Pregnancy results in dramatic surges in hormones during gestational development, with increases in steroids such as estrogens, progestins, and the glucocorticoids. These steroids have been linked to control and regulation of xenobiotic drug metabolism, primarily through activation of the nuclear xenobiotic receptors such as the pregnane X receptor (PXR) and the constitutive androstane receptor (CAR). PXR and CAR have been classified as steroid and xenobiotic sensors, so it can be anticipated that significant fluctuations in the pregnancy hormones will modulate transcriptional control of target genes such as those involved in xenobiotic or drug metabolism.

Clinical findings indicate that steroid fluctuations lead to changes in xenobiotic glucuronidation during pregnancy. For example, circulating unconjugated bilirubin is cleared from the circulation solely through UDP glucuronosyltransferase 1A1 (UGT1A1) metabolism. During pregnancy, total serum bilirubin (TSB) levels are lower in women, indicating that bilirubin metabolism is accelerated through induction of UGT1A1. Labetalol, an antihypertensive agent, which is metabolized primarily by UGT1A1 glucuronidation, shows increased clearance in the second and third trimesters of pregnancy compared to the postpartum period. Lamotrigine, an antiepileptic agent metabolized by UGT1A3 and UGT1A4, has a 50% decreased elimination half-life with an increased clearance of over 200% during pregnancy, leading to a closely correlated
higher incidence of epileptic seizures. Labetalol and lamotrigine clearance during pregnancy indicates that UGT1A1, UGT1A3, and UGT1A4 are induced and clearance is accelerated. It has been suggested that up-regulation of UGT1A4 during pregnancy may be mediated by 17β-estradiol and the estrogen receptor alpha (ERα). Clearly, hormonal sensors during pregnancy are leading to induction of human glucuronidation capacity.

The exact opposite is occurring during neonatal development, which is evident by the very high incidence of hyperbilirubinemia in newborn children. Because bilirubin is metabolized exclusively by UGT1A1,6 hyperbilirubinemia develops from the inability of liver glucuronidation to match the early rise in serum bilirubin that forms from the abundance of red blood cells needed to carry oxygen. Senescence of the erythrocytes leads to an accumulation of hemoglobin that is rapidly metabolized into bilirubin and released into the circulation, where it is transported to the liver for excretion following UGT1A1-dependent glucuronidation. Jaundice is directly linked to inadequate glucuronidation of serum bilirubin stemming from reduced expression of liver UGT1A1.11-13 It is unclear if the reduced expression of UGT1A1 in neonates is a controlled event through transcriptional silencing or simply a result of limited epigenetic factors that are eventually produced to positively regulate the UGT1A1 gene in a developmental fashion.

In this report it will be demonstrated that PXR is linked to both pregnancy-induced expression of the UGT1 locus as well as repression of the UGT1A1 gene in neonatal development. These findings were generated through the development of humanized UGT1 (hUGT1) mice,14,15 which express the entire human UGT1 locus in a murine Ugt1-null background.16

Taking advantage of the power of reverse genetics, it will be shown that PXR plays a crucial role in pregnancy-induced glucuronidation in addition to the early development of hyperbilirubinemia in neonatal hUGT1 mice.

To generate hUGT1/Pxr+/− mice, hUGT1*1 mice were crossed with Pxr−/− mice, producing Tg(UGT1*1)Ugt1+/− Pxr+/− mice. These mice were backcrossed in brother/sister matings to generate Tg(UGT1*1)Ugt−/− Pxr−/− (hUGT1*1/Pxr−/−) mice. The same breeding strategy was used to generate hUGT1*1/Car−/− mice.

**Primary Hepatocyte Isolation and PXR-Targeted Specific Small Interfering RNA (siRNA) Regulation.** Hepatocytes were isolated as described. The hepatocytes were then cultured in 6-well collagen-treated plates (Discovery Labware, Bedford, MA) in 2 mL of Dulbecco’s modified Eagle’s medium (DMEM) containing penicillin/streptomycin and supplemented with 10% fetal bovine serum. siRNA duplexes specific for mouse PXR were provided by Bioneer (Alameda, CA) and Santa Cruz Biotechnology. Four hours after primary hepatocytes were isolated from 14-day-old hUGT1*1 mice, cells were transfected in the presence of 20 nM of either siRNA or control RNA with Lipofectamine 2000 (Invitrogen) in a final volume of 0.5 mL of OPTI-MEM. After 5 hours cells were changed with fresh medium supplemented with 10% fetal bovine serum and penicillin-streptomycin. Forty-eight hours later, cells were used for RNA extraction. Reverse transcription (RT) and real-time polymerase chain reaction (Q-PCR) were carried out to examine gene expression levels of mouse Pxr, human UGT1A1 and mouse Cyp3a11.

**Immunoblot Analysis and Real-Time PCR.** Mice were sacrificed and livers were perfused with ice-cold 1.15% KCL and microsomes prepared as outlined. All western blots were performed using NuPAGE BisTris-polyacrylamide gels as described. For real-time quantitative Q-PCR analysis, ~100 mg of liver tissue was homogenized into 1 mL of TRIzol and RNA prepared. Using iScript Reverse Transcriptase (BioRad), 1 µg of total RNA was used for the generation of complementary DNA (cDNA) as outlined by the manufacturer in a total volume of 20 µL. Following synthesis of cDNA, 2 µL was used in real-time PCR conducted with a QuantiTect SYBR GreenPCR kit (Qiagen, Valencia, CA) using a MX4000 Multiplex Q-PCR (Stratagene, La Jolla, CA) programmed to take three fluorescence data points at the end of each annealing plateau. All PCR reactions were performed in triplicate as outlined. Ct values were normalized to mouse cyclophilin (CPH). The specific primers used to quantitate the respective gene transcripts are listed in Supporting Table 1.

**Materials and Methods**

**Animals.** The generation of Tg(UGT1*1)Ugt1−/− (hUGT1*1) and Tg(UGT1*1−28)Ugt1−/− (hUGT1*28) mice has been reported.15 Pxr−/− mice were generated as described and Car−/− mice were generously provided by Dr. Masahiko Negishi (NIH). All genetically modified strains were bred for over five generations with C57BL/6 wildtype mice before inbreeding.
based on the EZ-CHIP kit (Millipore). Liver tissue (100 mg) was minced and cross-linked in DMEM (Invitrogen) containing 1% formaldehyde. The procedures for cell lysis and sonication to shear DNA were followed according to the manufacturer's protocol (EZ-CHIP kit, Millipore). One mL of cell extract was precleared by incubation with 60 L of protein A Agarose/Salmon sperm DNA overnight at 4°C. The cleared cellular extract was incubated with anti-PXR antibody (Santa Cruz, sc-25381) for 2 hours at 4°C. Following precipitation with protein A agarose, the antibody-chromatin complex was then washed as outlined.19 The protein-DNA complexes were eluted in 200 L elution buffer and DNA was then reverse cross-linked and released from the complex as indicated in the EZ-CHIP instructions. Following the DNA purification with spin columns (Qiagen), the purified DNA was further analyzed by PCR with a pair of primers (forward 5'-TTGTGGGGCAATA CACTAGTA-3', reverse 5'-GTCCGGGTTTCAAGT TATGTA-3') for the amplification of the UGT1A1 promoter region containing the PXR binding site.3

Results

Expression of the Human UGT1A Genes in TgUGT1 Mice During Pregnancy. Heterozygous female TgUGT1*28 mice were mated with wildtype mice and the presence of the vaginal plug in the morning was set as gestation day 1 (GD1). Starting at GD4, gravid mice were sacrificed at various times during pregnancy and liver microsomes were prepared from female wildtype and TgUGT1 mice that were pregnant for 16 days. Control samples were prepared from nonpregnant wildtype and TgUGT1 mice. (C) Total RNA was isolated from liver samples taken from the pregnant TgUGT1 mice and used in RT and Q-PCR analysis to examine murine UGT1A1 RNA (Ugt1a1) expression and human UGT1A1 RNA expression. Student's t test was used to evaluate the statistical significance (**P < 0.01). (D) The same RNA samples were used to quantitate murine Ugt1a6 and human UGT1A6 gene expression (**P < 0.01, t test).
UGT1A6 genes were evaluated by RT and Q-PCR analysis using species-specific primers for each of these genes. Throughout pregnancy, we observed no induction of murine Ugt1a1 or Ugt1a6 gene expression (Fig. 1C, D), findings that correlated with the lack of wild-type UGT1A protein expression. Consistent with induction of UGT1A protein in TgUGT1*28 mice during pregnancy, UGT1A1 and UGT1A6 gene expression is induced at GD14, with the induction being sustained throughout the remainder of the gestational period. Introduction of the human UGT1 locus and the UGT1A genes in TgUGT1*28 mice is regulated throughout pregnancy in a pattern that is not replicated by the murine Ugt1 locus. Emerging principles of regulatory evolution strongly favor genetic diversity in cis-regulatory DNA and not trans-regulation of gene expression to explain interspecies differences in gene expression. The differences in transcriptional regulation between the murine and human UGT1 locus during pregnancy may be credited to important genetic differences in the regulatory regions of these genes.

**Induction of the UGT1 Locus in Humanized UGT1 Mice.** We generated hUGT1*1 and hUGT1*28 mice, which differ predominantly in expression levels of UGT1A1 in adult liver. Adult hUGT1*28 mice are hyperbilirubinemic, with TSB levels that average 1 mg/dL. During pregnancy and late gestation, the TSB levels in hUGT1*28 mice averaged 0.4 mg/dL, over 50% lower than in nonpregnant mice (Fig. 2A). The reduction in TSB can be accounted for by elevated levels of liver UGT1A1.

In hUGT1*1 mice, expression of UGT1A1, -1A3, -1A4, and -1A6 are 2 to 4-fold greater in female liver than male liver (Fig. 2B). The sole exception to the female dominance is UGT1A9, which has minimal expression in female liver. Examination of the fold increase in gene expression of each of the UGT1A genes shows that UGT1A9 expression increases to nearly 70-fold over nonpregnant values (Fig. 2C). This large increase in UGT1A9 expression accounted for by fold induction during pregnancy results in part from the very low basal levels observed in nonpregnant female mice. Along with UGT1A9 gene expression, UGT1A1 and UGT1A6 gene expression are found to dominate the induction process during pregnancy. These increases are also reflected in microsomal protein abundance as determined by western blot analysis using isozyme-specific antibodies (Fig. 2D).

**Pregnancy Steroids and Induction of UGT1A1.** We hypothesized that hormone surges in late pregnancy play an important role in the gestational regulation of the human UGT1 locus. To examine if selective steroids are capable of regulating the UGT1A genes, primary hepatocytes from hUGT1*1 mice were isolated, placed in culture, and exposed to 17β-estradiol, progesterone, or the synthetic glucocorticoid, dexamethasone (DEX). We measured induction of the UGT1A1 gene because the other UGT1A genes are refractive to expression in hepatocytes in culture. Progesterone (50 μg/mL) and estradiol (20 μg/mL) exposure for 24 hours resulted in a minimal 2 to 3-fold induction of the UGT1A1 gene (Fig. 3A). These concentrations were found to be optimal for UGT1A1 induction. The synthetic glucocorticoid DEX (10 μM) induced UGT1A1 gene expression up to 60-fold (Fig. 3B). This induction of gene expression and UGT1A1 induction is dose-dependent, with transcriptional
induction of the UGT1A1 gene in hepatocytes being substantial at 0.1 \mu M. Because corticosterone is the primary form of glucocorticoids in mouse, 20 \mu M of corticosterone was used to treat freshly isolated hepatocytes (Fig. 3C). Twenty-four hours after exposure, increased UGT1A1 protein levels were detected in whole cell lysates by western blot analysis, indicating that glucocorticoids play an important role in UGT1A gene expression.

Xenobiotic Receptors and Induction of the UGT1 Locus During Pregnancy. The progestins, corticosterone, and estradiols are low-affinity substrates for PXR\(^{23-25}\) and 17\(\beta\)-estradiol has been shown to activate CAR.\(^{4}\) Thus, experiments were conducted to examine precisely the role of PXR and CAR toward induction of the UGT1 locus during pregnancy.

To undertake these studies, \(h\)UGT1*1 mice were crossed with Car-null mice to create \(h\)UGT1*1/Car\(^{-/-}\) mice. On GD16, liver samples from \(h\)UGT1*1/Car\(^{-/-}\) mice were processed for total RNA along with microsomal extracts. RT and Q-PCR analysis for liver UGT1A gene products were conducted with specific oligonucleotide primers for each gene (Fig. 4A). In \(h\)UGT1*1/Car\(^{-/-}\) mice, gestational induction of the UGT1A1, -1A3, and -1A6 genes was found to be similar to that observed in \(h\)UGT1*1 mice. Using western blot analysis to examine UGT1A1 expression in liver microsomes, UGT1A1 was induced during pregnancy in \(h\)UGT1*1 and \(h\)UGT1*1/Car\(^{-/-}\) mice (Fig. 4B). However, CAR does play a role in the induction of UGT1A4 and UGT1A9. We observed approximately a 50% reduction in the 8-fold increase in UGT1A4 RNA accumulation observed in \(h\)UGT1*1 mice. When UGT1A9 expression was analyzed, the robust induction in \(h\)UGT1*1 pregnant mice was reduced over 75% during pregnancy in \(h\)UGT1*1/Car\(^{-/-}\) mice, indicating an important role for CAR in the induction of the UGT1A9 gene (Fig. 4A).

When \(h\)UGT1*1 mice are placed into a Pxr-null background, there was substantially reduced induction of each of the UGT1A genes during pregnancy when compared to expression in \(h\)UGT1*1 mice (Fig. 4A). The UGT1A1 gene, robustly induced around 15-fold in \(h\)UGT1*1 and \(h\)UGT1*1/Car\(^{-/-}\) mice during pregnancy, displays reduced expression at GD16 in \(h\)UGT1*1/Pxr\(^{-/-}\) mice. A similar pattern of expression was observed when UGT1A1 was detected by western blot analysis, showing little expression in \(h\)UGT1*1/Pxr\(^{-/-}\) mice (Fig. 4B). An important role for PXR binding to the UGT1A1 gene during pregnancy was reinforced when we examined PXR binding by CHIP analysis to the PXR binding site that flanks the UGT1A1 promoter.\(^3\) PXR is activated during pregnancy and binds to the UGT1A1 gene as demonstrated by CHIP analysis (Fig. 4C), indicating that endogenous ligands are participating in regulation of this gene. Coupled with CHIP analysis showing induced binding of PXR to the UGT1A1 gene following DEX treatment (Fig. 4C) along with previous experiments demonstrating that PXR binding to this region of the
UGT1A1 gene stimulates transactivation of the promoter,3 these findings confirm that induction of UGT1A1 is closely linked to activation of PXR during pregnancy.

**Glucocorticoids Induce the UGT1 Locus in a PXR-Dependent Fashion.** Because regulation of the UGT1 locus during pregnancy is linked to PXR, we examined if the genes associated with the UGT1 locus in humanized mice could be activated in a PXR-dependent fashion by glucocorticoids. We treated 8-week-old hUGT1*1 and hUGT1/Pxr−/− mice by the intraperitoneal route with 20 mg/kg DEX for 4 days and measured UGT1A gene expression in liver 48 hours after treatment (Fig. 5). Each of the five UGT1A genes expressed in liver was induced in hUGT1*1 mice. Although activation of the glucocorticoid receptor by DEX has been shown to activate UGT1A1 reporter gene constructs in HepG2 cells,26 DEX treatment had no effect on induction of UGT1A1 in hUGT1/Pxr−/− mice, indicating that induction of the UGT1A genes by glucocorticoids is facilitated solely by activation of the PXR in vivo.

In neonatal hUGT1 mice, UGT1A1 expression controls the levels of TSB, with significant hyperbilirubinemia developing due to limited expression of hepatic UGT1A1. We examined if glucocorticoids could regulate UGT1A1 gene expression during the neonatal period in a PXR-dependent fashion. In hUGT1*1 mice,

![Fig. 4. The impact of PXR and CAR deletion on gestational regulation of the human UGT1 locus in liver tissue. Humanized UGT1/Pxr−/− or hUGT1/Car−/− mice were obtained by backcrossing hUGT1*1 mice with Pxr−/− or Car−/− mice. Female hUGT1*1, hUGT1/Pxr−/−, and hUGT1/Car−/− mice at 8 weeks old were used for timed pregnancy experiments. Age-matched nonpregnant female mice from each strain were used as controls. Mice were sacrificed at GD16. (A) RNA was isolated from pooled liver samples followed by RT and Q-PCR analysis. Primers specific for human UGT1A1, UGT1A3, UGT1A4, UGT1A6, and UGT1A9 gene products were used (Supporting Table 1). Q-PCR results from pregnant mice were normalized by the housekeeping gene CPH and described as fold of induction over the nonpregnant control. (B) Western blot analysis using liver microsomes and anti-UGT1A1 and anti-GAPDH antibodies. (C) CHIP analysis of PXR associated with the human UGT1A1 gene in nonpregnant and 16 day pregnant hUGT1 mice, and DEX-treated adult females.](image)

![Fig. 5. Induction of the UGT1 locus by DEX in adult hUGT1*1 and hUGT1/Pxr−/− mice. Adult hUGT1*1 and hUGT1/Pxr−/− mice were treated with DEX by intraperitoneal injection for 4 consecutive days at 20 mg/kg per dose. Nontreated mice received solvent and were used as controls. Twenty-four hours after the last dose, liver RNA was prepared and RT and Q-PCR analysis of human UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, and murine Cyp3a11 gene expression was performed. Fold induction reflects the change in gene expression between solvent-treated and DEX-treated mice.](image)
TSB peaks 14 days after birth and ranges from 12-15 mg/dL (Fig. 6A). Within the same litters, half of the newborn hUGT1*1 mice were treated with 8 mg/kg DEX by oral gavage, whereas the other half received just vehicle. Serum bilirubin levels were determined 48 hours after treatment. DEX treatment led to a dramatic reduction in TSB (Fig. 6A). Analysis of UGT1A1 gene expression in hepatic tissue following DEX treatment confirmed a 40-fold induction of RNA in hUGT1*1 neonatal mice (Fig. 6B). When we examined TSB levels in response to 8 mg/kg DEX treatment in hUGT1*1/Pxr−/− mice, the serum bilirubin levels did not change when compared to vehicle-treated neonatal mice (Fig. 6A). There was also no induction of hepatic UGT1A1 as determined by RT and Q-PCR analysis (Fig. 6B). Thus, DEX induces hepatic UGT1A1 leading to bilirubin metabolism through a PXR-dependent mechanism.

**PXR Represses Human UGT1A1 Gene Expression in Neonatal hUGT1 Mice.** As we undertook these experiments, we also noted that TSB levels in neonatal hUGT1*1/Pxr−/− peak to only 4-6 mg/dL, almost 10 mg/dL lower than observed in hUGT1*1 mice (Fig. 6A). When we examined liver UGT1A1 gene expression at 14 days after birth, there was over a 10-fold increase in gene expression in hUGT1*1/Pxr−/− mice when compared to hUGT1*1 mice (Fig. 7A). This finding indicates that nonliganded PXR during development in hUGT1*1 mice is serving to repress liver UGT1A1 gene expression, because in PXR-deficient mice we observe induction of liver UGT1A1, which correlates with a reduction in serum bilirubin.

To further examine the developmental properties of PXR on UGT1A1 gene repression, we performed PXR CHIP analysis by using liver samples from both neonatal and adult hUGT1*1 mice. As shown in Fig. 7B, intensified PXR signals were observed in neonatal livers in comparison to adult livers. Abundant PXR binding to the UGT1A1 gene is concordant with reduced UGT1A1 gene expression, indicating that PXR is repressing gene expression. To examine this possibility, we isolated primary hepatocytes from 14-day-old hUGT1*1 mice and transfected them with PXR-specific siRNAs from two sources (Fig. 8). Forty-eight hours later, UGT1A1 gene expression was quantitated by using RT and Q-PCR. The fold induction was tied to the extent of the PXR mRNA knockdown. When the PXR knockdown was 50% (siPXR_b), ~2-fold induction of human UGT1A1 expression was observed. When the PXR knockdown was 70%, UGT1A1 gene induction was 4-fold. In contrast, Cyp3a11, another PXR target gene, showed no change. These findings confirm that transcriptional silencing of the UGT1A1 gene by PXR occurs during neonatal development in liver tissue.
Employing recently developed \( TgUGT1 \) mice with the \( UGT1A \) genes expressed in a \( Ugt1 \)-null background, the function of PXR and CAR during pregnancy was investigated using reverse genetics to examine induction of the \( UGT1A \) genes in xenobiotic receptor-defective mice. Previous experiments with \( TgUGT1 \) and \( hUGT1 \) mice have shown that chemical treatment with activators of either PXR or CAR leads to induction of \( UGT1A1, -1A3, -1A4, -1A6, \) and \(-1A9\).\(^{14}\) The mechanisms by which PXR and CAR control the induction of all these genes have not been determined, although PXR and CAR-responsive elements have been shown to play an important role in PXR/CAR binding and induction of the \( UGT1A1 \) gene. In primary hepatocytes from \( hUGT1 \) mice, selective treatment with \( 17\beta\)-estradiol, progesterone, and DEX each led to induction of the \( UGT1A1 \) gene, with glucocorticoid treatment maximizing the induction response. In timed pregnancy experiments, each of the maternal liver-specific \( UGT1A \) genes was induced in \( hUGT1 \) mice, with \( UGT1A1, UGT1A6, \) and \( UGT1A9 \) gene expression being the most prominent. During pregnancy, gestational induction of the \( UGT1A \) genes was mostly conserved in \( hUGT1/Car^{-/-} \) mice but greatly diminished in \( hUGT1/Pxr^{-/-} \) mice, suggesting that PXR participates in a global fashion to regulate the \( UGT1 \) locus during fetal development. The sole exception to this appears to be with \( UGT1A9 \) gene expression, which displayed reduced expression during pregnancy in \( hUGT1/Car^{-/-} \) mice. Because both PXR/CAR appear to be necessary for induction of \( UGT1A9 \) during pregnancy, there may be crosstalk occurring between the two xenobiotic receptors to facilitate regulation of the \( UGT1A9 \) gene.

The contribution of PXR toward induction of the human \( UGT1 \) locus is not conserved with the murine \( Ugt1 \) locus, because pregnancy has no effect on regulation of the murine \( Ugt1a \) genes as determined by gene expression profiling and protein accumulation. This was surprising because it has been demonstrated previously that overexpression of the human PXR in humanized PXR mice or treatment of mice with PXR ligands, such as PCN, leads to induction of the murine \( Ugt1a1 \) gene.\(^5,27,28\) Our results indicate that PXR is the central modulator of the human \( UGT1A \) genes during pregnancy, but additional regulatory events specifically toward control of the human \( UGT1 \) locus, and not the murine \( Ugt1 \) locus, are in place during pregnancy. Because an increase in \( UGT1A1 \)-dependent glucuronidation occurs in humans during pregnancy, the ability to reproduce this event in transgenic mice indicates that the genetic sequence specific to the \( UGT1 \) locus is largely responsible for directing the transcriptional program of the human \( UGT1A \) genes during pregnancy. Thus, the differences observed in the induction patterns between the human \( UGT1A \) genes and the murine \( Ugt1a \) genes are not the result of interspecies differences in epigenetic machinery or the cellular environment. From this result we can infer that transcriptional factors play a secondary role in dictating the differences observed in the human \( UGT1 \) and murine \( Ugt1 \) locus during pregnancy. Such a pattern expressing conserved genes between humans and mice has been demonstrated,\(^{27-29}\) reinforcing the hypothesis that differences in gene expression between species are controlled by changes in \( cis \)-acting transcriptional binding sequences.\(^{21,32,30}\)
Among the UGT1A isoforms, UGT1A1 is of special physiological importance because it is the only enzyme that catalyzes the glucuronidation of bilirubin. Accumulation of bilirubin leads to benign levels of hyperbilirubinemia shortly after birth, but if bilirubin levels continue to rise, the more serious symptoms associated with bilirubin-induced neurological dysfunction (BIND) can develop. Phenobarbital, a CAR agonist, has been used clinically for the treatment of neonatal hyperbilirubinemia in infants at risk for severe jaundice (TSB levels more than 16 mg/dL), therefore reducing the need for exchange transfusion. However, phenobarbital treatment is not effective immediately, and it diminishes the oxidative metabolism of bilirubin, increasing the risk of neurotoxic effects. Glucocorticoids have also been used to treat hyperbilirubinemia. The initial intent of glucocorticoid therapy is to help fetal lung maturation and reduce neonatal mortality in women at high risk for preterm labor before 35 gestational weeks. During these treatments, it has been observed that hyperbilirubinemia is significantly lower in the DEX-treated groups compared to untreated control groups. Our studies indicate that PXR serves as a major regulator following glucocorticoid treatment by inducing liver UGT1A1 expression, leading to reduction of hyperbilirubinemia. Identification of PXR as a key regulator of the UGT1A1 gene during neonatal development can be exploited as a potential therapeutic target in the treatment of hyperbilirubinemia.

Analysis of TSB levels in neonatal hUGT1 and hUGT1/Pxr−/− mice demonstrate that PXR plays a key role in controlling serum levels during development. Neonatal hUGT1 mice develop severe hyperbilirubinemia due to a reduction in liver UGT1A1 gene expression. In hUGT1/Pxr−/− mice, liver UGT1A1 is induced when compared to expression in hUGT1 mice. The increased levels of liver UGT1A1 in hUGT1/Pxr−/− mice lead to reduced levels of TSB. This finding indicates that in the absence of endogenous/exogenous ligands, the physiological role of PXR leads to repression of the UGT1A1 gene during early development. It may also be an underlying regulator of the UGT1A1 gene in newborns and responsible in part for neonatal hyperbilirubinemia. Hence, PXR acts as a repressor of UGT1A1 expression in the absence of ligand. It is known that the repressive function of PXR works in part through the recruitment of the corepressor Silencing Mediator of Retinoid and Thyroid Hormone Receptors (SMRT). SMRT binds to nuclear receptors in the absence of ligand and alters the chromatin structure through histone modification.

Clearly, deletion of PXR releases the repression (de-repression) allowing for spontaneous induction of UGT1A1 gene expression. This finding may be useful in future studies to identify PXR modulators that might directly influence bilirubin homeostasis and accelerate bilirubin metabolism and clearance in children with abnormally high levels of TSB.

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