A genes. Quantitative RT-PCR was conducted on RNA extracted from prenatal (n=60, 76 d to 32 wks EGA) and pediatric (n=20, 4 d to 18 yrs of age) liver tissue with primers for nuclear receptors implicated in regulating CYP3A gene expression. PXR and CAR were expressed at low (and highly variable) levels in pre- and neonatal liver relative to liver tissue derived from older children. CAR was expressed at higher levels relative to PXR in prenatal liver (757 ± 480 molecules CAR/ng RNA *vs* 271 ± 190 molecules PXR/ng RNA after correction for 18S rRNA). In contrast, mRNA expression of the heterodimer partner, RXRα, was less variable (33-fold) and did not differ appreciably between pre- and postnatal liver samples (219 ± 101 molecules/ng RNA prenatal versus 253 ± 232 molecules/ng RNA postnatal). Expression of HNF4α1 mRNA was similar to that of RXRα. LogCYP3A7 mRNA expression was significantly correlated with PXR (r^2=0.372) and CAR (r^2=0.380) mRNA in fetal liver but associations were weaker than those observed with CYP3A4 mRNA in postnatal liver (r^2=0.610 and 0.723 for PXR and CAR, respectively). In conclusion, nuclear receptor mRNA expression demonstrates considerable interindividual variability in human fetal and pediatric liver and is significantly correlated with CYP3A expression.

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Introduction

The human cytochrome P450 3A (CYP3A) enzymes are the most abundant cytochrome P450 enzymes expressed in liver and are clinically important for the metabolism of 45-60% of drugs as well as for maintenance of steroid homeostasis. The CYP3A family is comprised of four members, CYP3A4, CYP3A5, CYP3A7, and CYP3A43 (Gellner et al., 2001). CYP3A4 is the predominant P450 enzyme found in adult liver while CYP3A7 is primarily expressed in fetal liver. CYP3A5 is polymorphically expressed in liver while the contributions of CYP3A43, the most recently identified member of the CYP3A family, to drug metabolism is less certain (Domanski et al., 2001).

CYP3A expression and catalytic activity is highly variable among individuals and much of the interindividual variability in CYP3A can be attributed to genetic factors (Ozdemir et al., 2000; Hustert et al., 2001). Polymorphic expression of CYP3A5 is the result of the presence or absence of the *CYP3A5*3* allele, which results in alternative splicing and a truncated protein (Kuehl et al., 2001). Single nucleotide polymorphisms (SNP) in the coding regions of *CYP3A4* and *CYP3A7* have been identified (http://www.imm.ki.se/CYPalleles/), however, none of the variants described thus far have been able to account for the extent of observed variability in expression. Furthermore, the mechanisms regulating the developmental switch between CYP3A7 and CYP3A4 remain elusive.

It is hypothesized that variability in CYP3A4 and CYP3A7 may be the result of polymorphisms in the *cis*- and *trans*-acting factors controlling gene expression (Schuetz, 2004). The *CYP3A4* and *CYP3A7* gene promoters contain binding sites for numerous transcription factors, including the ubiquitous Sp1/Sp3 transcription factors, HNF3β, NF1, YY1 and E-box binding proteins (Itoh et al., 1992; Ourlin et al., 1997; Saito et al., 2001; Burk et al., 2002), and

polymorphisms have been identified in both gene promoters. The most common promoter variant of *CYP3A4* is *CYP3A4*1B* (Rebbeck et al., 1998; Westlind et al., 1999), however, the relationship between expression and the *CYP3A4*1B* allele is not clear (Lamba et al., 2002). *CYP3A7*1C*, in which 60 nucleotides of the *CYP3A7* gene promoter are replaced with corresponding sequences from the *CYP3A4* regulatory region, has been linked to expression of CYP3A7 in adult livers (Burk et al., 2002). Additional genetic variation in the *CYP3A7* gene promoter has been described (*CYP3A7*1B*, **1D*, and **1E*) (Kuehl et al., 2001) but the functional consequences for expression of CYP3A7 in fetal liver appear limited (Leeder et al., 2005).

Studies of the transcriptional contribution to CYP3A variability are complicated by modulation of constitutive and inducible expression by endogenous and exogenous compounds acting through the nuclear receptors, PXR and CAR (Pascussi et al., 1999; Bertilsson et al., 2001). The nuclear receptor (NR) superfamily regulates the expression of genes involved in metabolism, differentiation, proliferation and reproduction in response to small lipophilic molecules, including thyroid and steroid hormones, retinoids, and bile acids (Mangelsdorf et al., 1995). In addition to endogenous ligands, nuclear receptors such as the pregnane X receptor (PXR, NR1I2) and the constitutive androstane receptor (CAR, NR1I3) induce gene expression in response to xenobiotic compounds, including therapeutically important compounds such as phenobarbital and rifampin. Target genes of PXR and CAR include Phase I and II drug metabolizing enzymes and ABC transporters, leading to the suggestion that these nuclear receptors act as xeno-sensors coordinating the metabolism and elimination of xenobiotic compounds (for review, see Handschin and Meyer, 2003).

The developmental changes and interindividual variability in CYP3A in human liver may be attributable, at least in part, to the ontogeny of the transcription factors that regulate gene

expression, including the xenobiotic receptors. The present study was conducted to determine the developmental expression in fetal and pediatric livers of nuclear receptors associated with the regulation of *CYP3A* gene promoters using quantitative, real-time PCR methods. Nuclear receptor mRNA levels in fetal and pediatric liver are compared to the expression of CYP3A7 and CYP3A4 mRNA.

Materials and Methods

Liver samples. Sixty fetal (estimated gestational ages from 76d to 32wks) and 20 pediatric (ages 4d to 18yrs) liver samples were obtained from the University of Maryland Brain and Tissue Bank for Developmental Disorders (Baltimore, MD) or the University of Washington Central Laboratory for Human Embryology (Seattle, WA). Available demographic data for postnatal liver samples are presented in Table 1. Demographic data for prenatal tissue samples has been previously reported (Leeder et al., 2005). All tissues were maintained at -80º C prior to analysis. The use of these tissues was approved by the University of Missouri-Kansas City Pediatric Health Sciences Review Board.

RNA extraction. Total RNA was extracted from approximately 20-30 mg of liver tissue using the RNeasy RNA extraction kit from Qiagen (Valencia, CA) according to manufacturer's protocol. The quality of the RNA was assessed by agarose gel electrophoresis and quantity determined spectrophotometrically.

Quantitative RT-PCR. One-step quantitative, real-time RT-PCR for all nuclear receptors was performed using the QuantiTect SYBR Green RT-PCR kit (Qiagen, Valencia, CA) according to manufacturer's recommendations. Gene specific primer pairs, input RNA amounts and cycling conditions for each nuclear receptor are listed in Table 2. Reactions were performed at least two times in triplicate on a DNA Engine Opticon 2 (MJ Research, Boston, MA). Standard curves were generated by serial dilutions of PCR amplicons for each nuclear receptor from 10 to 10^7 molecule copies. Quantitative RT-PCR for 18S ribosomal RNA was performed using the TaqMan Ribosomal RNA Control Reagent kit (Applied Biosystems, Foster City, CA). Nuclear

receptor expression levels are expressed as copies/ng total RNA corrected for 18S rRNA. Quantitative RT-PCR assays for CYP3A4 and CYP3A7 have been previously described (Leeder et al., 2005).

Statistical analysis. Results are reported as mean ± standard deviation after correction for 18S rRNA. Univariate linear regression analysis was performed using SPSS version 12.0 (SPSS Inc., Chicago IL).

Results

Expression and variability of xenobiotic receptors in fetal and pediatric liver. CAR mRNA was detected in all 60 fetal liver samples tested (Fig. 1A, closed squares) at 757 ± 480 copies/ng total RNA after correction for 18S ribosomal RNA (mean ± standard deviation). CAR mRNA expression varied 89-fold between individual samples and ranged from 23.4 to 2070 copies/ng total RNA. In postnatal livers, CAR was expressed, on average, at higher levels relative to prenatal livers (Table 3). However, CAR expression levels in the liver were closer to that of prenatal liver in neonates and infants for the first six months of life (578 ± 511 copies/ng total RNA versus 2940 ± 1810 copies/ng total RNA in liver samples from older children) and varied up to 325-fold for all postnatal liver samples but varied only 22-fold in samples from donors over six months of age (n=12).

PXR expression levels (Fig. 1A, open circles) ranged from undetectable (n=1) to 853 copies/ng total RNA (mean ± standard deviation: 271 ± 190) and varied 345-fold in fetal livers expressing detectable levels of PXR. In contrast, PXR expression in postnatal liver averaged 1440 ± 1650 copies/ng total RNA and varied 230-fold. Similar to CAR mRNA levels, PXR expression exhibited developmental changes with PXR levels being similar to prenatal for samples from donors less than six months of age. In pediatric livers from children over six months of age (n=12), PXR was detected at 2260 ± 1680 copies/ng total RNA (mean ± standard deviation) and varied only 23-fold (range: 242 to 5510 copies/ng total RNA.).

Expression of RXRα. RXRα is the heterodimer partner for PXR and CAR as well as numerous other nuclear receptors including PPAR, RAR, VDR and TR. Quantitative RT-PCR was performed to detect RXRα to determine if the heterodimer partner for PXR and CAR displayed similar levels of variability and developmental changes in gene expression. RXRα

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mRNA levels (Fig. 1B) were 219 ± 101 (mean ± standard deviation) copies/ng RNA (Table 3) in fetal liver and was comparable to expression levels observed in postnatal liver (Table 3). Overall, RXRα expression was less variable versus PXR or CAR mRNA levels with less than 16- and 23-fold differences observed in pre- and postnatal liver, respectively.

Expression and variability of HNF4α. HNF4α (NR2A1) is expressed as several isoforms due to of the presence of multiple promoters and alternative splicing. Promoter P1 initiates transcripts containing exon 1A (HNF4α1) while promoter P2 initiates transcripts containing exon 1D (HNF4α7). Quantitative RT-PCR was performed to separately detect exon 1A- and exon 1D-containing transcripts in RNA extracted from human fetal and pediatric liver (Fig. 1C). Exon 1A-containing transcripts were more abundantly expressed in human fetal and pediatric liver (Table 3). No differences were observed in the levels of expression for either isoform between fetal and pediatric liver. HNF4α expression was highly variable in fetal liver but varied only 16-fold in postnatal liver (Table 3). The primers used in this study did not distinguish HNF4α1 transcripts from HNF4α2 or HNF4α3. HNF4α2 and HNF4α3 are products of alternative splicing of exons 9 and 8, respectively, and differ from HNF4α1 at the 3' end of the coding region affecting the functional activity of HNF4α protein. Therefore, the actual variability in functional HNF4α may be underrepresented and additional studies are required to determine the level of alternative splicing of HNF4α.

Coexpression of nuclear receptors in human liver. Recent studies have demonstrated functional interactions between the xenobiotic receptors and HNF4α (Kamiya et al., 2003; Tirona et al., 2003). Our investigations showed that the expression of HNF4α1 was positively correlated with RXRα (r^2=0.53, $p<0.001$), CAR (r^2=0.34, $p<0.001$) and PXR (r^2=0.38, $p<0.001$) expression levels in fetal liver (Table 4). In postnatal liver, positive correlations between

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HNF4α1 and PXR (r^2=0.68, p<0.001), CAR (r^2=0.76, p<0.001) and RXRα (r^2=0.76, p<0.001) were also observed (Table 4). However, the slope of the lines defining the correlations of RXRα, PXR and CAR with HNF4α1 differed statistically between pre- and postnatal liver samples (Fig. 2).

Expression of RXRα, the heterodimer partner for PXR and CAR, was positively correlated with the expression of CAR (r^2=0.41, p<0.001) and PXR (r^2=0.43, p<0.001) in both prenatal and postnatal liver (Table 4). Similar to HNF4α1, the correlation of RXRα with PXR or CAR differed statistically in pre- and postnatal livers (data not shown). Positive correlations were also observed between CAR and PXR expression in fetal (r^2=0.40, p<0.001) and pediatric (r^2=0.63, p<0.001) liver but no statistically significant difference was observed between pre- and postnatal livers.

Correlation of nuclear receptors and CYP3A4 expression in pediatric liver. Expression of CYP3A4 mRNA was determined in the pediatric liver samples. CYP3A4 message varied 9600-fold overall, but only 24-fold after 6 months of age, similar to PXR and CAR. Furthermore, CYP3A4 message levels correlated strongly with the expression of CAR (Fig. 3A, r^2=0.61, p<0.001), PXR (Fig. 3B, r^2=0.73, p<0.001), RXRα (r^2=0.56, p<0.001), and HNF4α (r^2=0.69, p<0.001), individually. The CYP3A4 expression was better predicted when all four receptors were included in the analysis (r^2=0.83, p<0.001). In contrast, CYP3A5 expression in postnatal liver did not correlate with the expression of any of the nuclear receptors studied (data not shown).

Correlation of nuclear receptor and CYP3A7 expression in fetal liver. CYP3A7 gene expression has previously been reported for a subset (n=54) of the fetal liver samples used in the current study (Leeder et al., 2005). To determine if nuclear receptor mRNA levels are

significantly related to CYP3A7 expression in fetal liver, nuclear receptor expression was compared to CYP3A7 mRNA. Weak, but statistically significant ($p<0.01$), correlations were observed between CYP3A7 mRNA and expression of CAR and PXR (Fig. 4). As with CYP3A4 expression in postnatal liver, PXR, CAR, RXRα, and HNF4α1 in combination produced a stronger correlation with CYP3A7 mRNA levels ($r^2=0.25$, $p<0.01$) than any receptor alone. CYP3A7 expression levels were highly variable and the data are skewed towards zero (Fig. 5A). Logarithmic transformation of the CYP3A7 mRNA levels produced a normal distribution (Fig. 5B) and a stronger correlation was observed between the expression of the four nuclear receptors and CYP3A7 mRNA expression ($r^2=0.5$, $p<0.001$).

Discussion

This study reports the ontogeny of nuclear receptors in human fetal and pediatric liver involved in the regulation of CYP3A gene expression. Limited data exist regarding the expression of PXR and CAR in human fetal tissues with most studies recording expression in one or pooled samples (Bertilsson et al., 1998; Huang et al., 2003; Arnold et al., 2004; Phillips et al., 2005). A recent study reported the expression of PXR in eight fetal liver samples of 14 to 20 weeks estimated gestational age and four neonates (Miki et al., 2005). This study investigated the expression of PXR, CAR, RXRα, and HNF4α in 60 fetal (11 to 32 weeks estimated gestational age) and 20 pediatric liver samples (4d to 18 yrs of age).

In contrast to their function in xenobiotic metabolism and elimination in postnatal liver, phase I and phase II enzymes, including CYP3A7, appear to play a critical role in steroid biosynthesis during fetal development. Modulation of CYP3A7 by drugs or environmental compounds through PXR and/or CAR may disrupt steroid biosynthesis, affecting normal fetal development and maintenance of pregnancy. During pregnancy, it is likely that endogenous ligands (*e.g.*, DHEA and DHEA-3-sulfate (Ripp et al., 2002) regulate constitutive CYP3A7 expression as fetal exposure to xenobiotics generally is limited as a consequence of functional maternal xenobiotic biotransformation and transport as well as placental barriers. However, the increase in xenobiotic receptor expression during the first year of life may reflect exposure to xenobiotics from environmental, dietary (maternal exposures via breast milk; introduction of solid oral foods) or therapeutic sources.

No developmental changes in RXRα expression were observed and RXRα mRNA levels displayed less variability relative to the xenobiotic receptors. The relatively low level of variability in RXRα mRNA in human liver observed in this study is consistent with the critical

role RXRα plays during development and in the maintenance of liver function (Kastner et al., 1994; Wan et al., 2000). Excess or insufficient exposure to retinoids during fetal development induces dramatic malformations and disruption of RXRα function would be expected to have deleterious effects on fetal development as retinoid levels, and the subsequent response mediated by retinoid receptors, are tightly regulated in the fetus (Collins and Mao, 1999; Ross et al., 2000; Perlmann, 2002). In addition, RXRα acts as the heterodimer partner for numerous nuclear receptors beyond CAR and PXR to regulate diverse physiological processes including glucose, fatty acid, calcium, and cholesterol homeostasis (Mangelsdorf and Evans, 1995; Chawla et al., 2001).

In addition to directly activating transcription through the *CYP3A* gene promoters, PXR, CAR, RXRα, and HNF4α are part of a larger, interacting network of transcription factors. This study demonstrates statistical relationships between the expression levels of nuclear receptors (Table 4) and with CYP3A gene expression (Figs 3 and 4). However, the relative role of each of the four transcription factors in regulating CYP3A7 expression in fetal liver and CYP3A4 expression in postnatal liver demonstrates important qualitative differences. This observation is consistent with previous studies showing HNF4α regulation of PXR in fetal but not adult hepatocytes (Kamiya et al., 2003). Additional studies are required to determine the hierarchy of the signaling cascade regulating developmental changes and variability in the expression of nuclear factors. These studies may also provide insight into the regulation of the developmental switch from CYP3A7 to CYP3A4 expression.

This study presents positive correlations between nuclear receptor mRNA levels and CYP3A7 and CYP3A4 mRNAs in human fetal and pediatric liver, respectively suggesting that interindividual variability in CYP3A activity may be influenced by variability in nuclear receptor

expression. It has yet to be determined if variable expression of nuclear receptor mRNA resulted in variable protein levels. Furthermore, alternatively spliced transcripts and allelic variants previously described for PXR, CAR and HNF4α were not investigated in this panel of fetal and pediatric livers (Sladek et al., 1999; Ryffel, 2001; Lamba et al., 2005). Nevertheless, the data presented indicate that *PXR* and *CAR* genes are indeed transcriptionally active during fetal development. Although the positive correlations between nuclear receptors and CYP3A7 or CYP3A4 mRNAs suggest that nuclear receptor networks act as important determinants of CYP3A expression in human liver, a causal relationship between CAR/PXR and CYP3A7 can not be established definitively at this time. In previous reports, PXR and CAR expression have individually been associated with CYP3A4 and CYP2B6 expression in adult liver based on similar experimental (quantitative RT-PCR) approaches (Pascussi et al., 2001; Chang et al., 2003). The data presented here suggest that combination of two or more nuclear factors is a better determinant of CYP3A expression. However, due to the limited exposure data available for the tissue samples used within this study, xenobiotic induction can not be eliminated as a significant contributor to CYP3A expression. An important extension of the current work will be to characterize further variability in expression of the regulatory cascade as a determinant of variability in the expression of additional targets, including phase II enzymes and transport proteins, involved in drug metabolism and elimination.

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Footnotes

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Legends for Figures

Figure 1. Ontogeny of nuclear receptor expression in human liver. Nuclear receptor expression was determined using quantitative RT-PCR as described in the Materials and Methods. A) PXR (open circles) and CAR (solid squares), B) RXRα, C) HNF4α 1A (solid squares) and HNF4α 1D (open circles). Data are expressed as molecules/ng total RNA after correction for 18S rRNA relative to estimated gestational age and postnatal age.

Figure 2. Correlation between HNF4α and other NR in fetal and pediatric liver. HNF4α 1A-containing transcript levels are plotted versus A) PXR mRNA, B) CAR mRNA, and C) RXRα mRNA expression for prenatal (open circles, dashed lines) and postnatal (closed circles, solid lines) liver samples.

Figure 3. A) Correlation between CYP3A4 and CAR mRNA expression in postnatal liver (r^2=0.73, $p<0.001$). B) Correlation between CYP3A4 and PXR mRNA expression in postnatal liver (r^2=0.61, $p<0.001$).

Figure 4. A) Correlation between CYP3A7 mRNA and CAR mRNA expression in prenatal liver (r^2=0.20, $p<0.001$). B) Correlation between CYP3A7 mRNA and PXR mRNA expression in prenatal liver (r^2=0.15, $p<0.01$).

Figure 5. A) Frequency distribution of CYP3A7 mRNA expression levels in prenatal liver. CYP3A7 mRNA expression determined previously by quantitative RT-PCR (Leeder et al., 2005). B) Frequency distribution of CYP3A7 mRNA expression from A) after logarithmic transformation.

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Table 1. Demographic data for postnatal liver samples

ID#	Age	Gender	Race	PMI*	Exposure**
CMM0297	15y 130d	F	CA	4	NR
CMM0398	16d	F	AA	3	NR
CMM0431	16d	M	CA	4	NR
CMM0432	4d	M	CA	2	NR
CMM0446	3y 338d	F	CA	1	NR
CMM0495	141d	M	AA	5	Albuteral
CMM0527	53d	M	CA	3	Epinephrine, dopamine, isupril, insulin, glucose
CMM0612	17y 8d	M	CA	5	Lorenzo's oil
CMM0615	163d	M	CA	2	NR
CMM0734	40d	F	CA	5	NR
CMM0779	5d	M	AA	5	NR
CMM1144	12y 232d	F	Unk	6	NR
CMM1203	7y 153d	M	CA	2	NR
CMM1281	206d	M	AA	6	NR
CMM1325	182d	F	AA	1	NR
CMM1380	338d	F	CA	6	NR
CMM1409	18y 38d	M	CA	6	NR
CMM1465	17y 189d	M	CA	4	Alcohol
CMM1482	244d	F	CA	2	NR
CMM1624	3y 60d	F	CA	3	NR

* Post-mortem interval in hours
**NR None reported

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Table 2. Quantitative RT-PCR Primers

Gene	Forward Primer	Reverse Primer	Annealing Temp	Input RNA	Amplicon
PXR	ctggtcctcgatgggcaagtc	caagcggaagaaaagtgaacg	58.0	120 ng	441 bp
CAR	cacatgggcaccatgtttga	aagggctggtgatggatgaa	57.0	20 ng	73 bp
RXRα	gagcggcagcgtggcaagg	ggcaaatgttggtgacaggg	60.0	15 ng	187 bp
HNF4α 1A	acatggacatggccgactac	ctcgaggcaccgtagtgttt	60.0	15 ng	225 bp
HNF4α 1D	gcgggcccctgctcctccat	ctcgaggcaccgtagtgttt	60.0	15 ng	213 bp

Table 3. Summary of Nuclear Receptor Expression in Pre- and Postnatal Liver

	PXR		CAR		RXRα		HNF4α	
	Prenatal (n=60)	Postnatal (n=20)	Prenatal (n=59)	Postnatal (n=20)	Prenatal (n=60)	Postnatal (n=20)	Prenatal (n=60)	Postnatal (n=20)
Mean	271	1140	757	1990	219	254	167	148
Std Dev	190	1650	480	1840	101	232	96.6	112
Minimum	2.47*	23.7	23.4	19.9	30.5	43.7	1.91	24.3
Maximum	853	5510	2070	6470	478	1010	415	398

*Lowest detectable levels of PXR mRNA. n=1 with PXR below level of detection

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Table 4. Pairwise correlation of nuclear receptor expression

	PXR			CAR			RXRα		
	Pre	Post	All	Pre	Post	All	Pre	Post	All
CAR	0.40 (p<0.001)	0.63 (p<0.001)	0.67 (p<0.001)						
RXRα	0.43 (p<0.001)	0.62 (p<0.001)	0.41 (p<0.001)	0.41 (p<0.001)	0.77 (p<0.001)	0.56 (p<0.001)			
HNF4α	0.38 (p<0.001)	0.68 (p<0.001)	0.18 (p<0.001)	0.34 (p<0.001)	0.76 (p<0.001)	0.27 (p<0.001)	0.53 (p<0.001)	0.76 (p<0.001)	0.54 (p<0.001)

Figure 1

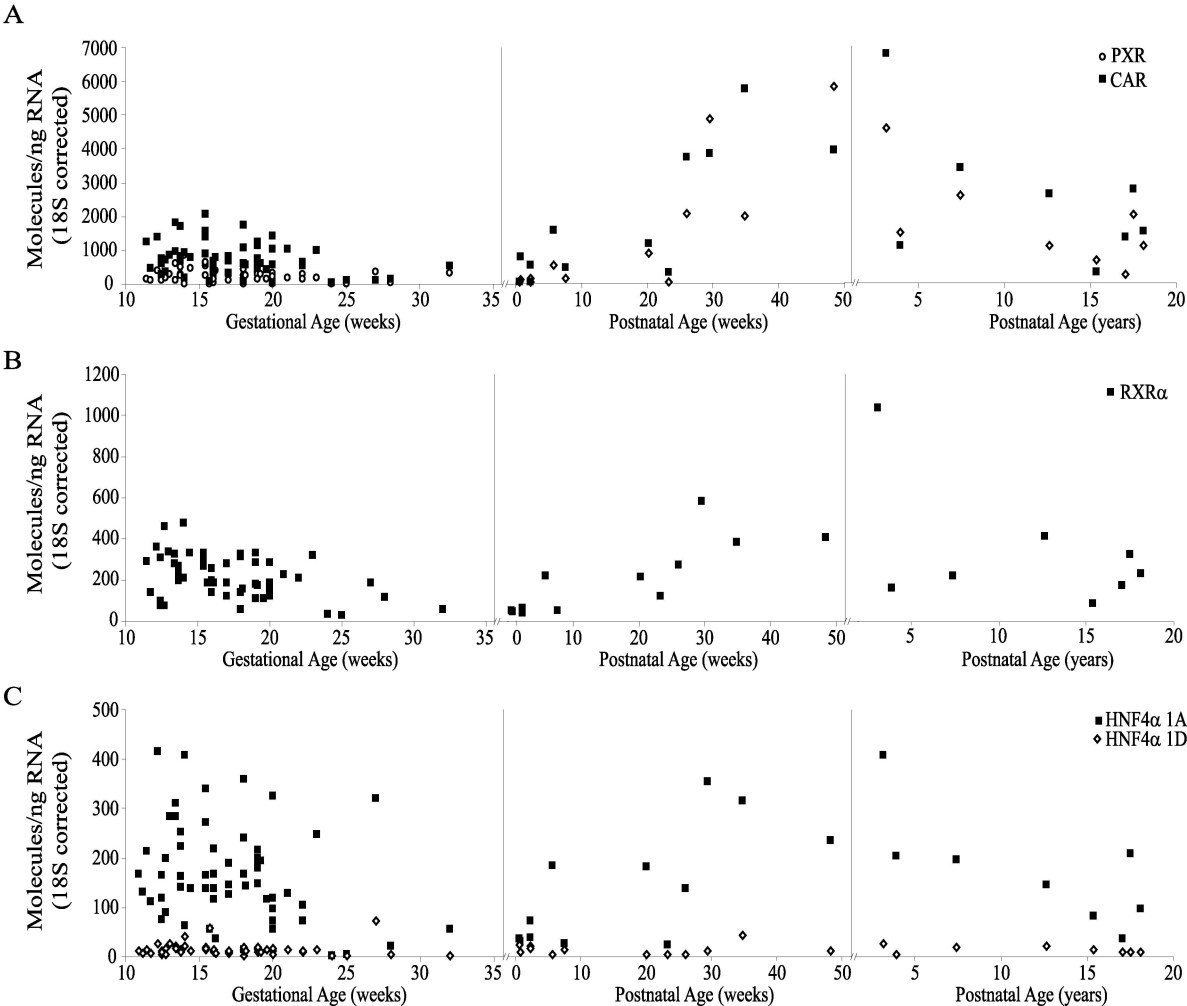

A
Molecules/ng RNA (18S corrected)
7000
6000
5000
4000
3000
2000
1000
0
PXR
CAR
10 15 20 25 30 35
Gestational Age (weeks)
0 10 20 30 40 50
Postnatal Age (weeks)
5 10 15 20
Postnatal Age (years)
B
Molecules/ng RNA (18S corrected)
1200
1000
800
600
400
200
0
RXRα
10 15 20 25 30 35
Gestational Age (weeks)
0 10 20 30 40 50
Postnatal Age (weeks)
5 10 15 20
Postnatal Age (years)
C
Molecules/ng RNA (18S corrected)
500
400
300
200
100
0
HNF4α 1A
HNF4α 1D
10 15 20 25 30 35
Gestational Age (weeks)
0 10 20 30 40 50
Postnatal Age (weeks)
5 10 15 20
Postnatal Age (years)

Figure 2

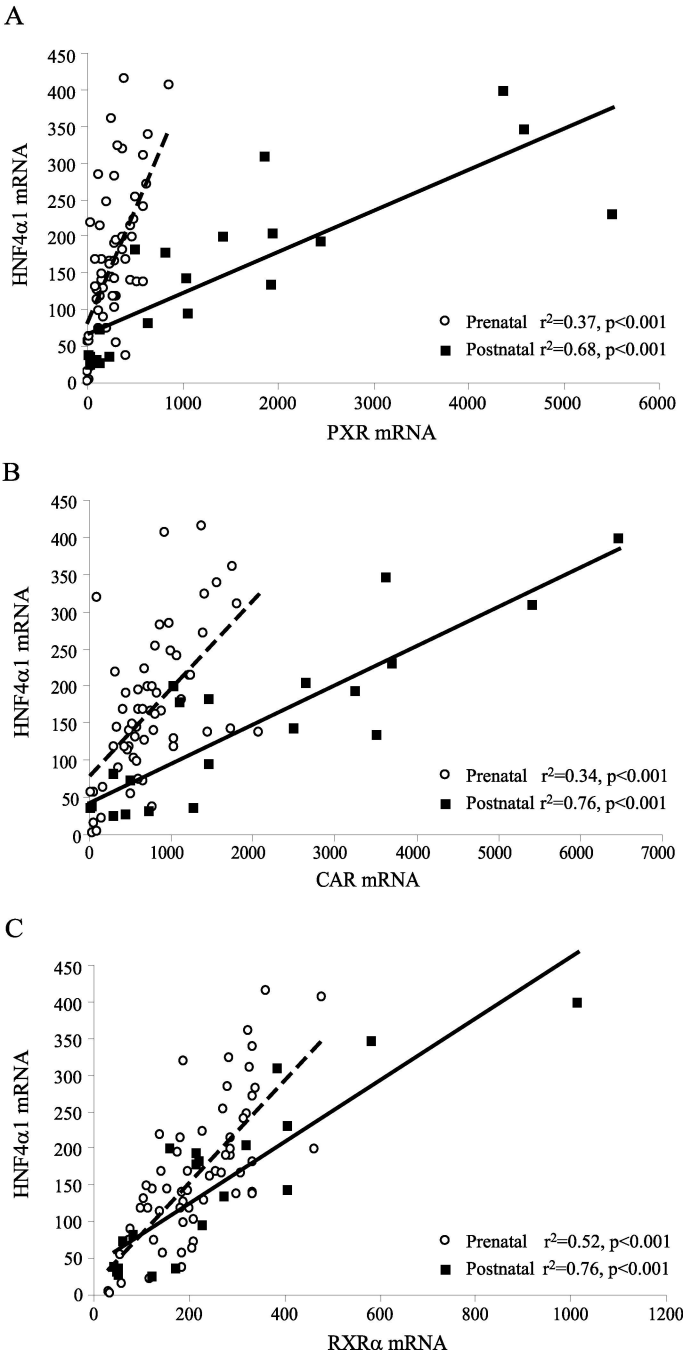
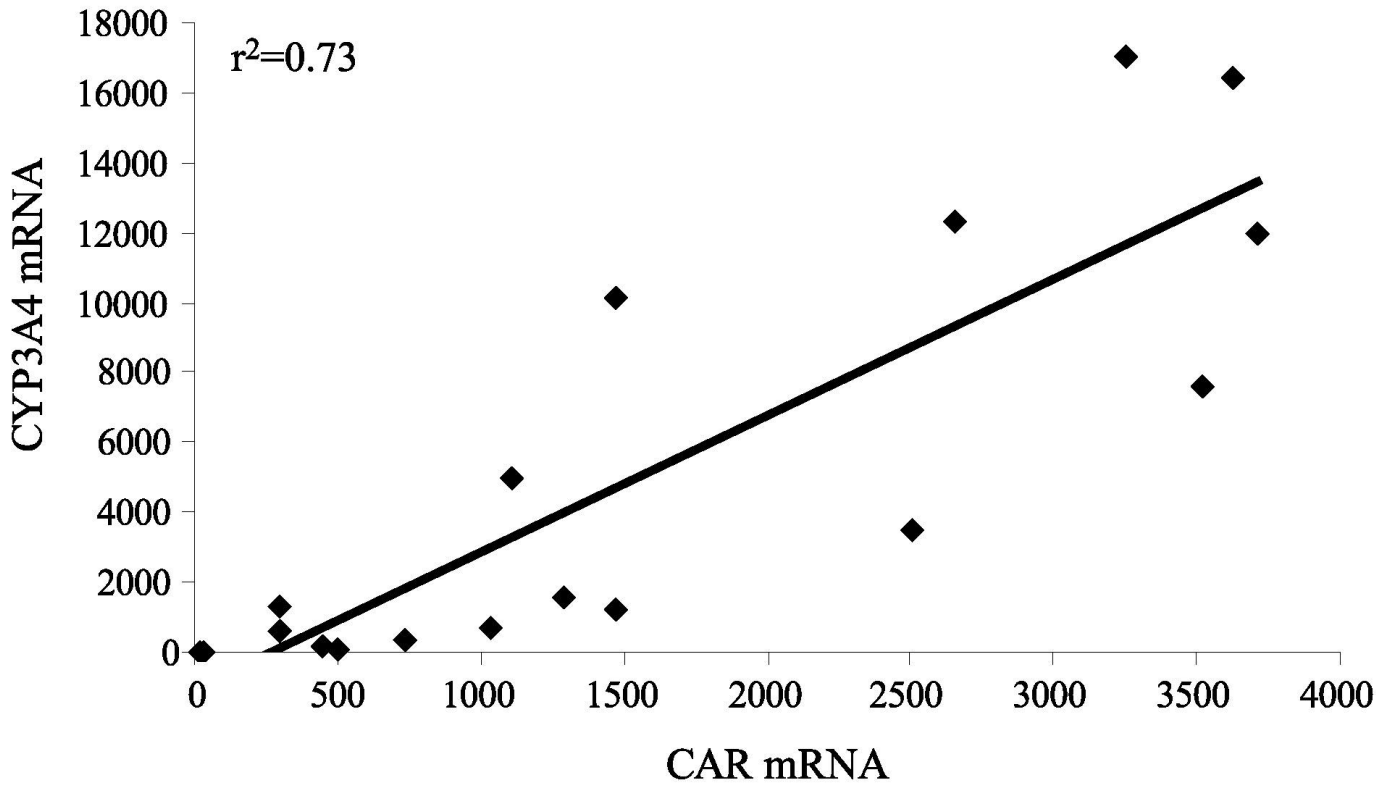

A
HNF4α1 mRNA
PXR mRNA
Prenatal r²=0.37, p<0.001
Postnatal r²=0.68, p<0.001
B
HNF4α1 mRNA
CAR mRNA
Prenatal r²=0.34, p<0.001
Postnatal r²=0.76, p<0.001
C
HNF4α1 mRNA
RXRα mRNA
Prenatal r²=0.52, p<0.001
Postnatal r²=0.76, p<0.001

Figure 3

A


r2=0.73
CYP3A4 mRNA
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
0
500
1000
1500
2000
2500
3000
3500
4000
CAR mRNA

B

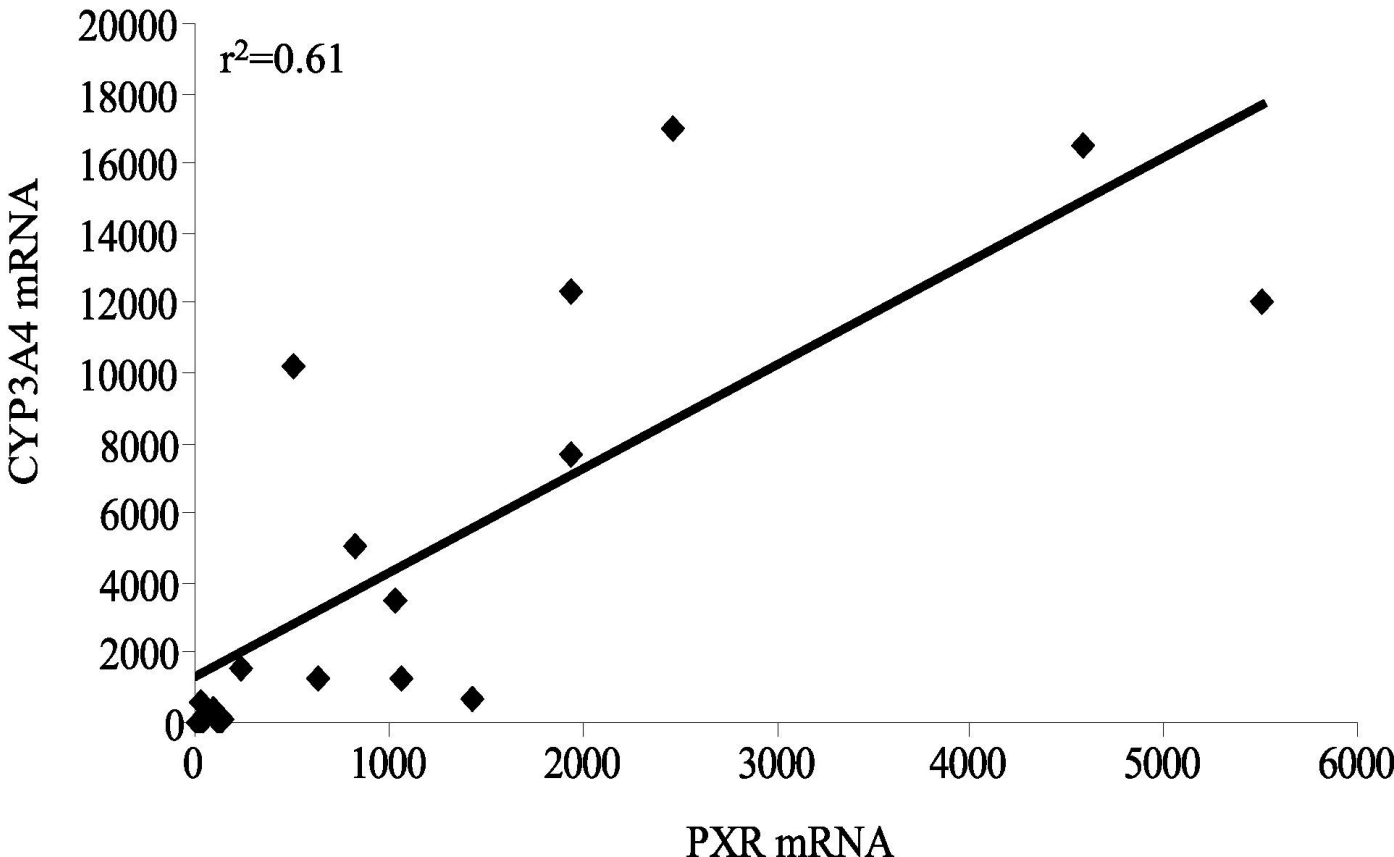
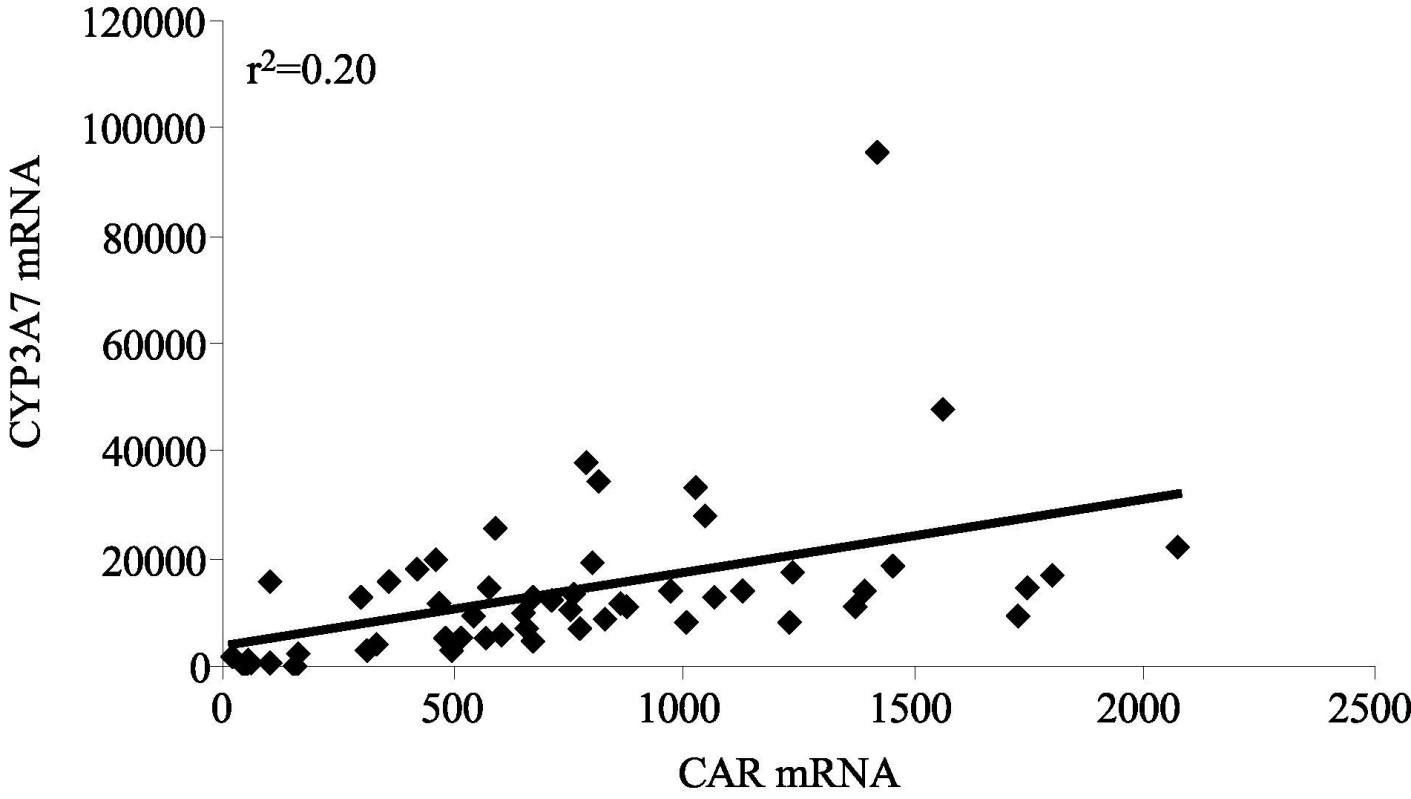

r2=0.61
CYP3A4 mRNA
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
0
1000
2000
3000
4000
5000
6000
PXR mRNA

Figure 4

A


120000
100000
80000
60000
40000
20000
0
r2=0.20
CYP3A7 mRNA
0
500
1000
1500
2000
2500
CAR mRNA

B

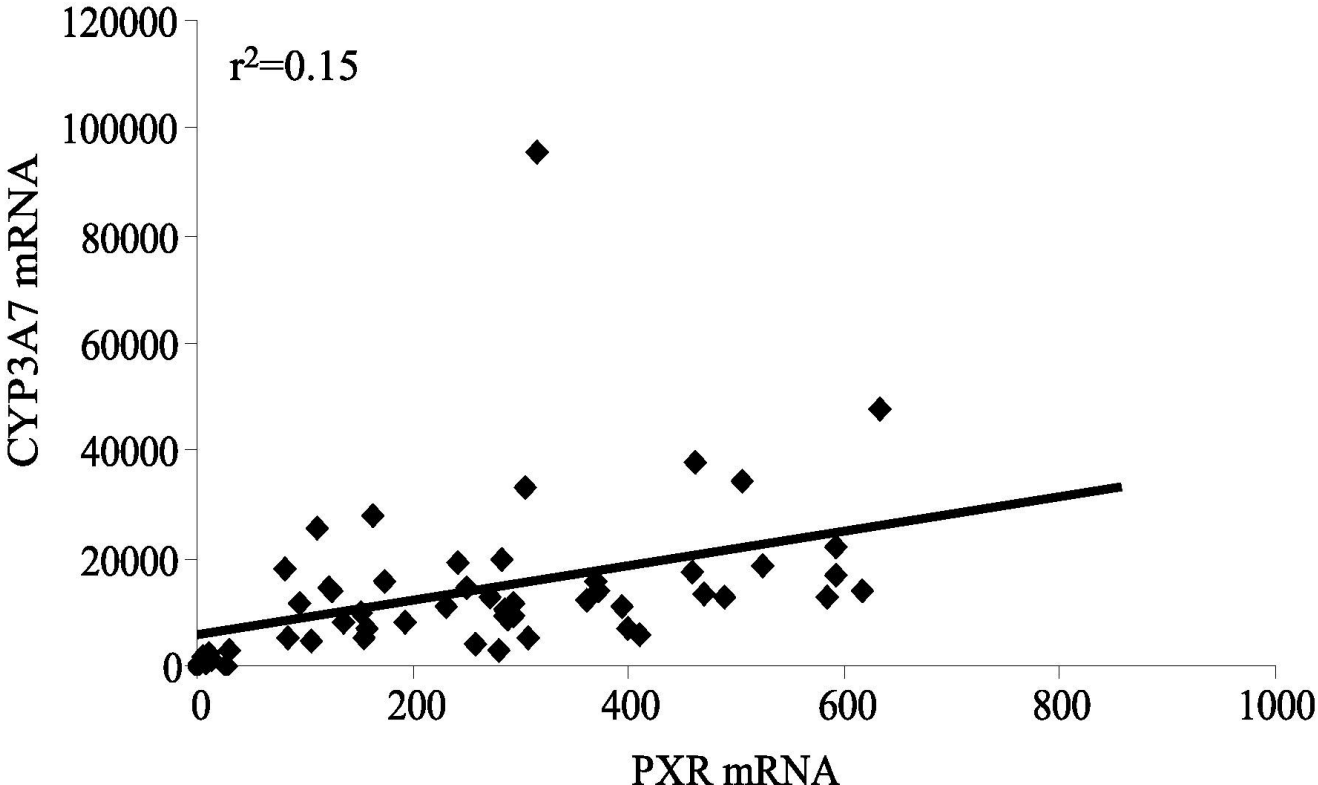
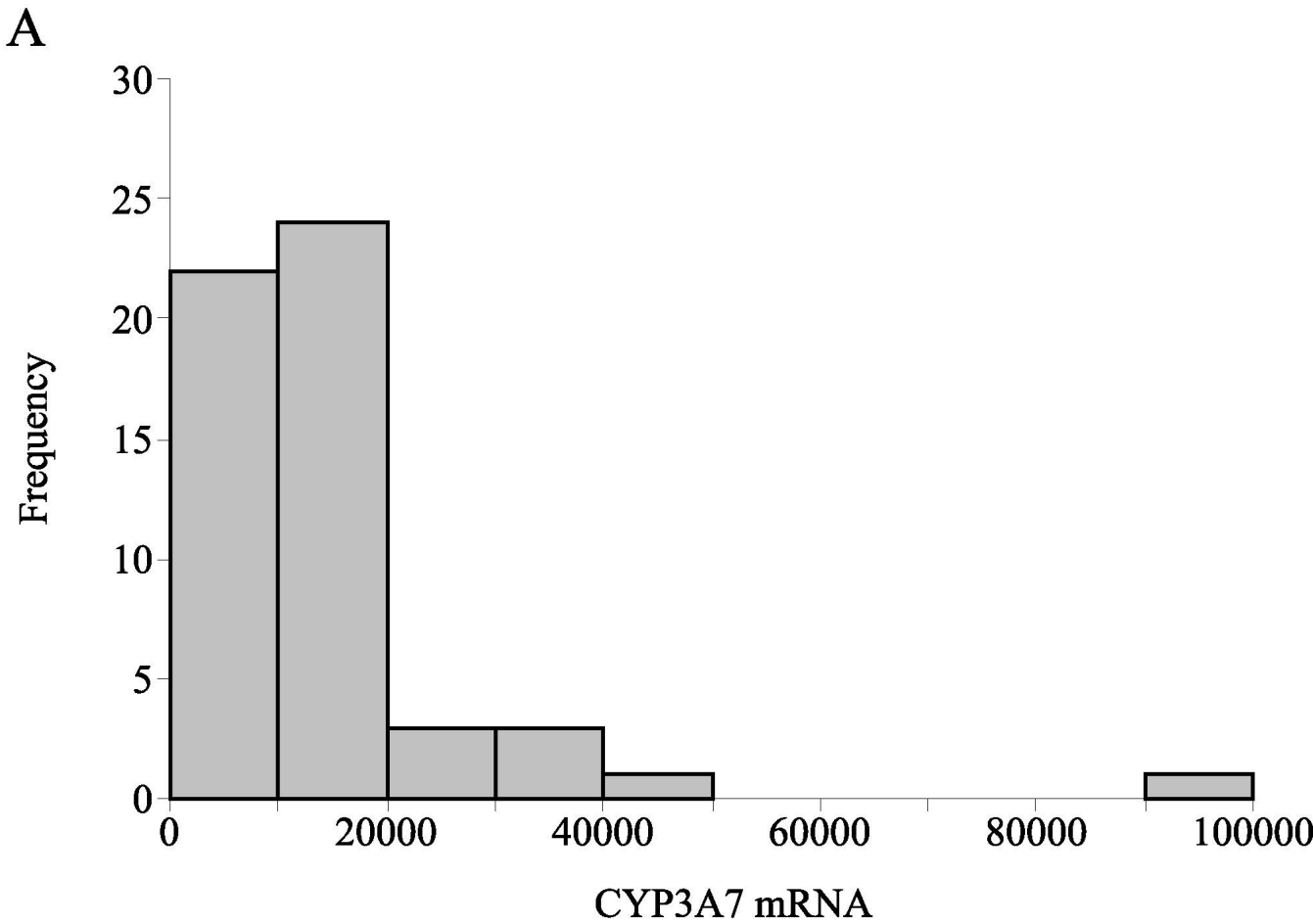
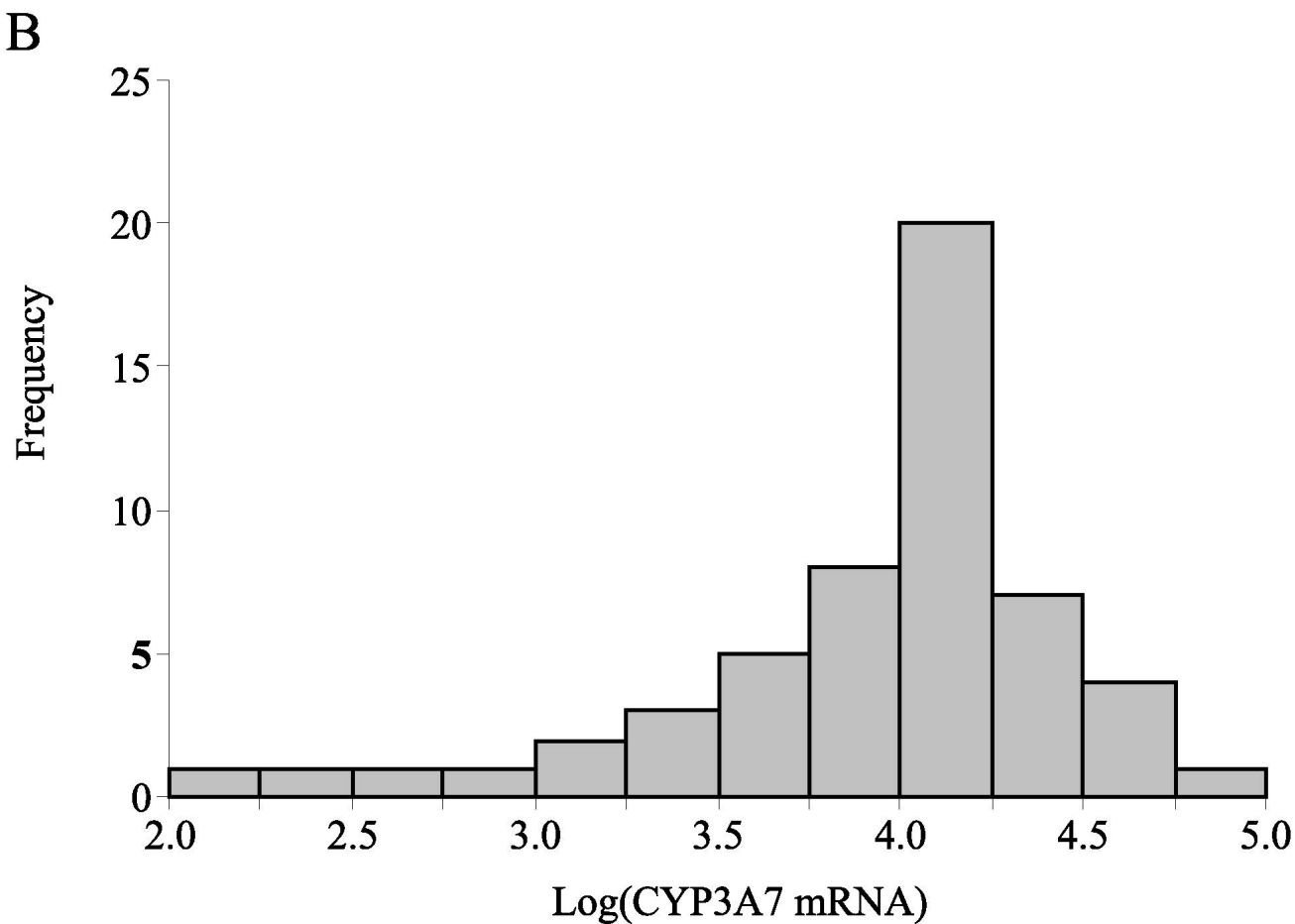

120000
100000
80000
60000
40000
20000
0
r2=0.15
CYP3A7 mRNA
0
200
400
600
800
1000
PXR mRNA

Figure 5


A
30
25
20
15
10
5
0
Frequency
0
20000
40000
60000
80000
100000
CYP3A7 mRNA


B
25
20
15
10
5
0
Frequency
2.0
2.5
3.0
3.5
4.0
4.5
5.0
Log(CYP3A7 mRNA)