Altered Cytochrome P450 Expression in Mice during Pregnancy

Kwi Hye Koh, Hui Xie, Ai-Ming Yu, and Hyunyoung Jeong

Department of Biopharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago,
Chicago, IL, USA (K.H.K, H.J.)
Department of Epidemiology & Biostatistics, University of Illinois at Chicago, IL, USA (H.X.)
Department Pharmaceutical Sciences, University at Buffalo, The State University of New York, Buffalo,
NY, USA (A-M.Y.)
Running title page

a) Running title: Cyp expression during mouse pregnancy

b) Address correspondence to:
Hyunyoung Jeong, PharmD, PhD
Department of Pharmacy Practice (MC 886)
College of Pharmacy, University of Illinois at Chicago
833 S. Wood Street, Chicago, IL 60612
Phone: 312-996-8639
Fax: 312-996-0379
E-mail: yjeong@uic.edu

c) 12 text page
   2 tables
   2 figures
   24 references
   190 words in Abstract
   559 words in Introduction
   427 words in Results
   763 words in Discussion

d) List of abbreviations: AhR, Aryl hydrocarbon receptor; CAR, constitutive androstane receptor; CYP, cytochrome P450; ER, estrogen receptor; IL, interleukin; IFN, interferon; PPAR, peroxisome proliferator-activated receptor; PXR, pregnane X receptor; TNF, tumor necrosis factor
Abstract

Human pregnancy is known to influence hepatic drug metabolism in a CYP-specific manner. However, the underlying mechanisms remain unknown in part due to a lack of experimental models to study altered drug metabolism during pregnancy. In this study, we examined how pregnancy influences expression of major Cyp isoforms in mice. Liver tissues were isolated from female FVB/N mice at different gestational time points: pre-pregnancy, 7, 14 and 21 days of pregnancy, and 7 days post partum. mRNA expression levels of major Cyp isoforms (Cyp1a2, Cyp2a5, Cyp2b10, Cyp2c37, Cyp2d22, Cyp2e1, Cyp3a11, and Cyp3a41) in the liver tissues were determined by quantitative real-time PCR. While Cyp2a5 expression was unchanged, Cyp3a41 expression was significantly increased during pregnancy. In contrast, expression of Cyp1a2, Cyp2c37, Cyp2d22, Cyp2e1, and Cyp3a11 was all decreased. Expression of Cyp2d22 and Cyp2e1 isoforms correlated with that of PPARα in the mouse livers, suggesting potential involvement of PPARα in downregulation of the Cyp expression during pregnancy. Effects of pregnancy on expression of other Cyp mouse isoforms as well as on in vivo drug disposition remain to be characterized. These results provide a guide for future studies on CYP regulation during pregnancy.
Introduction

Human pregnancy affects hepatic drug metabolism in a CYP pathway-specific manner. During pregnancy, elimination of drugs metabolized by CYP2A6, CYP3A4, CYP2D6 and CYP2C9 is increased, while elimination of CYP1A2 and CYP2C19 substrate drugs is decreased (Dempsey et al., 2002; Anderson, 2005; Hodge and Tracy, 2007). The underlying mechanisms remain unknown, in part due to a lack of experimental models to study altered drug metabolism during pregnancy.

The effects of pregnancy on hepatic drug metabolism have been extensively examined in rats, the commonly used animal model for pharmacological studies (Guarino et al., 1969; Neale and Parke, 1973; Borlakoglu et al., 1993; He et al., 2007). Pregnancy generally decreases CYP contents and activities, as well as mRNA or protein expression of many other drug-metabolizing enzymes (DME), per gram rat liver. For example, pregnancy reduced activities of ethoxyresorufin-O-deethylation and aminopyrine N-demethylation (Borlakoglu et al., 1993) as well as aniline hydroxylation and ethylmorphine N-demethylation (Guarino et al., 1969). Results from cDNA microarray experiment also revealed downregulation of CYP2A1, CYP2D2, CYP2C23, and CYP2E1 in livers of pregnant rats (He et al., 2007). These findings, in general, do not correspond to the drug metabolism changes shown in pregnant women (Dempsey et al., 2002; Anderson, 2005; Hodge and Tracy, 2007).

Mice are another commonly used animal model in pharmacology and genetics studies based on their litter size and available technologies to manipulate their genome. Potential interspecies differences between rats and mice have been reported in regards to regulation of Cyp expression, especially pertaining to ligand specificity for transcriptional regulators of Cyp (Graham and Lake, 2008); however, the effects of pregnancy on mouse Cyp expression have not been studied extensively. A recent study has demonstrated that pregnancy increases mRNA expression of Cyp3a16, Cyp3a41, and Cyp3a44 in mice (Zhang et al., 2008). The increased Cyp3a expression was found to be in part responsible for enhanced metabolism of glyburide in pregnant mice (Zhou et al., 2010), providing a mechanistic basis for the
increased elimination of glyburide in pregnant women. This suggests that mice may serve as a potential animal model to study changes in Cyp-mediated drug metabolism during pregnancy. The effects of pregnancy on expression of other mouse Cyp isoforms remain unknown.

Expression of CYP enzymes are modulated by actions of nuclear receptors or immunomodulators. Cytokines released during systemic inflammation, such as IL-6, IL-1β, tumor necrosis factor (TNF)α, and interferon (IFN)γ, are known to downregulate hepatic expression of major Cyp isoforms (Aitken et al., 2006). On the other hand, hepatic transcription factors, such as pregnane X receptor (PXR) or constitutive androstane receptor (CAR), play critical roles in upregulation of hepatic Cyp expression. Results from a previous study have shown decreased CAR expression during mouse pregnancy (Zhang et al., 2008). The second trimester of human pregnancy is characterized by an inflammatory environment manifested by T helper type 2 cell cytokines, such as IL-6 (Creasy et al., 2009) although the inflammatory environment of mouse pregnancy is yet to be defined. Involvement of these Cyp regulators in altered drug metabolism during pregnancy remains unknown.

The aim of this study was to examine how pregnancy influences expression of major Cyp isoforms in mice to establish mice as a potential animal model to study altered drug metabolism during pregnancy. We also explored potential mechanisms underlying altered Cyp expression during mouse pregnancy by examining how pregnancy influences expression of major transcriptional regulators for Cyp expression.
Materials and Methods

Animals. All pregnant and virgin FVB/N mice were housed under controlled temperature (20 ± 2°C), relative humidity (50-60%) and lighting (lights on 6:00 a.m. - 6:00 p.m.), with food and water provided ad libitum. All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at University at Buffalo. Adult female (8 weeks old) mice were mated with male mice of same age. The second day after mating was assumed as gestational day 1 for those female mice demonstrating sperm plug. At gestational day 7, 14, 21, and 7 days after delivery, the female mice were sacrificed and liver tissues were collected. Age-matched virgin female mice were used as control (gestational day 0). All tissues were gently washed in 4°C saline (kept on ice) and then stored at -80°C before use.

RNA Isolation and Quantitative Real-time (qRT) PCR. Approximately one third of a whole liver tissue from each mouse was pulverized in liquid nitrogen, and total RNAs were isolated using Trizol (Invitrogen, Carlsbad, CA). The quality of total RNA was determined by measuring the A260/A280 ratio (greater than 1.8), and RNA integrity was further confirmed on agarose gels. One μg of total RNA was used as template for cDNA synthesis using High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA). With the cDNA as template, qRT-PCR was performed using StepOnePlus Real-Time PCR System and TaqMan® Gene expression assays (Applied Biosystems). TaqMan probes for mouse Cyp1a2 (Mm00487224_m1), Cyp2a4 / Cyp2a5 (Mm00487248_g1), Cyp2b10 (Mm00456588_mH), Cyp2c37 (Mm00833845_m1), Cyp2d22 (Mm00530542_m1), Cyp2e1 (Mm00491127_m1), Cyp3a11 (Mm00731567_m1), Cyp3a41 (Mm00776855_mH), and β-actin (Mm00607939_s1) were purchased from Applied Biosystems. For immune modulators and transcription factors, we used SYBR® Green RT-PCR Reagents Kit (Applied Biosystems). Primers used for the detection were shown in Table 1. The fold change in mRNA levels during pregnancy was determined after normalizing the gene expression levels by those of β-actin (2^−ΔΔCt method) (Schmittgen and Livak, 2008). All qRT-PCR experiments were

This article has not been copyedited and formatted. The final version may differ from this version.
performed in duplicate for each gene, and the results were verified by using at least two different sets of cDNA made from the same RNA.

Statistical Analysis. All data were analyzed using SAS (version 9.1, Cary, NC). One-way analysis of variance (ANOVA) was performed, followed by unpaired Student’s t-test for comparison with the pre-pregnancy level. Correlation between mRNA expression of Cyp isoforms and transcription factors (or inflammatory markers) was determined by the Spearman rank analysis and expressed as the corresponding correlation coefficient ($r$). Post-hoc Bonferroni correction was performed to adjust for the Type I error rate ($= 0.05$) for 20 ANOVA and 96 correlation testings. Our study had 80% power to detect a change greater than 82% from the pre-pregnancy values at a level of significance of 5% [PASS software (Kaysville, Utah)].
Results

**Isoform-specific Effects of Pregnancy on Cyp Expression.** Mouse Cyp enzymes in drug-metabolizing Cyp1, 2, and 3 families amount to over 30 different isoforms as compared to 20 isoforms in humans (Martignoni et al., 2006). We examined mRNA expression of 8 major Cyp isoforms (Cyp1a2, Cyp2a5, Cyp2b10, Cyp2c37, Cyp2d22, Cyp2e1, Cyp3a11, and Cyp3a41) in livers of virgin, pregnant (7-, 14-, and 21-days of pregnancy; P7, P14, and P21, respectively), and 7-days postpartum (PP7) mice. Their expression was normalized by that of β-actin, which was not influenced by pregnancy (p = 0.08, based on comparison of C_T values). Results from ANOVA testing showed that significant differences existed among different gestational stages for all Cyp isoforms except Cyp2a5: Cyp1a2 (p = 0.0002), Cyp2b10 (p = 0.0007), Cyp2c37 (p = 0.0003), Cyp2d22 (p < 0.0001), Cyp2e1 (p = 0.0001), Cyp3a11 (p = 0.0024), and Cyp3a41 (p = 0.0001). Pregnancy decreased expression of 5 Cyp isoforms as compared to pre-pregnancy levels: Cyp1a2, Cyp2c37, Cyp2d22, Cyp2e1, and Cyp3a11 (Fig. 1). Expression of Cyp2d22 and Cyp2e1 was decreased as early as P7 and had not recovered by PP7. In contrast, expression of Cyp2c37 and Cyp3a11, after reaching a nadir at P14 or P21, showed a trend toward recovery to the pre-pregnancy level at PP7. Expression of Cyp3a41 was upregulated in mouse pregnancy, a result consistent with a previous report (Zhang et al., 2008). Cyp2b10 expression was increased after delivery. Taken together, these results indicate that, while pregnancy in general decreases expression of major Cyp isoforms in mice, the effect of pregnancy and delivery on Cyp expression is apparently isoform-specific.

**Changes in Expression of Inflammatory Markers and Hepatic Transcription Factors.** To explore the potential mechanisms underlying pregnancy-mediated changes in Cyp expression, we examined the expression levels of inflammatory markers (IL-2, IL-4, IL-6, IL-10, IL-1β, IFNγ, and TNFα) and hepatic transcription factors [PXR, CAR, PPARα, AhR, and ERα] in mouse livers at different gestational stages. Results from ANOVA testing showed that significant differences existed among different gestational stages for PPARα (p < 0.0001) (Fig. 2); pregnancy had insignificant effects on expression levels of inflammatory markers or other transcription factors (data not shown).
Next we examined whether the expression levels of inflammatory markers or transcription factors correlated with those of Cyp isoforms in mouse livers at different gestational stages (n = 20). Data obtained from Spearman rank analysis are summarized in Table 2. This preliminary examination (including 96 sets of correlation) revealed that expression of Cyp2d22 and Cyp2e1 is correlated with that of PPARα, suggesting that PPARα may be involved in altered Cyp expression during mouse pregnancy.
Discussion

Our results indicate that pregnancy generally represses expression of major Cyp isoforms in mice. Among eight Cyp isoforms examined in this study, only Cyp3a41 showed an increased expression during pregnancy (Fig. 1). Expression of the rest of Cyp isoforms was either decreased (Cyp1a2, Cyp2c37, Cyp2d22, Cyp2e1, and Cyp3a11) or not affected (Cyp2a5) by pregnancy. Although it remains to be determined how these changes in mRNA expression lead to altered pharmacokinetics of drugs in mice, the mRNA results suggest that changes in drug metabolism during mouse pregnancy may differ from the clinically observed changes in humans.

The apparent discrepancy in directional changes in Cyp-mediated drug metabolism between mouse and human pregnancy may be explained by a lack of clear orthologues between the two species, especially for CYP2Cs, CYP2Ds, and CYP3As (Nelson et al., 2004). For example, compared to humans, which have only one isoform in the CYP2D subfamily (i.e., CYP2D6), the mouse has at least 9 different isoforms in the Cyp2d subfamily (Martignoni et al., 2006). This is due to significant expansion of Cyp gene families in mice (relative to humans) since the divergence of human and rodent lineages at ~75 million years ago (MGSC, 2002). Considering the large number of Cyp isoforms in mice, it appears plausible that Cyp isoforms in the same subfamily may respond to pregnancy differently, some of which may better reflect the changes in drug metabolism during human pregnancy than the other Cyp isoforms. In fact, pregnancy upregulates Cyp3a41 expression while it downregulates Cyp3a11 in mice (Fig. 1). As the net result is increased protein expression of Cyp3a (Zhang et al., 2008), mice may serve as an animal model to study enhanced CYP3A4-mediated drug metabolism during human pregnancy (Zhou et al., 2010). Further studies are needed to examine how expression of other mouse Cyp isoforms are influenced during pregnancy to establish mice as the animal model to investigate altered drug metabolism during pregnancy. Alternatively, transgenic mice that harbor upstream regulatory region of human CYP genes (Corchero et al., 2001) may present a useful animal model in that interspecies differences in gene regulatory sequences can be overcome.
Underlying mechanisms for the global downregulation of major Cyp isoforms during mouse pregnancy remain unknown. Pregnancy-related physiological changes are likely responsible, which include (1) rising levels of pregnancy hormones, (2) potentially heightened inflammatory response, and (3) possible changes in activity and/or expression of key transcription factors involved in regulation of Cyp expression. Pregnancy is accompanied by increasing plasma concentrations of female hormones (estrogens and progesterone), cortisol, and placental growth hormones (Barkley et al., 1979; Masuyama et al., 2001), which may potentially modulate hepatic Cyp expression. For example, growth hormone (released in a continuous pattern) increases expression of Cyp3a41 (Sakuma et al., 2002), and estradiol and glucocorticoid can potentiate the inducing effects of growth hormones on Cyp3a41 expression (Sakuma et al., 2004). As suggested previously (Zhang et al., 2008), upregulation of Cyp3a41 may be attributed to the combined effects of pregnancy hormones on Cyp expression. On the other hand, estrogen downregulates human CYP2C19 expression in an ERα-dependent manner (Mwinyi et al., 2010). These regulatory effects of hormones on CYP expression support potentially significant roles of pregnancy hormones in the reduced Cyp expression during mouse pregnancy.

Our results indicate that mouse pregnancy has insignificant effects on hepatic expression of immunomodulators but downregulates expression of PPARα (Fig. 2). The lack of effects on other transcription factors or immunomodulators may be due to the small sample size used in this exploratory study. The decreased PPARα expression strongly correlated with the reduced expression levels of Cyp2d22 and Cyp2e1 (Table 2), suggesting that PPARα potentially participates in regulation of Cyp expression during mouse pregnancy. In fact, it has been shown that a peroxisome proliferator activator (such as WY-14,643) downregulates CYP2C11 and CYP2C12 while upregulating CYP4A in rats (Corton et al., 1998; Graham and Lake, 2008) and mice (Savas et al., 2009). It is uncertain, however, whether these results can be extrapolated to humans. Significant interspecies difference between rodents and humans has been reported in the PPARα target genes and subsequent physiological outcomes of PPARα
activation (Graham and Lake, 2008). The physiological roles of reduced PPARα expression in mouse pregnancy, as well as clinical implications of such changes, remain to be determined.

Taken together, we have shown in this study that pregnancy downregulates the expression of major Cyp isoforms in mice. The information obtained from the current study should be of great value in better understanding the mouse as an animal model and guiding future studies for identification of an appropriate model system and approaches to investigate CYP regulation during pregnancy.

**Acknowledgements.** We would like to thank Liam Fischer and Dr. Hye Jin Chung for reading of this manuscript.

**Authorship Contributions**

*Participated in research design:* Koh, Yu, and Jeong

*Conducted experiments:* Koh and Yu

*Performed data analysis:* Koh, Xie, and Jeong

*Wrote or contributed to the writing of the manuscript:* Koh, Yu, and Jeong
References


Footnotes

This work was supported by the National Institute of Child Health and Human Development [Grant HD055313]. H. J. is a recipient of a fellowship from National Institute of Child Health and Human Development [Grant K12HK055892]. H. X. is supported by National Center for Research Resources [Grant UL1RR029879].

Please send reprint requests to

Hyunyoung Jeong, PharmD, PhD

Department of Pharmacy Practice (MC 886)

College of Pharmacy, University of Illinois at Chicago

833 S. Wood Street, Chicago, IL 60612

E-mail: vjeong@uic.edu
FIGURE LEGENDS

Fig. 1. Effects of pregnancy on mRNA expression of major Cyp enzymes. mRNA expression levels of mouse CYP isoenzymes (Cyp1a2, Cyp2a5, Cyp2b10, Cyp2c37, Cyp2d22, Cyp2e1, Cyp3a11 and Cyp3a41) were determined by qRT-PCR in mouse livers at different gestational time points (n = 4/group; mean ± S.D.). Data shown are expression levels of each CYP isozyme relative to the expression in mouse no. 1 of the virgin group (arbitrarily set as 1). *, p < 0.05; **, p < 0.01 compared with the virgin group.

Fig. 2. Effects of pregnancy on mRNA expression of PPARα. mRNA expression level of PPARα was determined by qRT-PCR in mouse livers at different gestational time points (n = 4/group; mean ± S.D.). Data shown are relative expression of PPARα as compared to mouse no. 1 of the virgin group (arbitrarily set as 1). *, p < 0.05 compared with the virgin group.
**TABLE 1** Sequences of primers used in qRT-PCR for detection of immunomodulators and transcription factors

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence</th>
<th>Gene</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>(forward) 5’-GCAACTGTTCCTGAACCTCAACT-3’ (reverse) 5’-ATCTTTTGGGTCCGTCACCT-3’</td>
<td>PXR</td>
<td>(forward) 5’-GATGGAGGTTCTTCAAATCTGCC-3’ (reverse) 5’-GGCCCTTCTGAAACACCCCT-3’</td>
</tr>
<tr>
<td>IL-2</td>
<td>(forward) 5’-GTTGCTCTTTGTAACACAGC-3’ (reverse) 5’-GGGGAGTTTCAGGATCTC-3’</td>
<td>CAR</td>
<td>(forward) 5’-CCCTGACAGACCAGTTA-3’ (reverse) 5’-GCCGAGACTGTGTTCCATAAT-3’</td>
</tr>
<tr>
<td>IL-4</td>
<td>(forward) 5’-GGTCTCAACCCCCAGCTAGT-3’ (reverse) 5’-GGCGATGATCTCCTCAAGTGAT-3’</td>
<td>PPARα</td>
<td>(forward) 5’-AGAGCCCACTGTCCTTCCTC-3’ (reverse) 5’-CTGGTGATGACTGCAAAACCAA-3’</td>
</tr>
<tr>
<td>IL-6</td>
<td>(forward) 5’-TAGTCTCTTCTACCCAAATTCC-3’ (reverse) 5’-TTGGCTCCTAGCCACTCTCC-3’</td>
<td>ERα</td>
<td>(forward) 5’-CCTCCCCCTTCTACAGGT-3’ (reverse) 5’-CACACGGCACAGTAGGAG-3’</td>
</tr>
<tr>
<td>IL-10</td>
<td>(forward) 5’-GCTCTTACTGACTGCGATGAG-3’ (reverse) 5’-CGCAGCTCTAGGACATG-3’</td>
<td>AhR</td>
<td>(forward) 5’-AGCCGCTGCAAAAAACAGTA-3’ (reverse) 5’-AGGCCGTCTAAGCTGTTGTCC-3’</td>
</tr>
<tr>
<td>IFNγ</td>
<td>(forward) 5’-ATGAACGCTACACTGACAC-3’ (reverse) 5’-CCATCTCTTGGCCAGTCCC-3’</td>
<td>β-actin</td>
<td>(forward) 5’-GGCTGTATTTCCCCTCCCATCG-3’ (reverse) 5’-CCAGTGGTTACAATGCCATGT-3’</td>
</tr>
<tr>
<td>TNFα</td>
<td>(forward) 5’-CCCTCACAACCTAGTCTCTTCTC-3’ (reverse) 5’-GCTACGACGGGTGCTACAG-3’</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE 2 Correlation of mRNA expression levels between immunomodulators/transcription factors and Cyp isoforms (n = 20). Statistically significant correlation is marked: *, p < 0.0005 after Bonferroni correction.

<table>
<thead>
<tr>
<th>Immunomodulator</th>
<th>Cyp1a2</th>
<th>Cyp2a5</th>
<th>Cyp2b10</th>
<th>Cyp2c37</th>
<th>Cyp2d22</th>
<th>Cyp2e1</th>
<th>Cyp3a11</th>
<th>Cyp3a41</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>0.048</td>
<td>0.077</td>
<td>-0.220</td>
<td>0.143</td>
<td>0.178</td>
<td>0.147</td>
<td>0.071</td>
<td>0.068</td>
</tr>
<tr>
<td>p-value</td>
<td>0.842</td>
<td>0.746</td>
<td>0.351</td>
<td>0.548</td>
<td>0.453</td>
<td>0.537</td>
<td>0.766</td>
<td>0.776</td>
</tr>
<tr>
<td>IL-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>0.149</td>
<td>0.039</td>
<td>-0.052</td>
<td>0.013</td>
<td>0.025</td>
<td>-0.032</td>
<td>-0.049</td>
<td>0.167</td>
</tr>
<tr>
<td>p-value</td>
<td>0.544</td>
<td>0.875</td>
<td>0.834</td>
<td>0.958</td>
<td>0.918</td>
<td>0.898</td>
<td>0.841</td>
<td>0.494</td>
</tr>
<tr>
<td>IL-4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>0.389</td>
<td>0.214</td>
<td>0.037</td>
<td>0.444</td>
<td>0.578</td>
<td>0.539</td>
<td>0.516</td>
<td>-0.082</td>
</tr>
<tr>
<td>p-value</td>
<td>0.090</td>
<td>0.365</td>
<td>0.878</td>
<td>0.048</td>
<td>0.008</td>
<td>0.014</td>
<td>0.020</td>
<td>0.731</td>
</tr>
<tr>
<td>IL-6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>-0.158</td>
<td>0.095</td>
<td>-0.547</td>
<td>-0.076</td>
<td>0.048</td>
<td>0.191</td>
<td>-0.103</td>
<td>0.383</td>
</tr>
<tr>
<td>p-value</td>
<td>0.506</td>
<td>0.692</td>
<td>0.013</td>
<td>0.749</td>
<td>0.842</td>
<td>0.420</td>
<td>0.665</td>
<td>0.095</td>
</tr>
<tr>
<td>IL-10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>-0.289</td>
<td>-0.115</td>
<td>-0.539</td>
<td>-0.218</td>
<td>-0.104</td>
<td>0.014</td>
<td>-0.325</td>
<td>0.422</td>
</tr>
<tr>
<td>p-value</td>
<td>0.216</td>
<td>0.629</td>
<td>0.014</td>
<td>0.355</td>
<td>0.662</td>
<td>0.955</td>
<td>0.162</td>
<td>0.064</td>
</tr>
<tr>
<td>IFN-γ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>-0.040</td>
<td>-0.018</td>
<td>-0.310</td>
<td>-0.034</td>
<td>0.091</td>
<td>0.113</td>
<td>-0.187</td>
<td>0.074</td>
</tr>
<tr>
<td>p-value</td>
<td>0.868</td>
<td>0.940</td>
<td>0.183</td>
<td>0.886</td>
<td>0.703</td>
<td>0.634</td>
<td>0.430</td>
<td>0.758</td>
</tr>
<tr>
<td>TNFα</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>-0.312</td>
<td>0.078</td>
<td>-0.535</td>
<td>-0.011</td>
<td>0.019</td>
<td>0.200</td>
<td>-0.166</td>
<td>0.213</td>
</tr>
<tr>
<td>p-value</td>
<td>0.181</td>
<td>0.744</td>
<td>0.015</td>
<td>0.962</td>
<td>0.935</td>
<td>0.399</td>
<td>0.485</td>
<td>0.366</td>
</tr>
<tr>
<td>Transcription factor</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PXR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>0.370</td>
<td>0.587</td>
<td>0.278</td>
<td>0.382</td>
<td>0.333</td>
<td>0.361</td>
<td>0.551</td>
<td>-0.026</td>
</tr>
<tr>
<td>p-value</td>
<td>0.109</td>
<td>0.007</td>
<td>0.235</td>
<td>0.097</td>
<td>0.152</td>
<td>0.118</td>
<td>0.012</td>
<td>0.913</td>
</tr>
<tr>
<td>CAR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This article has not been copyedited and formatted. The final version may differ from this version.
<table>
<thead>
<tr>
<th></th>
<th>PPARα</th>
<th>ERα</th>
<th>AhR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r-value</td>
<td>p-value</td>
<td>r-value</td>
</tr>
<tr>
<td></td>
<td>0.385</td>
<td>0.094</td>
<td>0.475</td>
</tr>
<tr>
<td></td>
<td>0.171</td>
<td>0.471</td>
<td>0.457</td>
</tr>
<tr>
<td></td>
<td>-0.115</td>
<td>0.628</td>
<td>-0.421</td>
</tr>
<tr>
<td></td>
<td>0.214</td>
<td>0.365</td>
<td>0.499</td>
</tr>
<tr>
<td></td>
<td>0.472</td>
<td>0.036</td>
<td>0.773</td>
</tr>
<tr>
<td></td>
<td>0.477</td>
<td>0.033</td>
<td>0.827</td>
</tr>
<tr>
<td></td>
<td>0.358</td>
<td>0.121</td>
<td>0.494</td>
</tr>
<tr>
<td></td>
<td>-0.121</td>
<td>0.611</td>
<td>0.063</td>
</tr>
</tbody>
</table>

*Significant at the 0.05 level.
Fig. 1.

Cyp1a2

Cyp2a5

Cyp2b10

Cyp2c37

Cyp2d22

Cyp2e1

Cyp3a11

Cyp3a41

Relative expression

Virgin P7 P14 P21 PP7

Virgin P7 P14 P21 PP7

Virgin P7 P14 P21 PP7

Virgin P7 P14 P21 PP7

Virgin P7 P14 P21 PP7

Virgin P7 P14 P21 PP7

Virgin P7 P14 P21 PP7

Virgin P7 P14 P21 PP7
Fig. 2.

**PPARα**

Relative expression

Virgin P7 P14 P21 PP7

* * **