TITLE PAGE

Inhibition of Human *UGT2B7* Gene Expression in Transgenic Mice by the Constitutive Androstane Receptor

MF Yueh, PL Mellon, and RH Tukey

Laboratory of Environmental Toxicology

Departments of Chemistry & Biochemistry, and Pharmacology -(MFY, RHT)

Department of Reproductive Medicine -(PLM)

University of California San Diego

9500 Gilman Drive MC 0722

La Jolla, CA 92093

Molecular Pharmacology Fast Forward. Published on March 17, 2011 as DOI: 10.1124/mol.110.070649
This article has not been copyedited and formatted. The final version may differ from this version.
MOL #70649

RUNNING TITLE PAGE

CAR and *UGT2B7* Expression in Transgenic Mice

Address Correspondence to Robert H. Tukey

9500 Gilman Drive MC 0722

La Jolla, CA 92093

Phone-858-822-0286

Fax-858-822-0363

Email- rtukey@ucsd.edu

Number of text pages= 24

Number of Tables= 0

Number of Figures= 6

Number of References=38

Abstract word count= 229

Introduction word count= 596

Discussion word count= 1002

Nonstandard Abbreviations:

UGT= UDP-glucuronosyltransferase

TCPOBOP= 1,4-b-s [2-(3, 5,-dichloropyridyloxy)]

CAR= Constitute androstane receptors

PXR= Pregnane X receptor

HNF= Hepatocyte nuclear factor

HDCA= Hydodeoxycholu acid

XenRs= Xenobiotic receptors

Molecular Pharmacology Fast Forward. Published on March 17, 2011 as DOI: 10.1124/mol.110.070649
This article has not been copyedited and formatted. The final version may differ from this version.
MOL #70649

ABSTRACT

The xenobiotic receptors (XenRs), constitutive androstane receptor (CAR), and pregnane X receptor (PXR) regulate and alter the metabolism of xenobiotic substrates. Among the 19 functional UDP-glucuronosyltransferases (UGTs) in humans, UGT2B7 is involved in the metabolism of many structurally diverse xenobiotics and plays an important role in the clearance and detoxification of many therapeutic drugs. To examine if this gene is regulated by CAR and PXR in vivo, transgenic mice expressing the entire *UGT2B7* gene (*TgUGT2B7*) were created. Gene expression profiles revealed that *UGT2B7* is differentially expressed in liver, kidney, adipocytes, brain, and estrogen-sensitive tissues, such as ovary and uterus. Liver *UGT2B7* expression levels were decreased when *TgUGT2B7* mice were treated with the CAR ligand 1, 4-b-s-[2-(3, 5,- dichloropyridyloxy)] (TCPOBOP), but not the PXR ligand pregnenalone 16 α-carbonitrile. While TCPOBOP decreased the levels of UGT2B7 mRNA in *TgUGT2B7* mice it had no impact on *Tg(UGT2B7)Car*$^{-/-}$ mice, adding support for a CAR-dependent mechanism contributing towards *UGT2B7* gene suppression. Expression of promoter constructs in HepG2 cells showed the CAR dependent inhibition was linked to HNF4α-mediated transactivation of the *UGT2B7* promoter. The inhibitory effect of CAR on *UGT2B7* gene expression was validated in CHIP assays in which TCPOBOP treatment blocked HNF4α binding to the *UGT2B7* promoter. These results suggest that HNF 4α plays an important role in the constitutive expression of hepatic UGT2B7, and CAR acts as a negative regulator by interfering with HNF 4α binding activity.

Molecular Pharmacology Fast Forward. Published on March 17, 2011 as DOI: 10.1124/mol.110.070649
This article has not been copyedited and formatted. The final version may differ from this version.
MOL #70649

INTRODUCTION

Located in the cellular endoplasmic reticulum, the family of UDP-glucuronosyltransferases (UGTs) plays a vital role in the metabolism and detoxification of numerous endogenous and exogenous compounds. There are 19 functional UGTs in humans, nine are encoded by the *UGT1* locus on chromosome 2, and the other *UGT2* genes on chromosome 4 (Mackenzie et al., 2005). The expression of these genes in human tissues is highly organized, with each tissue comprising its own complement of the UGTs (Gregory et al., 2004;Tukey and Strassburg, 2000). Among the human UGTs, UGT2B7 is expressed in many tissues and conveys broad substrate specificity. Some estimates indicate that UGT2B7 is responsible for the metabolism of 35% of all clinical drugs (Williams et al., 2004). In addition, UGT2B7 participates in the metabolism of bile acids, fatty acids, and steroids (Ritter et al., 1992).

Since UGT2B7 plays a key role in drug metabolism and is abundant in human liver (Izukawa et al., 2009) and intestine, efforts are underway to investigate the mechanisms leading to *UGT2B7* gene control. In human liver, there is large interindividual variability in the expression of UGT2B7 (Izukawa et al., 2009), part of which has been linked to hepatocyte nuclear factor-1α (HNF1α) expression (Toide et al., 2002;Ormrod et al., 1999). In human Caco-2 cells, exposure to farnesoid X receptor (FXR) ligands, such as lithocholic acid, suppressed constitutive expression of UGT2B7 (Lu et al., 2005b). Retinoic acids, which are also metabolized by UGT2B7 (Samokyszyn et al., 2000) but play a key role in nuclear receptor function by activating the retinoid X receptor (RXR), have also been shown to suppress *UGT2B7* expression in Caco-2 cells (Lu et al., 2008). These results indicate that the family of xenobiotic nuclear receptors (XenRs), including FXR and possibly others that are expressed in liver and intestine such as the constitutive androstane receptor (CAR) and pregnane X receptor (PXR) may also be implicated in control of the *UGT2B7* gene.

Molecular Pharmacology Fast Forward. Published on March 17, 2011 as DOI: 10.1124/mol.110.070649
This article has not been copyedited and formatted. The final version may differ from this version.
MOL #70649

The placement of human genes into mice that are expressed as transgenes serves as a powerful tool to examine the influence of hormones, steroids and nuclear receptors towards influencing transcriptional control and function of the gene products. The generation of transgenic *UGT1* (*TgUGT1*) mice expressing the human *UGT1* locus has confirmed that the 9-*UGT1A* genes are expressed in a coordinated fashion (Chen et al., 2005) that resembles their expression pattern as mapped in human tissues (Strassburg et al., 1997a;Strassburg et al., 1997b;Tukey and Strassburg, 2000). The treatment of *TgUGT1* mice with ligands that activate the XenRs is a powerful tool to examine the role of these receptors in control and expression of the *UGT1A* genes, as the genes are regulated both through induction and tissue specificity (Chen et al., 2005;Verreault et al., 2006;Yueh and Tukey, 2007;Senekeo-Effenberger et al., 2007). The functional role of the human *UGT1A1* gene in homeostatic control of serum bilirubin was recently demonstrated in humanized *UGT1* mice, which expresses the *UGT1A* genes in a complete *Ugt1*-null background (Fujiwara et al., 2010). We undertook a similar approach to examine the regulation of the human *UGT2B7* gene.

The *UGT2B7* gene spans 16 kb on chromosome 4 (Monaghan et al., 1994). We generated *UGT2B7* transgenic mice (*TgUGT2B7*) with a bacterial artificial chromosome encoding the human *UGT2B7* gene. Tissue-specific expression demonstrated by transcriptional levels revealed that the pattern of expression in *TgUGT2B7* mice is comparable with what has been found for UGT2B7 expression in human tissues (Turgeon et al., 2001). Here we describe experiments which suggest functional inhibitory crosstalk between HNF 4α in liver of mice exposed to TCPOBOP, confirming a role for HNF 4α and CAR towards the regulation of *UGT2B7*.

Molecular Pharmacology Fast Forward. Published on March 17, 2011 as DOI: 10.1124/mol.110.070649
This article has not been copyedited and formatted. The final version may differ from this version.
MOL #70649

MATERIALS AND METHODS

Animals: The *TgUGT2B7* mice were generated at the University of California San Diego Superfund Research Program Mouse Genetics Core Facility. A bacterial artificial chromosome (BAC) encoding the *UGT2B7* gene (Genbank accession number RP13-644M16) was purified, microinjected into the pronucleus of CB6F1 mouse eggs, and transplanted into the oviduct of pseudopregnant C57BL/6N mice. For genotyping, DNA was isolated from tail clippings, and a 418-bp DNA fragment in exon 1 or a 292-bp DNA fragment in Exon 6 were identified by PCR (Exon 1 forward: 5' GATTAAGAGATGGTCAGACC, Exon 1 reverse: 5' CCACTTCTTCATGTCAAATATTTC; Exon 6 forward: AATTCAACATGATCAACCAGTG, Exon 6 reverse: GTCTCACCTATCAGGTTTTCC). Founders containing the *UGT2B7* gene were bred with *Car*-null mice (Dr. M Negishi, NIEHS, Research Triangle Park, NC), and *Tg(UGT2B7)Car*$^{+/-}$ mice backcrossed to produce *Tg(UGT2B7)Car*$^{-/-}$ mice (genotyping for *Car*-null mice as described previously) (Ueda et al., 2002). All animals received food and water *ad libitum* and were housed in constant temperature rooms with a 12hr light/12 hr dark cycle. Mouse handling and experimental procedures were conducted in accordance with institutional guidelines.

UGT2B7 promoter activity: A 4-kb *UGT2B7* promoter element was cloned by PCR from the BAC DNA containing the *UGT2B7* gene (Genbank accession number: PR13-644M16) and subcloned into a pGL3 luciferase reporter plasmid. The primers for PCR-cloning of the *UGT2B7* promoter element were: -4kb (forward KpnI: 5'ATTTGGTACCCAGTTCTCAGTA, reverse BglII: 5' atttagatc ttcagtctgacac); -2.8kb (forward KpnI: 5' atttggtac ctttgtgtgtcag,; reverse BglII: 5' aaagaagatcttctatgggta); -1.5kb (forward KpnI: 5' taaaggtac caacagtttcata, reverse BglIII: 5' tgacagatcttgtttctgcag); -0.4kb (forward KpnI: 5' attaggtac catgtttagtcatt, reverse BglII atttagatctggtgcaatgcaatg). Using the DNA fragment spanning from –1.0 kb to the translation start site (-1.0kb forward KpnI: 5' atttggtac ctaatgattaatgc, reverse XhoI: 5'

Molecular Pharmacology Fast Forward. Published on March 17, 2011 as DOI: 10.1124/mol.110.070649
This article has not been copyedited and formatted. The final version may differ from this version.
MOL #70649

attactcgagacatcctggtgcaa), site-directed mutagenesis was carried out, altering two bases (underlined) on the HNF4α core sequences (HNF4α mutant, forward: 5' tatgtactttgcattataagggtt, reverse: 5' aacccttataatgcaaagtacata). For transient transfection experiments, HepG2 cells were seeded on 12-well plates 24 hrs before transfection. Cells were transfected with luciferase plasmids along with either pcDNA (Invitrogen, Carlsbad CA), HNF4α-pcDNA, or VP-CAR expression vectors (Xie et al., 2003) using lipofectamine 2000 (Invitrogen) based on the manufacturer's instructions. Cells were harvested with a lysis buffer (Promega, WI) 48 hours after the transfection, and the supernatant was collected by a brief centrifugation. The promoter activities were measured by the expression of firefly luciferase and were normalized to the renilla luciferase levels using a dual luciferase reporter assay kit (Promega, WI).

Chromatin Immunoprecipitation (CHIP): CHIP analysis was performed using the modified protocol based on the EZ-CHIP kit (Upstate Biotechnology). HepG2 cells were transfected either with an HNF4α expression vector (HNF4α-pcDNA) or an HNF4α expression vector along with an activated CAR expression vector, VP-CAR (Xie et al., 2003). HepG2 cells were collected 48 hrs after the transfections 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, Upstate Biotechnology). One ml of cell extract in CHIP dilution buffer was pre-cleared by incubation with 60 µl of Protein A Agarose/Salmon sperm DNA (Upstate Biotechnology) overnight at 4 ^{o}C. The cleared cellular extract was incubated with anti-HNF4α antibody (Santa Crutz, CA) for 2 hr at 4 ^{o}C. Following precipitation with Protein A Agarose for 1h at 4 ^{o}C, the antibody-chromatin complex was then transferred to a spin column (Qiagen) for three 400 µl washes with each of the following buffers: low-salt immune complex wash buffer, high-salt immune complex wash buffer, LiCl immune complex wash buffer, high-salt LiCl immune complex wash buffer (Okino et al., 2007), and Tris-

Molecular Pharmacology Fast Forward. Published on March 17, 2011 as DOI: 10.1124/mol.110.070649
This article has not been copyedited and formatted. The final version may differ from this version.
MOL #70649

EDTA buffer. The protein-DNA complexes were eluted in 200 µl 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, the purified DNA was further analyzed by real time PCR with a pair of primers (HNF4α CHIP, forward 5': gtgtgaacagttcatttaccttc; HNF4α CHIP, reverse: 5' ctggtgcaatgcaatgctgt) for the amplification and quantification of the UGT2B7 promoter region containing the HNF4α binding site.

Quantification of UGT2B7 gene transcripts by real time PCR: Total RNA was isolated from tissues using Trizol (Invitrogen). One microgram of total RNA was used for the generation of cDNA with iScript cDNA synthesis kit (Bio-Rad, Hercules, CA). Following the cDNA synthesis, real time PCRs were conducted to determine Ct values using the MX4000 Multiplex QPCR (Stratagene, La Jolla CA). Briefly, one microlitter of the cDNA template from the RT-PCR reaction was used in a 20 µl of reaction mixture containing 10 µl of 2X MESA GREEN qPCR MasterMix (Eurogentec, San Diego) and 0.4 µM of a pair of primers for the detection of the mRNA of UGT2B7 or internal control gene cyclophilin (qPCR UGT2B7 forward: 5' gacttttggttcgaaatatttgaca, qPCR UGT2B7 reverse: 5' gaggaaactgaaaattccagg; qPCR cyclophilin forward: 5' cagacgccactgtcgcttt, qPCR cyclophilin reverse: 5' tgtctttgg aactttgtctgcaa). The thermal profile is the following: 95°C for 10 min, 40 Cycles of 95°C for 40 sec, 58°C for 40 sec, and 72°C for 60 sec. After the amplification cycles were completed, the dissociation curve was generated at 95°C for 1 min followed by a 41-dissociation cycle starting at 55°C and increasing by 1°C every 30 sec per cycle. Each sample was performed in triplicate and was quantified based on the formula $\Delta Ct = Ct_{(UGT2B7)} - Ct_{(cyclophilin)}$.

In vivo studies with TgUGT2B7 and Car-null mice: Age-matched groups of 8-10 week old animals were used for all experiments. Wild type, Tg-*UGT2B7*, *Car*$^{-/-}$ or *Tg(UGT2B7)Car*$^{-/-}$ (n= 3

Molecular Pharmacology Fast Forward. Published on March 17, 2011 as DOI: 10.1124/mol.110.070649
This article has not been copyedited and formatted. The final version may differ from this version.

or 4) mice were treated intraperitoneally every 24 hrs for 2 days with DMSO, PCN (10 mg/kg), dexamethasone (15 mg/kg), or TCPOBOP (4 mg/kg). All the chemicals were purchased from Sigma and dissolved in 100 µl DMSO for each injection. After 48 hrs, the liver tissues, from each treatment group, were pulverized in liquid nitrogen and used for preparation of microsomes and total RNA. Microsomal fractions for UGT2B7 catalytic assay were prepared as described previously (Yueh et al., 2003).

Glucuronidation activity assay. UDP-glucuronyltransferase activities were determined using HDCA and 4-hydroxylestrone as substrates by TLC assay according to the method of Bansal and Gessner with modification (Bansal and Gessner, 1980). Briefly, liver tissues were homogenized in a five-fold volume of 1.15% ice-cold KCl and microsomal fractions were prepared in buffer (50 mM Tris-HCl (pH 7.6), and 10 mM $MgCl_2$) as described previously (Yueh et al., 2003). Each UGT assay was in a total volume of 100 µl reaction mixture containing 50 mM Tris-HCl (pH 7.6), 10 mM $MgCl_2$, 100 µM substrate, 500 µM uridine 5-diphosphoglucuronic acid (UDPGA), 0.04 µCi of UDP[^{14}C]glucuronic acid, 8.5 mM saccharolactone and 75 µg of microsomal protein. The reactions were performed at 37^{o}C in a shaking water bath for 45 min. At the end of the reaction, 100 µl of ethanol was added and the cell debris was pelleted by centrifugation. The supernatant was applied to TCL plates and chromatography performed in a mixture of (35:35:10:20 v/v) of n-butanol:acetone:acetic acid:water. The resulting glucuronides were visualized with a phosphorimager (Molecular Dynamics Storm 820) and were removed and placed in scintillation fluid for quantification with a liquid scintillation counter (Beckman Instruments, Palo Alto, CA).

Reagents: The BAC DNA containing *UGT2B7* gene (PR13-644M16) was from Children's Hospital Oakland Research Institute (CHORI). 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene

Molecular Pharmacology Fast Forward. Published on March 17, 2011 as DOI: 10.1124/mol.110.070649
This article has not been copyedited and formatted. The final version may differ from this version.
MOL #70649

(TCPOBOP), pregnenolone-16α-carbonitrile (PCN), dexamethasone, and DMSO were from Sigma. Restriction enzymes and T4 DNA ligase for subcloning were from New England Biolabs. The Bradford assay for protein concentration analysis was from Bio-Rad. Taq polymerase, the dual-luciferase reporter assay system and reporter plasmids, pGL3-basic vector, pGL3 promoter vector and pRL-SV40 vector were from Promega (Madison, WI). The expression vector for HNF4α (pcDNA-HNF4α) was a kind gift provided by Dr. Barbier at Laval University Hospital Research Center, Quebec, Canada. The construct for the expression vector VP-CAR was described previously (Xie et al., 2003). Thin-layer chromatography plates for the catalytic assay were from Whatman (Clifton, NJ).

Molecular Pharmacology Fast Forward. Published on March 17, 2011 as DOI: 10.1124/mol.110.070649
This article has not been copyedited and formatted. The final version may differ from this version.
MOL #70649

RESULTS

Expression of UGT2B7 in transgenic mice

The organization of the *UGT2B7* gene in the BAC DNA, consisting of a 5' promoter region and 6 exons and introns, is shown in Fig 1. The BAC clone was purified and microinjected into fertilized CB6F1 mouse eggs, and *TgUGT2B7* transgenic mice were produced. The genotype analysis from tail DNA identified founders carrying sequences of exons 1 through 6. Three founders were used for breeding experiments to generate F1 progeny.

To determine if expression of the human gene in liver produced an intact mRNA, highly specific oligonucleotides were used to clone from reverse transcriptase product the full length UGT2B7 RNA into pcDNA followed by expression in COS-1 cells. Cell lysates prepared from UGT2B7 pcDNA transfected COS-1 cells displayed catalytic activity towards hyodeoxycholic acid (Fig 2A), a known substrate for UGT2B7. Enhanced levels of HDCA glucuronidation in liver microsomes from *TgUGT2B7* mice when compared to wild-type mice were also observed (Figure 2B), confirming that expression of the *UGT2B7* gene in transgenic mice produces a functional gene transcript.

Examination of the constitutive expression pattern of the *UGT2B7* gene was conducted by reverse transcription followed by PCR (RT-PCR) with UGT2B7 specific oligonucleotides to assess gene expression profiles. The oligonucleotides used in these experiments did not amplify gene transcripts from wild-type mouse liver RNA. Total RNA from different tissues was isolated from both male and female *TgUGT2B7* mice. The intense *UGT2B7* gene transcript was observed in liver and kidney tissues with liver being the most prominent (Fig 3). Lower levels of *UGT2B7* gene expression products were shown in large and small intestines, adipose tissue, brain, muscle, ovary, and uterus. When we quantitated *UGT2B7* gene expression using real time-PCR procedures, the expression levels matched the intensity of the banding patterns observed by RT-PCR. In experiments using human tissues, it has been demonstrated that the

Molecular Pharmacology Fast Forward. Published on March 17, 2011 as DOI: 10.1124/mol.110.070649
This article has not been copyedited and formatted. The final version may differ from this version.
MOL #70649

UGT2B7 is expressed abundantly in various tissues including liver, kidney, small intestine, large intestine, mammary gland, and uterus (Ohno and Nakajin, 2009;Turgeon et al., 2001;Izukawa et al., 2009) . O verall, the ti ssue ex pression pr ofile of the *UGT2B7* gene i n tr ansgenic mi ce corresponds well with that of humans indicating that the *TgUGT2B7* mice could be useful as an *in vivo* model to characterize *UGT2B7* gene expression.

Regulation of hepatic UGT2B7 expression by PXR or CAR ligands:

The effec t of PX R and CAR ac tivation of the *UGT2B7* gene i n *TgUGT2B7* mic e w as evaluated fol lowing tr eatment w ith the P XR l igand PCN (10 mg/k g) or the CAR l igand TCPOBOP (4 mg/k g). F ollowing a dministration by the i .p. r oute, Q -RT-PCR anal ysis to quantitate *UGT2B7* gene ex pression w as c onducted w ith RNA pr epared fr om l iver. PCN, a prototypical ligand of murine PXR, produced no effe ct on *UGT2B7* gene e xpression. However, treatment with TCPOBOP, a potent l igand of the mous e CAR, i nhibited hepatic *UGT2B7* gene expression (Fig 4A).

In effor ts to deter mine i f CAR i s ti ed to r egulation o f the *UGT2B7* gen e, w e c rossed *TgUGT2B7* mice with *Car*$^{-/-}$ mice to generate *Tg(UGT2B7)Car*$^{-/-}$ mice. Wild- type, *TgUGT2B7*, or *Tg(UGT2B7)Car*$^{-/-}$ mice were treated with either DMSO or TCPOBOP. RNA was prepared from liver t issues an d the levels of *UGT2B7* gene ex pression quanti tated b y Q-RT-PCR. When compared w ith *TgUGT2B7* mi ce, the i nterruption of the *Car* gene i n DMSO -treated *Tg(UGT2B7)Car*$^{-/-}$ mice produced no change in *UGT2B7* gene ex pression (Fig 4B). TCPOBOP treatment to *TgUGT2B7* mice resulted in over an 80% reduction in gene expression. However, when *Tg(UGT2B7)Car*$^{-/-}$ mi ce w ere tr eated w ith T CPOBOP, *UGT2B7* gene ex pression remained unc hanged and w ere c omparable to untr eated mi ce. *Cyp2b10* gene ex pression, a well known TCPOBOP-inducible CAR target gene, were substantially increased by treatment of TCPOBOP in *TgUGT2B7* mi ce but n ot *Tg(UGT2B7)Car*$^{-/-}$ mice (Fig 4C). Overall, these studies demonstrate that CAR functions as a negative regulator of the *UGT2B7* gene in liver.

Molecular Pharmacology Fast Forward. Published on March 17, 2011 as DOI: 10.1124/mol.110.070649
This article has not been copyedited and formatted. The final version may differ from this version.
MOL #70649

Hepatocyte nuclear factor 4α (HNF4α) is crucial for constitutive UGT2B7 expression in liver:

To study the molecular mechanisms that control constitutive expression of *UGT2B7* in liver, 4kb of the *UGT2B7* promoter was cloned from the BAC DNA and subsequently subcloned into a luciferase reporter plasmid. HepG2 cells were transfected with the *UGT2B7* promoter luciferase plasmids and high promoter activity was observed in the 400 bp proximal promoter region (-367/+12) adjacent to the transcription start site (Fig 5). Sequence analysis indicated there is one consensus DR1 core sequence (TGTACT X TGACTT) for HNF4α binding within this region. When HepG2 cells were co-transfected with both a -0.4 kb *UGT2B7* promoter-containing reporter plasmid (-0.4 kb/+0) and an HNF4α expression vector, the promoter activity was induced significantly, suggesting the presence of an HNF4α binding site in this region (Fig 5A). A two-base mutation in the DR1 core sequence blocked HNF4α-mediated transactivation, confirming the involvement of HNF4α in constitutive *UGT2B7* promoter activity (Fig 5B).

To explore the suppressive effect by CAR activation, HepG2 cells were transfected with an HNF4α expression vector with or without cotransfection of a CAR expression vector (VP-CAR). Interestingly, transfection with HNF4α alone increased promoter activity, and co-transfection of VP-CAR produced suppression of promoter activity (Fig 5C). Similar results were observed when HNF4α transfected HepG2 cells were co-transfected with a CAR expression vector and treated with TCPOBOP for 48 hours, indicating that CAR might interact with HNF4α and inhibit HNF4α-directed transactivation. To gain further insight into the possible interaction of CAR and HNF4α in regulating *UGT2B7* transcription, HepG2 cells were transfected with an HNF4α expression vector with or without VP-CAR co-transfection followed by chromatic immunoprecipitation (CHIP) analysis. In CHIP studies using an HNF4α antibody, the precipitation of the DR1 element that contains the HNF4α binding site (-181/+11), quantitated by real time PCR, was decreased in VP-CAR co-transfected HepG2 cells (Fig 6), indicating that the

Molecular Pharmacology Fast Forward. Published on March 17, 2011 as DOI: 10.1124/mol.110.070649
This article has not been copyedited and formatted. The final version may differ from this version.

inhibition of HNF4α by CAR requires the inhibition in the binding of HNF4α to the direct repeat 1 site in the *UGT2B7* promoter.

Molecular Pharmacology Fast Forward. Published on March 17, 2011 as DOI: 10.1124/mol.110.070649
This article has not been copyedited and formatted. The final version may differ from this version.
MOL #70649

DISCUSSION

Recent studies have indicated that the *UGT2B7* gene plays an important role in drug metabolism and steroid homeostasis (Barbier et al., 2000;Coffman et al., 1998;Thibaudeau et al., 2006). The concern of species differences and lack of comprehensive knowledge regarding rodent *UGT* gene families prompted us to create a transgenic animal model containing a full length human *UGT2B7* gene. The present study delineates the use of this transgenic animal model to study the regulatory properties of the *UGT2B7* gene. The expression pattern of UGT2B7 in various organs in *TgUGT2B7* mice indicates that humoral and transcription factors meditating *UGT2B7* gene expression resemble those patterns found in humans. The observation that liver tissue had the highest expression levels of UGT2B7 suggested that liver-specific factors were required for physiological transcriptional responses. It has been shown that HNF4α plays an important role in regulating hepatic expression of phase II enzymes and transporters in mice (Lu et al., 2010). We provide evidence that HNF4α is the contributing factor responsible for constitutive expression of hepatic UGT2B7. HNF4α regulates *UGT2B7* gene expression by binding to a direct repeat motif of the AGGTCA sequence separated by one nucleotide (DR1) in the *UGT2B7* 5' flanking promoter region. The HNF4α specificity and requirement for *UGT2B7* gene activation was further confirmed by mutation of the DR1 core sequence, which eliminated the binding of HNF4α to the promoter and abolished promoter activity. Similar to our findings, mice lacking hepatic HNF4α had significantly lower gene expression of *Ugt2b1* when compared with wild type mice (Lu et al., 2010) indicating that both hepatic expressions of human *UGT2B7* and mouse *Ugt2b1* are controlled by HNF4α.

It is well documented that XenRs, PXR and CAR, act as xenobiotic sensors and mediate induction of numerous xenobiotic metabolizing enzymes. Induction of glucuronidation by xenobiotic receptors has been demonstrated using a number of clinical drugs and endogenous compounds. For example, CAR is a strong inducer of *UGT1A1* (Xie et al., 2003;Huang et al.,

Molecular Pharmacology Fast Forward. Published on March 17, 2011 as DOI: 10.1124/mol.110.070649
This article has not been copyedited and formatted. The final version may differ from this version.
MOL #70649

2003), which proceeds through binding to a phenobarbital response element flanking the *UGT1A1* gene promoter. We were surprised to observe that TCPOBOP treatment and activation of CAR in *TgUGT2B7* mice led to a reduction in *UGT2B7* gene expression. The specificity of CAR-mediated regulation is supported by findings that PXR-specific ligands, such as PCN and dexamethasone, had no effect on the repression of *UGT2B7* transcription in transgenic mice. Combined with evidence that overexpression of CAR produced a decrease in promoter activity of HNF4α transactivation in HepG2 cells, *UGT2B7* seems to be a candidate gene for CAR-associated transcriptional inhibition. In addition, the role for HNF4α in CAR-mediated inhibition of *UGT2B7* expression was validated as CHIP assays revealed that CAR activation reduced HNF4α bound to the *UGT2B7* chromatin. Activation of CAR inhibited HNF4α transactivation of *UGT2B7* gene, which suggested that these two regulators are able to cross talk in the regulation of *UGT2B7* expression. Finally, the use of *Car*-null mice proved that the suppressive effect of TCPOBOP is linked to CAR, which acts as a transcriptional repressor in response to chemical activation by TCPOBOP and blocks HNF4α activation of *UGT2B7* gene expression. By inhibiting HNF4α binding, CAR may prevent the changes in chromatin structure and consequent activation of *UGT2B7* by HNF4α. In comparison with the antagonism between HNF4α and CAR for *UGT2B7* gene regulation, a previous study showed that HNF4α inhibited PXR-mediated transactivation of CYP7A1 gene (Bhalla et al., 2004). The activated PXR did not affect the binding of HNF4α to CYP7A1. Instead, the association of HNF4α with cofactor PGC-1 (peroxisome proliferator activating receptor coactivator 1) bound to the promoter was inhibited. HNF4α-dependent transactivation of *UGT2B7* gene is mediated through the response element of the HNF4α binding site in the promoter region, and a two-base change in the response element drastically reduces the ability of HNF4α to bind DNA. When acting as a positive regulator, CAR binds to the regulatory region of the target genes. Without a functional binding site in the *UGT2B7* promoter region, CAR is able to interact with HNF4α through a yet-to-be

Molecular Pharmacology Fast Forward. Published on March 17, 2011 as DOI: 10.1124/mol.110.070649
This article has not been copyedited and formatted. The final version may differ from this version.
MOL #70649

identified mechanism that possibly involves contact with other associated transcription factors and cofactors which are specifically associated with the UGT2B7 promoter region. For example, CAR could be inhibitory by competing for binding to common coactivators for HNF4α, such as PGC-1. Thus, the UGT2B7 specific regulation of HNF4α and CAR may largely depend on the promoter context.

UGT2B7 regulation at the transcription level is largely unstudied. Recently, using human Caco-2 cells, UGT2B7 suppression by lithocholic acid was linked to negative regulation by farnesoid X receptor (FXR) (Lu et al., 2005a). Similarly, retinoids (i.e., all trans retinoic acid and 9-cis retinoic acid) were shown to inhibit UGT2B7 mRNA expression in this intestinal cell line. The fact that both lithocholic acid and retinoids are recognized as activators of CAR (Sakai et al., 2006;Chen et al., 2010) leads us to speculate that UGT2B7 down regulation in these human intestinal cells might be partially caused by CAR activation. This down regulation of CAR-dependent UGT2B7 gene expression might have implications in metabolism of therapeutic agents destined for glucuronidation by UGT2B7. Furthermore, CAR activation may lead to changes in the steady-state dynamics of steroids and bile acid homeostasis. A growing body of evidence shows the inhibitory effect of CAR on genes involved in hepatic glucose and lipid metabolism, bile acid biosynthesis (Ueda et al., 2002), such as phosphoenolpyruvate carboxykinase 1 (PEPCK1), glucose-6-phosphatase (G6P), and CYP7A1 activity (Miao et al., 2006). In combination, these studies implicate a diverse function of CAR as a negative regulator of genes associated with drug and xenobiotic, glucose, and lipid metabolism. Compared with DMSO-treated *Car*$^{-/-}$ and wild-type mice, TCPOBOP-treated *Car*$^{-/-}$ mice exhibited higher *UGT2B7* gene expression (~150%, Fig 7A); this finding is consistent with results from a number of other investigations (Bell and Michalopoulos, 2006;Tamasi et al., 2009) in which an induction of HNF4α by phenobarbital in the absence of CAR was observed. It is possible that TCPOBOP

Molecular Pharmacology Fast Forward. Published on March 17, 2011 as DOI: 10.1124/mol.110.070649
This article has not been copyedited and formatted. The final version may differ from this version.

is abl e to i nfluence HNF 4α ac tivity i n the abs ence of CAR, w ith i nduced l evels of HNF 4α contributing to greater *UGT2B7* gene expression.

Molecular Pharmacology Fast Forward. Published on March 17, 2011 as DOI: 10.1124/mol.110.070649
This article has not been copyedited and formatted. The final version may differ from this version.

ACKNOWLEGEMENTS

The authors wish to thank Autumn Bonner for assisting in formatting and assembling this manuscript.

Molecular Pharmacology Fast Forward. Published on March 17, 2011 as DOI: 10.1124/mol.110.070649
This article has not been copyedited and formatted. The final version may differ from this version.

AUTHORSHIP CONTRIBUTIONS

Participated in research design: Yeuh, MF, Mellon, PL, and Tukey, RH

Conducted experiments: Yueh, MF

Contributed new reagents or analytical tools: Yueh, MF and Mellon PL

Performed data analysis: Yueh, MF

Wrote or contributed to the writing of the manuscript: Yeuh, MF and Tukey RH

Molecular Pharmacology Fast Forward. Published on March 17, 2011 as DOI: 10.1124/mol.110.070649
This article has not been copyedited and formatted. The final version may differ from this version.

REFERENCES

Bansal SK and Gessner T (1980) A Unified Method for the Assay of Uridine Diphosphoglucuronyltransferase Activities Toward Various Aglycones Using Uridine Diphospho[U-14C]Glucuronic Acid. *Anal Biochem* **109**:321-329.

Barbier O, Girard C, Breton R, Belanger A and Hum D W (2000) N-Glycosylation and Residue 96 Are Involved in the Functional Properties of UDP-Glucuronosyltransferase Enzymes. *Biochemistry* **39**:11540-11552.

Bell AW and Michalopoulos G K (2006) Phenobarbital Regulates Nuclear Expression of HNF-4alpha in Mouse and Rat Hepatocytes Independent of CAR and PXR. *Hepatology* **44**:186-194.

Bhalla S, Ozalp C, Fang S, Xiang L and Kemper J K (2004) Ligand-Activated Pregnane X Receptor Interferes With HNF-4 Signaling by Targeting a Common Coactivator PGC-1alpha. Functional Implications in Hepatic Cholesterol and Glucose Metabolism. *J Biol Chem* **279**:45139-45147.

Chen S, Beaton D, Nguyen N, Senekeo-Effenberger K, Brace-Sinnokrak E, Argikar U, Remmel R P, Trottier J, Barbier O, Ritter J K and Tukey R H (2005) Tissue-Specific, Inducible, and Hormonal Control of the Human UDP-Glucuronosyltransferase-1 (UGT1) Locus. *J Biol Chem* **280**:37547-37557.

Chen S, Wang K and Wan Y J (2010) Retinoids Activate RXR/CAR-Mediated Pathway and Induce CYP3A. *Biochem Pharmacol* **79**:270-276.

Coffman BL, King C D, Rios G R and Tephly T R (1998) The Glucuronidation of Opioids, Other Xenobiotics, and Androgens by Human UGT2B7Y(268) and UGT2B7H(268). *Drug Metab Dispos* **26**:73-77.

Fujiwara R, Nguyen N, Chen S and Tukey R H (2010) Developmental Hyperbilirubinemia and CNS Toxicity in Mice Humanized With the UDP Glucuronosyltransferase 1 (UGT1) Locus. *Proc Natl Acad Sci U S A* **107**:5024-5029.

Gregory PA, Lewinsky R H, Gardner-Stephen D A and Mackenzie P I (2004) Regulation of UDP Glucuronosyltransferases in the Gastrointestinal Tract. *Toxicol Appl Pharmacol* **199**:354-363.

Huang W, Zhang J, Chua S S, Qatanani M, Han Y, Granata R and Moore D D (2003) Induction of Bilirubin Clearance by the Constitutive Androstane Receptor (CAR). *Proc Natl Acad Sci U S A* **100**:4156-4161.

Izukawa T, Nakajima M, Fujiwara R, Yamanaka H, Fukami T, Takamiya M, Aoki Y, Ikushiro S, Sakaki T and Yokoi T (2009) Quantitative Analysis of UDP-Glucuronosyltransferase (UGT) 1A and UGT2B Expression Levels in Human Livers. *Drug Metab Dispos* **37**:1759-1768.

Lu H, Gonzalez F J and Klaassen C (2010) Alterations in Hepatic MRNA Expression of Phase II Enzymes and Xenobiotic Transporters After Targeted Disruption of Hepatocyte Nuclear Factor 4 Alpha. *Toxicol Sci* **118**:380-390.

Lu Y, Heydel J M, Li X, Bratton S, Lindblom T and Radominska-Pandya A (2005a) Lithocholic Acid Decreases Expression of UGT2B7 in Caco-2 Cells: A Potential Role for a Negative Farnesoid X Receptor Response Element. *Drug Metab Dispos* **33**:937-946.

Lu Y, Bratton S, Heydel J M and Radominska-Pandya A (2008) Effect of Retinoids on UDP-Glucuronosyltransferase 2B7 MRNA Expression in Caco-2 Cells. *Drug Metab Pharmacokinet* **23**:364-372.

Lu Y, Heydel J M, Li X, Bratton S, Lindblom T and Radominska-Pandya A (2005b) Lithocholic Acid Decreases Expression of UGT2B7 in Caco-2 Cells: a Potential Role for a Negative Farnesoid X Receptor Response Element. *Drug Metab Dispos* **33**:937-946.

Mackenzie PI, Walter B K, Burchell B, Guillemette C, Ikushiro S, Iyanagi T, Miners J O, Owens I S and Nebert D W (2005) Nomenclature Update for the Mammalian UDP Glycosyltransferase (UGT) Gene Superfamily. *Pharmacogenet Genomics* **15**:677-685.

Miao J, Fang S, Bae Y and Kemper J K (2006) Functional Inhibitory Cross-Talk Between Constitutive Androstane Receptor and Hepatic Nuclear Factor-4 in Hepatic Lipid/Glucose Metabolism Is Mediated by Competition for Binding to the DR1 Motif and to the Common Coactivators, GRIP-1 and PGC-1alpha. *J Biol Chem* **281**:14537-14546.

Monaghan G, Clarke D J, Povey S, See C G, Boxer M and Burchell B (1994) Isolation of a Human YAC Contig Encompassing a Cluster of *UGT2* Genes and Its Regional Localization to Chromosome 4q13. *Genomics* **23**:496-499.

Ohno S and Nakajin S (2009) Determination of MRNA Expression of Human UDP-Glucuronosyltransferases and Application for Localization in Various Human Tissues by Real-Time Reverse Transcriptase-Polymerase Chain Reaction. *Drug Metab Dispos* **37**:32-40.

Okino ST, Quattrochi L C, Pookot D, Iwahashi M and Dahiya R (2007) A Dioxin-Responsive Enhancer 3' of the Human CYP1A2 Gene. *Mol Pharmacol* **72**:1457-1465.

Ormrod D, Holm K, Goa K and Spencer C (1999) Epirubicin: a Review of Its Efficacy As Adjuvant Therapy and in the Treatment of Metastatic Disease in Breast Cancer. *Drugs Aging* **15**:389-416.

Ritter JK, Chen F, Sheen Y Y, Lubet R A and Owens I S (1992) Two Human Liver CDNAs Encode UDP-Glucuronosyltransferases With 2 Log Differences in Activity Toward Parallel Substrates Including Hyodeoxycholic Acid and Certain Estrogen Derivatives. *Biochemistry* **31**:3409-3414.

Sakai H, Iwata H, Kim E Y, Tsydenova O, Miyazaki N, Petrov E A, Batoev V B and Tanabe S (2006) Constitutive Androstane Receptor (CAR) As a Potential Sensing Biomarker of Persistent Organic Pollutants (POPs) in Aquatic Mammal: Molecular Characterization, Expression Level, and Ligand Profiling in Baikal Seal (Pusa Sibirica). *Toxicol Sci* **94**:57-70.

Samokyszyn VM, Gall W E, Zawada G, Freyaldenhoven M A, Chen G, Mackenzie P I, Tephly T R and Radominska-Pandya A (2000) 4-Hydroxyretinoic Acid, a Novel Substrate for Human Liver Microsomal UDP-Glucuronosyltransferase(s) and Recombinant UGT2B7. *J Biol Chem* **275**:6908-6914.

Senekeo-Effenberger K, Chen S, Brace-Sinnokrak E, Bonzo J A, Yueh M F, Argikar U, Kaeding J, Trottier J, Remmel R P, Ritter J K, Barbier O and Tukey R H (2007) Expression of the Human UGT1 Locus in Transgenic Mice by 4-Chloro-6-(2,3-Xylidino)-2-Pyrimidinylthioacetic Acid (WY-14643) and Implications on Drug Metabolism Through Peroxisome Proliferator-Activated Receptor {Alpha} Activation. *Drug Metab Dispos* **35**:419-427.

Strassburg CP, Manns M P and Tukey R H (1997a) Differential Down Regulation of the *UDP-Glucuronosyltransferase 1A* Locus Is an Early Event in Human Liver and Biliary Cancer. *Cancer Res* **57**:2979-2985.

Strassburg CP, Oldhafer K, Manns M P and Tukey R H (1997b) Differential Expression of the UGT1A Locus in Human Liver, Biliary, and Gastric Tissue: Identification of UGT1A7 and UGT1A10 Transcripts in Extrahepatic Tissue. *Mol Pharmacol* **52**:212-220.

Tamasi V, Juvan P, Beer M, Rozman D and Meyer U A (2009) Transcriptional Activation of PPARalpha by Phenobarbital in the Absence of CAR and PXR. *Mol Pharm* **6**:1573-1581.

Thibaudeau J, Lepine J, Tojcic J, Duguay Y, Pelletier G, Plante M, Brisson J, Tetu B, Jacob S, Perusse L, Belanger A and Guillemette C (2006) Characterization of Common UGT1A8, UGT1A9, and UGT2B7 Variants With Different Capacities to Inactivate Mutagenic 4-Hydroxylated Metabolites of Estradiol and Estrone. *Cancer Res* **66**:125-133.

Toide K, Takahashi Y, Yamazaki H, Terauchi Y, Fujii T, Parkinson A and Kamataki T (2002) Hepatocyte Nuclear Factor-1alpha Is a Causal Factor Responsible for Interindividual Differences in the Expression of UDP-Glucuronosyltransferase 2B7 MRNA in Human Livers. *Drug Metab Dispos* **30**:613-615.

Tukey RH and Strassburg C P (2000) Human UDP-Glucuronosyltransferases: Metabolism, Expression, and Disease. *Annu Rev Pharmacol Toxicol* **40**:581-616.

Turgeon D, Carrier J S, Levesque E, Hum D W and Belanger A (2001) Relative Enzymatic Activity, Protein Stability, and Tissue Distribution of Human Steroid-Metabolizing UGT2B Subfamily Members. *Endocrinology* **142**:778-787.

Ueda A, Hamadeh H K, Webb H K, Yamamoto Y, Sueyoshi T, Afshari C A, Lehmann J M and Negishi M (2002) Diverse Roles of the Nuclear Orphan Receptor CAR in Regulating Hepatic Genes in Response to Phenobarbital. *Mol Pharmacol* **61**:1-6.

Verreault M, Senekeo-Effenberger K, Trottier J, Bonzo J A, Belanger J, Kaeding J, Staels B, Caron P, Tukey R H and Barbier O (2006) The Liver X-Receptor Alpha Controls Hepatic Expression of the Human Bile Acid-Glucuronidating UGT1A3 Enzyme in Human Cells and Transgenic Mice. *Hepatology* **44**:368-378.

Williams JA, Hyland R, Jones B C, Smith D A, Hurst S, Goosen T C, Peterkin V, Koup J R and Ball S E (2004) Drug-Drug Interactions for UDP-Glucuronosyltransferase Substrates: A Pharmacokinetic Explanation for Typically Observed Low Exposure (AUCI/AUC) Ratios. *Drug Metab Dispos* **32**:1201-1208.

Xie W, Yeuh M F, Radominska-Pandya A, Saini S P S, Negishi Y, Bottroff B S, Cabrera G Y, Tukey R H and Evans R M (2003) Control of Steroid, Heme, and Carcinogen Metabolism by Nuclear Pregnane X Receptor and Constitutive Androstane Receptor. *Proc Natl Acad Sci USA* **100**:4150-4155.

Yueh MF, Huang Y H, Hiller A, Chen S J, Nguyen N and Tukey R H (2003) Involvement of the Xenobiotic Response Element (XRE) in Ah Receptor-Mediated Induction of Human UDP-Glucuronosyltransferase 1A1. *Journal of Biological Chemistry* **278**:15001-15006.

Yueh MF and Tukey R H (2007) Nrf2-Keap1 Signaling Pathway Regulates Human UGT1A1 Expression in Vitro and in Transgenic UGT1 Mice. *J Biol Chem* **282**:8749-8758.

Molecular Pharmacology Fast Forward. Published on March 17, 2011 as DOI: 10.1124/mol.110.070649
This article has not been copyedited and formatted. The final version may differ from this version.

FOOTNOTES

This work was supported by the National Institute of Environmental Health Sciences [P42ES010337] and the National Institute of General Medicine [GM086713].

Molecular Pharmacology Fast Forward. Published on March 17, 2011 as DOI: 10.1124/mol.110.070649
This article has not been copyedited and formatted. The final version may differ from this version.
MOL #70649

LEGENDS FOR FIGURES

Fig 1. The gene arrangement of UGT2B7 in the BAC DNA: A 156 kb-bacterial artificial chromosome encoding the *UGT2B7* gene locus was used to generate the UGT2B7 transgenic mice. The drawing is a representation of the *UGT2B7* gene locus, ranging from 48 kb to 64 kb, in the BAC clone with 6 black boxes as 6 exons.

Fig 2. Determination of UGT activity: (A) RNA from *TgUGT2B7* mouse liver was isolated, reverse transcribed to cDNA, subcloned into a pcDNA3 expression vector, and heterologously expressed in COS-1 cells by transient transfections. Following preparation of cell lysates, UGT activity was determined using HDCA as a substrate. UGT1A4 specific substrate amitriptyline was as a negative control substrate. (B) UGT activity was determined in liver microsomes prepared from *TgUGT2B7* and wild type mice (n=4) using HDCA as substrate.

Fig 3. Tissue distribution of UGT2B7 transcript: Tissues from female and male *TgUGT2B7* mice were used to prepare total RNA. The UGT2B7 gene expression levels in various tissues were examined by RT-PCR (female tissues) and real time-PCR using oligonucleotides specific for *UGT2B7* gene products and normalized to cyclophilin RNA. The specificity of the PCR product was confirmed by direct sequence.

Fig 4. Inhibition of UGT2B7 expression by CAR ligand TCPOBOP and reversion of UGT2B7 inhibition in *Car* null mice: (A) Age-matched *TgUGT2B7* mice were treated with either DMSO, CAR ligand TCPOBOP or PXR ligand PCN by i.p. injection for 48 hrs. The liver tissues were used for preparation of total RNA. Following the reverse transcription for cDNA synthesis, real-time PCR was conducted to determine Ct value with cyclophilin as an internal control gene. (B) *TgUGT2B7*, *Tg(UGT2B7)Car*$^{-/-}$, and wild type mice were treated with DMSO or TCPOBOP by i.p.

Molecular Pharmacology Fast Forward. Published on March 17, 2011 as DOI: 10.1124/mol.110.070649
This article has not been copyedited and formatted. The final version may differ from this version.
MOL #70649

injection for 48 hrs. RNA was isolated from the liver tissues and the levels of UGT2B7 mRNA were measured by real time PCR. (C) The levels of mouse Cyp2b10 mRNA in liver tissues of treated mice were examined by RT-PCR (Cyp2b10 forward: 5' aaagtcccgtggcaacttcc, Cyp2b10 reverse: 5' catcccaaagtctctcatgg).

Fig 5. Transactivation of UGT2B7 promoter by HNF4α and inhibition of HNF4α-mediated transactivation by CAR: A 4 kb of the *UGT2B7* promoter was cloned, divided into 4 fragments, and subcloned into the luciferase reporter plasmids, pGL3 basic vector (BV) or promoter vector (PV). HepG2 cells were transiently transfected with *UGT2B7* promoter-containing reporter plasmids, and luciferase activity was determined in the cytosolic fraction 48 hrs after transfections. (A) *UGT2B7* promoter activities were compared between cotransfection with a pcDNA plasmid or a HNF4α-containing expression vector and values were normalized to renilla luciferase activity by using a luciferase dual assay kit (Promega) and were shown as fold induction. (B) Two bases were mutated, from AC to CA, in DR1-like core sequence within the *UGT2B7* promoter region (*UGT2B7* promoter -1.0 kb/+0) by PCR-directed mutagenesis. The luciferase reporter plasmids containing either wild type or mutated DR1 were transiently transfected into HepG2 cells. The promoter activities were normalized to renilla luciferase activity and shown as firefly luciferase levels. (C) HepG2 cells were transfected with the reporter plasmid containing the *UGT2B7* promoter region (*UGT2B7* promoter -1.0 kb/+0) and cotransfected with HNF4α, VP-CAR, or HNF4α plus VP-CAR. Forty eight hours following transfection, firefly luciferase activity was determined and values were normalized to renilla luciferase activity.

Molecular Pharmacology Fast Forward. Published on March 17, 2011 as DOI: 10.1124/mol.110.070649
This article has not been copyedited and formatted. The final version may differ from this version.
MOL #70649

Fig 6. Chromatic immunoprecipitation analysis of HNF4α associated with the *UGT2B7* 5' flanking region. HepG2 cells were either transfected with a HNF 4α expression vector or cotransfected with an activated CAR expression vector (VP-CAR). Transfected HepG2 cells were collected 48 hr after the transfections. Cells were fixed and sonicated for the preparation of sheared chromatin, and immunoprecipitations were performed using HNF 4α antibody, or nonspecific IgG, as a negative control. Following immunoprecipitation, associated DNA was amplified with a pair of primers targeting *UGT2B7* gene region -181 to +11, quantitated by real time PCR, and displayed by gel electorphorisis. Input and western blot of HNF4α indicate equal amounts of lysates used prior to immunoprecipitation.

Fig 1

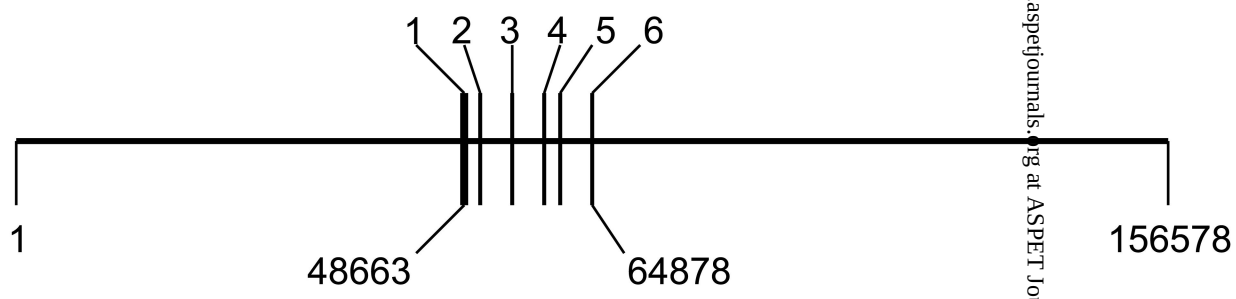


Fig 2A

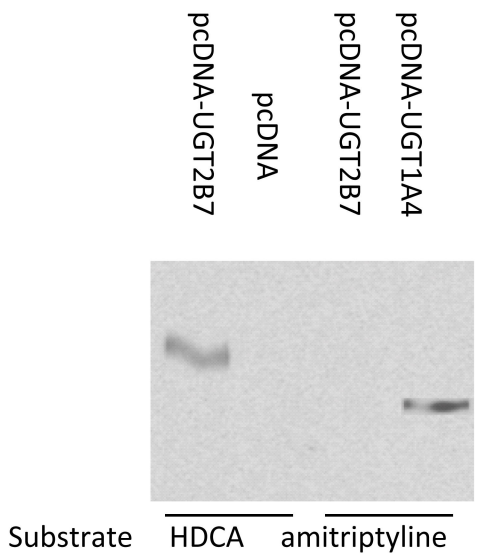


Fig 2B

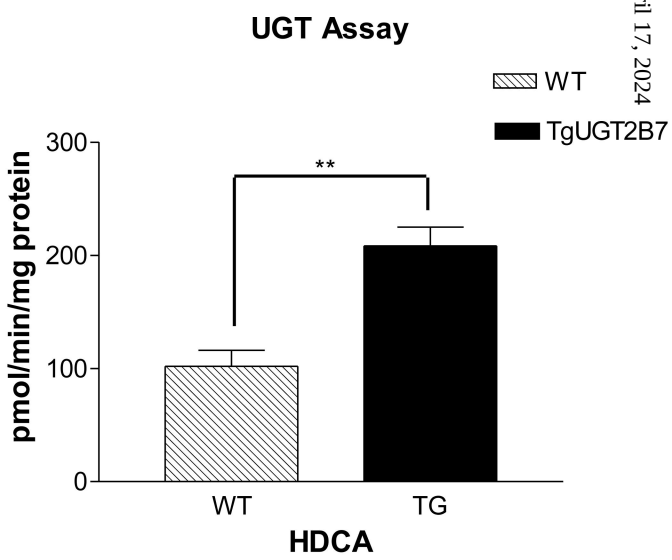
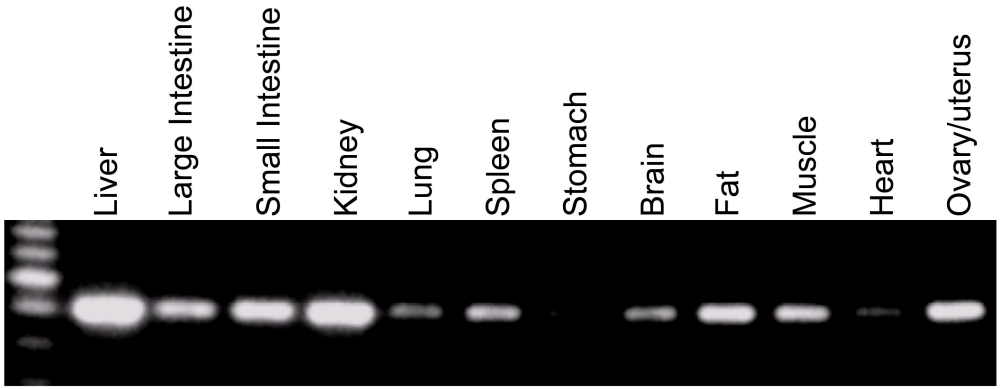
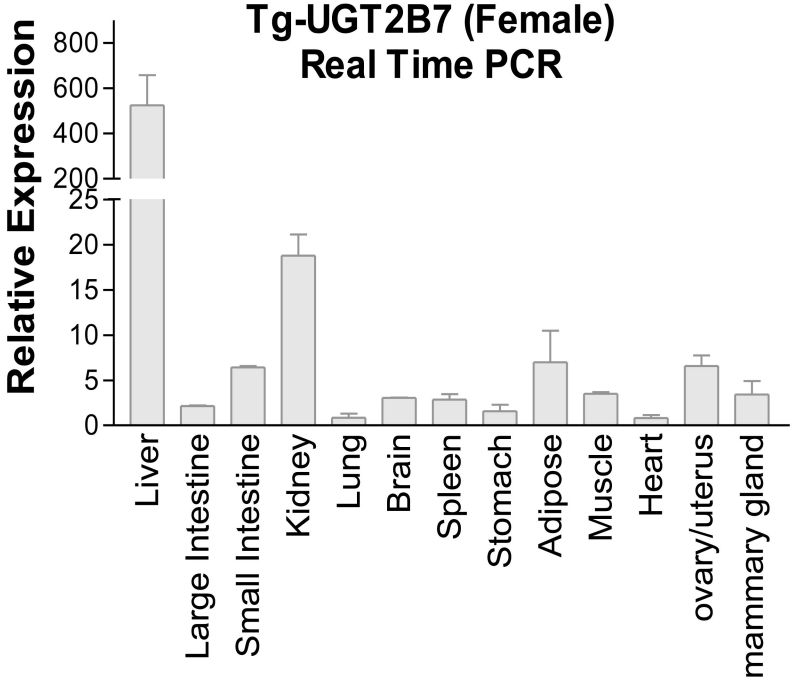


Fig 3





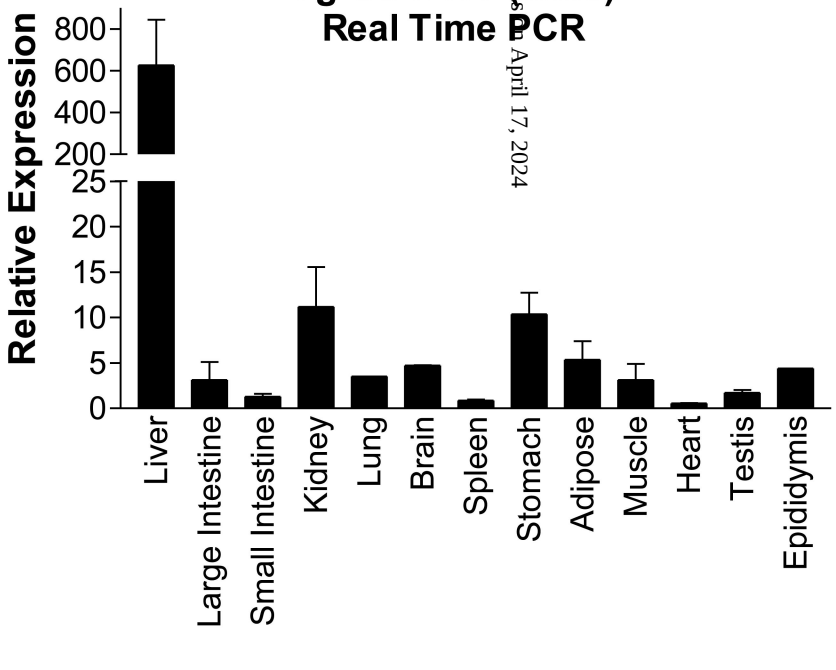


Fig 4A

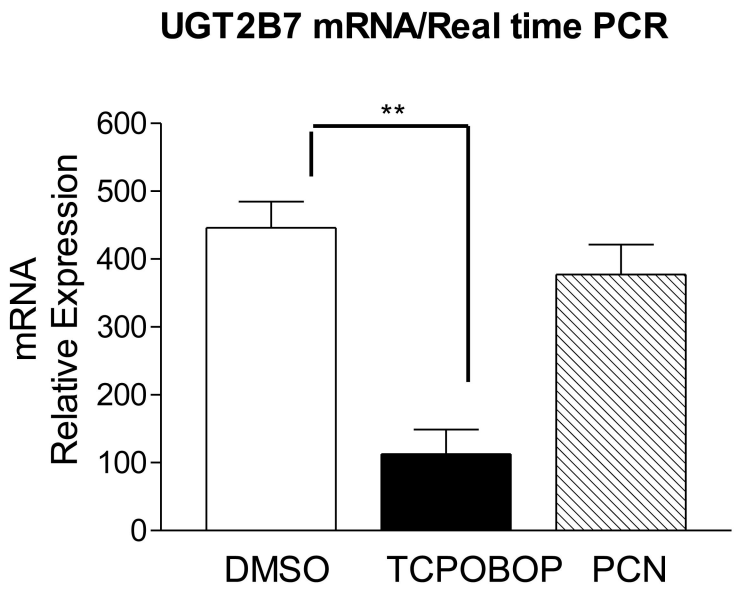


Fig 4B

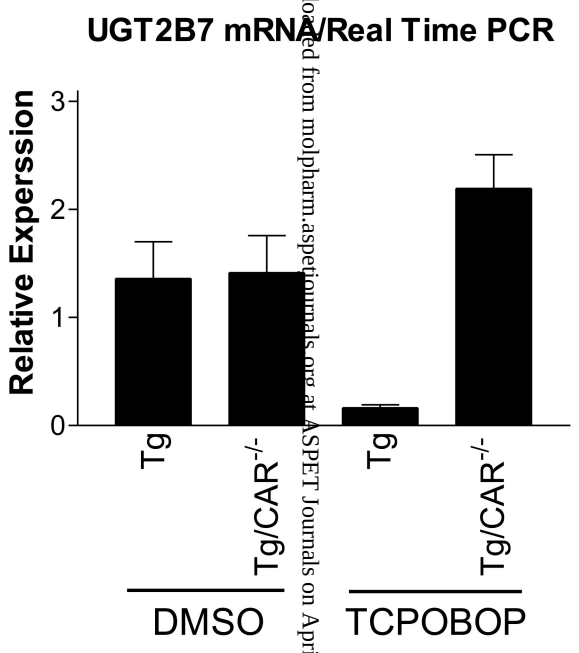


Fig 4C

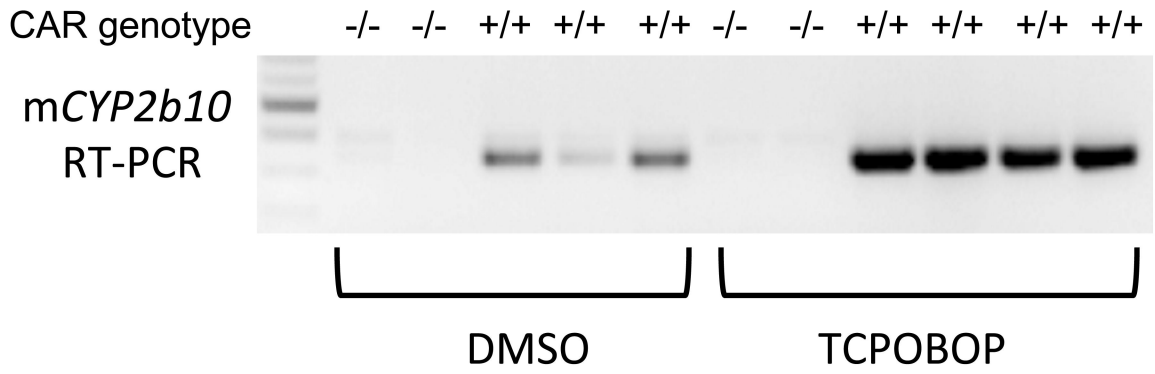


Fig 5A

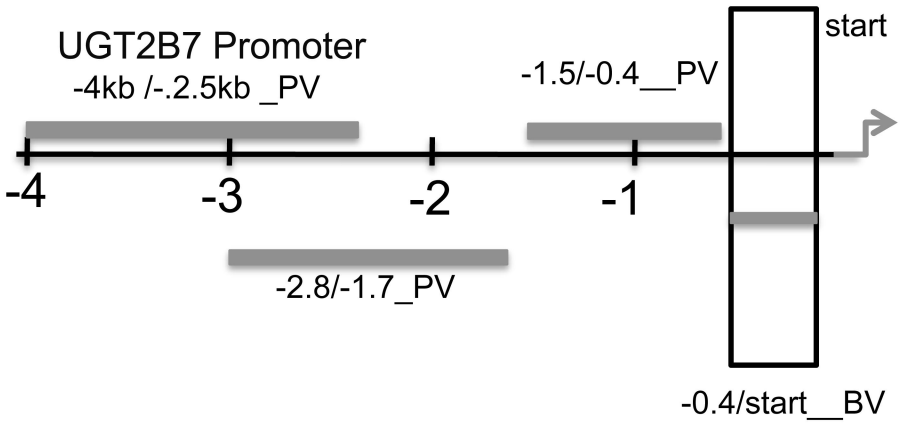
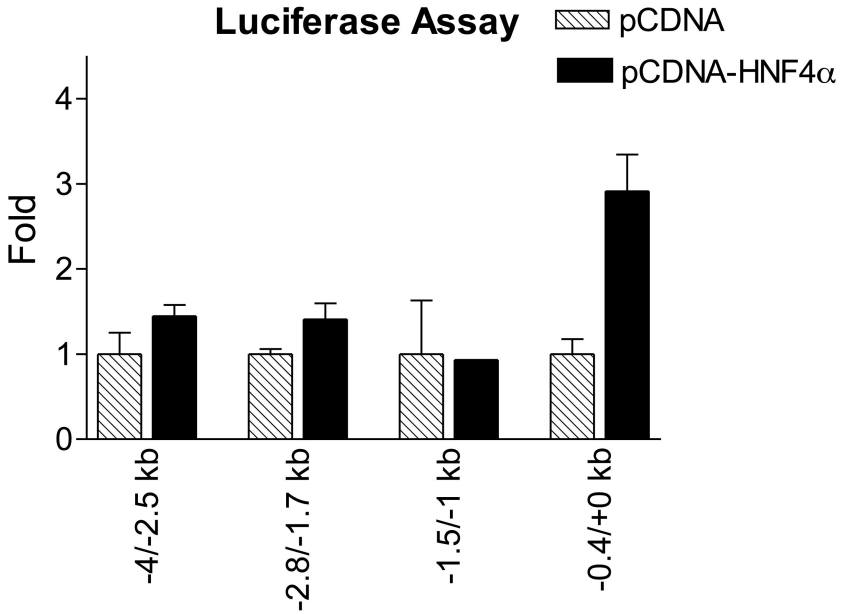


Fig 5B



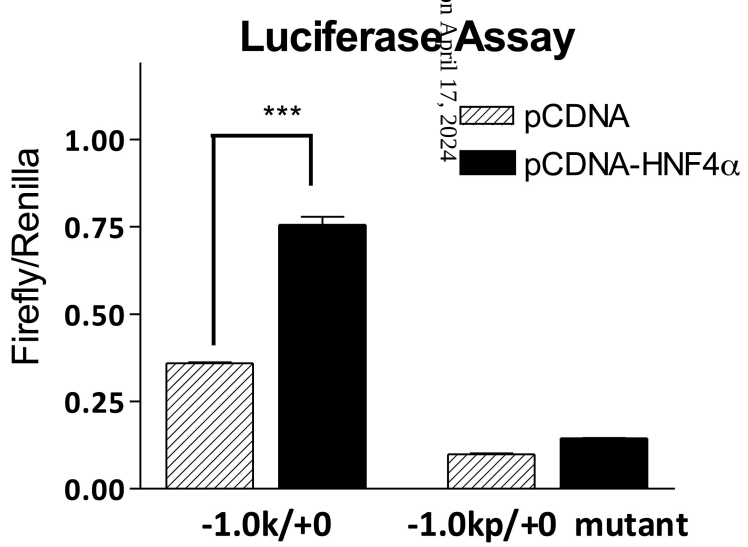


Fig 5C

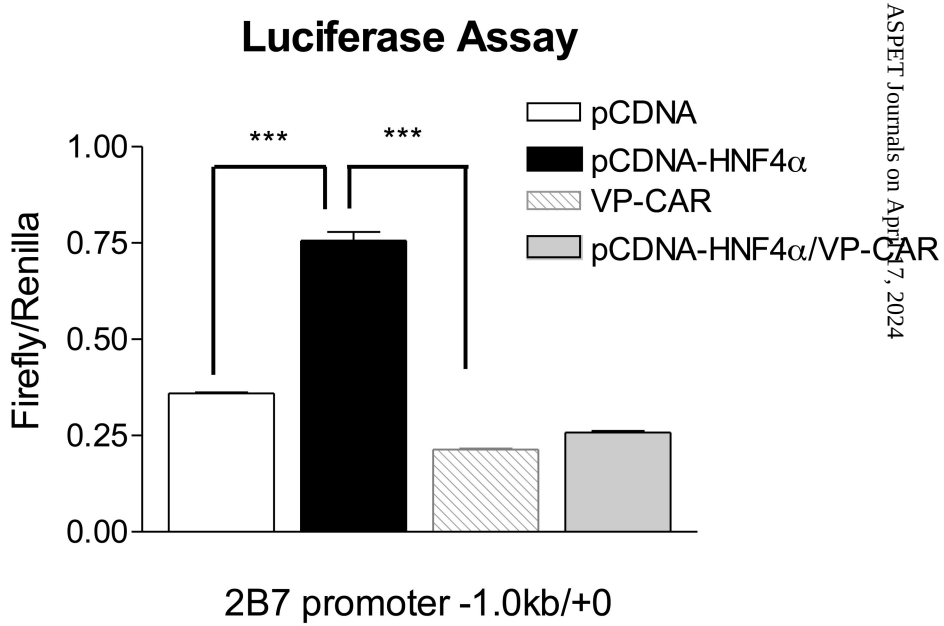


Fig 6