

UC San Diego

UC San Diego Previously Published Works

Title

Differential Role of Liver X Receptor (LXR) α and LXR β in the Regulation of UDP-Glucuronosyltransferase 1A1 in Humanized UGT1 Mice

Permalink

<https://escholarship.org/uc/item/1g75r63z>

Journal

Drug Metabolism and Disposition, 48(4)

ISSN

0090-9556

Authors

Hansmann, Eva

Mennillo, Elvira

Yoda, Emiko

et al.

Publication Date

2020-04-01

DOI

10.1124/dmd.119.090068

Peer reviewed

Differential Role of Liver X Receptor (LXR) α and LXR β in the Regulation of UDP-Glucuronosyltransferase 1A1 in Humanized *UGT1* Mice [§]

Eva Hansmann, Elvira Mennillo, Emiko Yoda, Mélanie Verreault, Olivier Barbier, Shujuan Chen, and Robert H. Tukey

Laboratory of Environmental Toxicology, Department of Pharmacology, University of California, San Diego, La Jolla, California (E.H., E.M., E.Y., S.C., R.H.T.); Division of Health Chemistry, Department of Healthcare and Regulatory Sciences, School of Pharmacy, Showa University, Shinagawa-ku, Tokyo, Japan (E.Y.); and Laboratory of Moléculaire Pharmacology, Centre de Recherche du CHU de Québec, Faculté de Pharmacie, Université Laval Québec, Québec, Canada (M.V., O.B.)

Received November 29, 2019; accepted January 14, 2020

ABSTRACT

Liver X receptors (LXRs), LXR α and LXR β , are nuclear receptors that regulate the metabolism of cholesterol and bile acids and are activated by oxysterols. Humanized *UGT1* (*hUGT1*) mice express the 9-human *UGT1A* genes associated with the *UGT1* locus in a *Ugt1*-null background. The expression of UGT1A1 is developmentally delayed in the liver and intestines, resulting in the accumulation of serum bilirubin during the neonatal period. Induction of UGT1A1 in newborn *hUGT1* mice leads to rapid reduction in total serum bilirubin (TSB) levels, a phenotype measurement that allows for an accurate prediction on UGT1A1 expression. When neonatal *hUGT1* mice were treated by oral gavage with the LXR agonist T0901317, TSB levels were dramatically reduced. To determine the LXR contribution to the induction of UGT1A1 and the lowering of TSB levels, experiments were conducted in neonatal *hUGT1/Lxr α ^{-/-}*, *hUGT1/Lxr β ^{-/-}*, and *hUGT1/Lxr $\alpha\beta$ ^{-/-}* mice treated with T0901317. Induction of liver UGT1A1 was dependent upon LXR α , with the induction pattern paralleling induction of LXR α -specific stearyl CoA desaturase 1. However, the actions of T0901317 were also shown to display a lack

of specificity for LXR, with the induction of liver UGT1A1 in *hUGT1/Lxr $\alpha\beta$ ^{-/-}* mice, a result associated with activation of both pregnane X receptor and constitutive androstane receptor. However, the LXR agonist GW3965 was highly selective toward LXR α , showing no impact on lowering TSB values or inducing UGT1A1 in *hUGT1/Lxr α ^{-/-}* mice. An LXR-specific enhancer site on the *UGT1A1* gene was identified, along with convincing evidence that LXR α is crucial in maintaining constitutive expression of UGT1A1 in adult *hUGT1* mice.

SIGNIFICANCE STATEMENT

It has been established that activation of LXR α , and not LXR β , is responsible for the induction of liver UGT1A1 and metabolism of serum bilirubin in neonatal *hUGT1* mice. Although induction of the human *UGT1A1* gene is initiated at a newly characterized LXR enhancer site, allelic deletion of the *Lxr α* gene drastically reduces the constitutive expression of liver UGT1A1 in adult *hUGT1* mice. Combined, these findings indicate that LXR α is critical for the developmental expression of UGT1A1.

Introduction

UDP-glucuronosyltransferases (UGTs) catalyze the transformation of xeno- and endobiotics into excretable glucuronides and thus participate in biologic inactivation and clearance of these compounds (Hu et al., 2014). Variations in UGT expression in addition to the induction or inhibition of these proteins can result in adverse drug effects as well as hormone imbalance (Yang et al., 2017). The *UGT1* gene family encodes

nine transferases (UGT1A1, UGT1A3, UGT1A4, UGT1A5, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10) (Ritter et al., 1992), which exhibit unique substrate specificity and are expressed in a tissue-specific as well as inducible manner (Tukey and Strassburg, 2000). Humanized *UGT1* (*hUGT1*) mice express the *UGT1A* genes in a tissue-specific pattern that was similar to expression patterns of the same genes in human tissues (Fujiwara et al., 2010). Interestingly, UGT1A1, which is the sole transferase responsible for the metabolism of serum bilirubin (Bosma et al., 1994), is developmentally regulated in newborn *hUGT1* mice and has been a valuable tool in examining the contribution of UGT1A1 toward the control of neonatal hyperbilirubinemia (Fujiwara et al., 2010, 2012; Yueh et al., 2014; Chen and Tukey, 2018). Neonatal *hUGT1* mice develop near-fatal levels of total serum bilirubin (TSB) during the first 2 weeks after birth (Fujiwara et al., 2010). The

This work was supported by the National Institutes of Health National Institute of Environmental Health Sciences [ES010337] and General Medicine [GM126074].

¹E.H. and E.M. contributed equally to these studies.

<https://doi.org/10.1124/dmd.119.090068>.

[§]This article has supplemental material available at dmd.aspetjournals.org.

ABBREVIATIONS: CAR, constitutive androstane receptor; ChIP, chromatin immunoprecipitation; CYP, cytochrome P450; EMSA, electrophoretic mobility shift assay; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HRP, horseradish peroxidase; *hUGT1*, humanized *UGT1*; LXR, liver X receptor; LXRE, LXR response element; NCoR1, nuclear corepressor 1; PCR, polymerase chain reaction; PPAR α , peroxisome proliferator-activated receptor α ; pRL renilla luciferase (pRL-NUL)PXR, pregnane X receptor; qPCR, quantitative polymerase chain reaction; SCD, stearyl CoA desaturase; TSB, total serum bilirubinTK-pGL3thymidine kinase luciferase reporter vector; UCSD, University of California, San Diego; UGT, UDP-glucuronosyltransferase.

administration of ligands known to activate either liver or intestinal constitutive androstane receptor (CAR) (Cai et al., 2010; Fujiwara et al., 2012), the pregnane X receptor (PXR) (Chen et al., 2005, 2012), or the peroxisome proliferator-activated receptor α (PPAR α) (Senekeo-Effenberger et al., 2007) result in the induction of UGT1A1 and the simultaneous metabolism and elimination of circulating TSB.

The liver X receptor (LXR) was first identified in the 1990s as an orphan member of the nuclear receptor superfamily and later found to be activated by endogenous oxysterols (Peet et al., 1998a). LXR consists of two isoforms, LXR α and LXR β . LXR α is expressed in all tissues (Song et al., 1994), in contrast, the expression of LXR β is restricted to the liver, intestine, kidney, spleen, macrophages, and adipose tissue (Willy et al., 1995). LXR is largely involved in the regulation of cholesterol homeostasis protecting the cells from cholesterol overload by stimulating reverse cholesterol transport (Millatt et al., 2003). Furthermore, LXR has been linked to other major physiologic functions, including lipid metabolism (Ulven et al., 2005; Cha and Repa, 2007), glucose homeostasis (Grefhorst et al., 2005; Oosterveer et al., 2010), steroidogenesis, immunity, and inflammation (Zelcer and Tontonoz, 2006).

LXR α and LXR β were cloned in 1995 (Mangelsdorf and Evans, 1995; Willy et al., 1995), and later confirmed that the ligand-binding and DNA-binding domains of the receptors were 77% homologous (Alberti et al., 2000). Following confirmation that expression of the LXRs displayed differential tissue specificity (Shinar et al., 1994; Willy et al., 1995) yet both bound to similar LXR enhancer sequences, it had been speculated and later demonstrated that activation of the LXR α /RXR and LXR β /RXR complexes have different roles in gene expression (Annicotte et al., 2004; Hong and Tontonoz, 2008). This was first demonstrated when Lxr α ^{-/-} and Lxr β ^{-/-} mice were placed on a normal or cholesterol high diet (Alberti et al., 2001). The cholesterol-enriched diet had a profound effect on LXR α ^{-/-} mice, inducing fatty liver, increased liver mass, and increased cholesterol levels, eventually leading to impaired liver function. The Lxr β ^{-/-} mice were resistant to cholesterol-induced fatty liver formation. There are not highly selective LXR α or LXR β ligands, making it difficult to isolate the actions of the different LXR receptors on gene expression or function. Yet, activation of LXR by nonselective LXR agonists in Lxr α ^{-/-} or Lxr β ^{-/-} mice can isolate the direct actions of these receptors on gene expression. For example, the LXR agonist T0901317-induced hepatic stearyl CoA desaturase (SCD1) expression only in wild-type or Lxr β ^{-/-} mice and not Lxr α ^{-/-} mice, demonstrating that LXR α controls SCD1 expression (Zhang et al., 2014). There is little information on the direct impact of LXR toward the induction and regulation of the UGT1A1 gene. We have now taken a direct approach to evaluate the role of the LXR receptors on the induction of the UGT1A1 gene by deleting LXR α , LXR β , or LXR α and LXR β in *hUGT1* mice.

Materials and Methods

Materials. UDP-glucuronic acid and bilirubin were obtained from Sigma (St. Louis, MO). SN-38 and T0901317 were obtained from Cayman Chemical (Ann Arbor, MI). SN-38 glucuronide was from Santa Cruz Biotechnology (Santa Cruz, CA). Internal standards deuterated d10-CPT-11 and d3-SN-38 were purchased from Toronto Research Chemicals (Toronto, Ontario, Canada). Rabbit anti-UGT1A1 monoclonal antibody was purchased from Abcam (Cambridge, UK). Mouse anti-GAPDH monoclonal antibody was obtained from Santa Cruz Biotechnology (Dallas, TX). Anti-mouse IgG horseradish peroxidase (HRP) conjugated antibody and anti-rabbit IgG HRP conjugated antibodies were obtained from Cell Signaling Technology, Inc. (Danvers, MA). Human hepatoma (HepG2) and human embryonic kidney (HK293) cells were obtained from the

American Type Culture Collection (Rockville, MD). The cells were cultured and passaged per standard protocol.

Animal Studies. Humanized *UGT1* mice were previously generated by introducing a human bacterial artificial chromosome (BAC) clone encoding the entire *UGT1* locus into mice [transgenic(*TG*)*UGT1* mice] (Chen et al., 2005) and crossing *TgUGT1* mice with *Ugt1*^{+/-} mice (Nguyen et al., 2008) until *TgUGT1/Ugt1*^{-/-} mice (*hUGT1* mice) were created (Fujiwara et al., 2010). A breeding strategy between *hUGT1*, *Lxr α* ^{-/-}, *Lxr β* ^{-/-}, and *Lxr $\alpha\beta$* ^{-/-} mice was developed that resulted in the generation of *hUGT1/Lxr α* ^{-/-}, *hUGT1/Lxr β* ^{-/-}, and *hUGT1/Lxr $\alpha\beta$* ^{-/-} mice. The *Lxr*-null mice were originally developed by the David Mangelsdorf laboratory (Repa et al., 2000) and were generously obtained from Christopher Glass at the University of California, San Diego (UCSD). *hUGT1/Car*^{-/-} mice were developed previously (Fujiwara et al., 2012). All mouse strains were housed in a pathogen-free UCSD Animal Care Facility and received food and water ad libitum. All animal protocols were reviewed and approved by the UCSD Animal Care and Use Committee. Neonatal male and female 10-day-old mice were treated orally with either propylene glycol/tween 4:1 (vehicle) or 50 mg/kg T0901317 dissolved in vehicle or GW3965 (75 mg/kg), and tissues were collected 48 hours after treatment.

Bilirubin Measurements. Blood was obtained from the submandibular vein and centrifuged at 16,000g for 2 minutes. Serum samples were immediately measured for TSB levels using a Unistat Bilirubinometer (Reichert, Depew, NY).

Liver Tissue Sections. Entire livers were dissected from mice, snap-frozen in liquid nitrogen, and stored at -80°C. Frozen tissues were pulverized and the powder aliquoted for further RNA and protein extraction.

Total RNA Preparation and Real-time Reverse Transcription Polymerase Chain Reaction. Total RNA was isolated from liver tissue of treated and control mice according to the TRIzol reagent protocol as specified by the supplier (Thermo Fisher Scientific, Waltham, MA). The samples were prepared for reverse transcription by using the iScript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, CA). Quantitative polymerase chain reaction (qPCR) using newly synthesized cDNA was carried out using a CFX96 QPCR system (Bio-Rad) by employing SsoAdvanced SYBR Green reagent (Bio-Rad). Primers used in these studies are shown in Table 1.

Western Blot Analysis. Tissues were homogenized in RIPA lysis buffer (EMD Millipore Corporation, Temecula, CA) supplemented with protease and phosphatase inhibitor cocktail (1:100 each). Protein solutions were pooled to have a total of four samples for each genotype and condition. Western blots were

TABLE 1
Sequence of primers used in qPCR analysis

Genes		Oligonucleotide Sequence (5'-3')
Human UGT1A1	Forward	CCATCATGCCCAATATGGTT
	Reverse	CCACAATTCATGTCTCCA
Human UGT1A3	Forward	AGGTGACTGTCCAGGACCTA
	Reverse	CAAATTCCTGGGATAGTGGATTT
Human UGT1A4	Forward	CAACGGGAAGCCACTATCTC
	Reverse	TGAGACCATTGATCCCAAAGA
Human UGT1A6	Forward	ACCGGGTCTATGAGATTGTA
	Reverse	TGGTCATACCGCACTGGATA
Human UGT1A9	Forward	GAACATTTATTATGCCACCG
	Reverse	CAACAACCAAAATTGATGTGTG
Scd-1	Forward	GCTCTACACTGCCTCTTCG
	Reverse	CAGCCGAGCCTTGTAAGTTC
Scd-2	Forward	TCCTGCAAGCTCTACACTG
	Reverse	TGCCTTGTATGTTCTGTGGC
LXR α	Forward	TACAACCGGGAAGACTTTGC
	Reverse	CAGAGAAGATGCTGATGGCA
LXR β	Forward	CTTGGTGGTGTCTTCTTGA
	Reverse	TGTGGTAGGCTGAGGTGTA
Cyp2b10	Forward	AAAGTCCCGTGGCAACTTCC
	Reverse	CATCCCAAAGTCTCTCATGG
Cyp3a11	Forward	TTCTGTCTTACAAAACCCGGC
	Reverse	GGGGGACAGCAAAGCTCTAT
Cyp4a10	Forward	GATGGACGCTCTTTACCCAA
	Reverse	AAGGGTCAAACACCTCTGGA
Cph	Forward	ATGGTCAACCCCACTGTGT
	Reverse	TTCTTGTCTCTTTGGAACCTTGTG

performed by using NuPAGE 4%–12% BisTris-polyacrylamide gels (Thermo Fisher Scientific), with the protocols described by the manufacturer. Protein (30 μ g) was electrophoresed at 170 V for 50 minutes and transferred at 20 V for 60 minutes to PVDF membranes (EMD Millipore Corporation). Membranes were blocked with 5% skim milk at room temperature for 1 hour and incubated with primary antibodies [rabbit anti-human UGT1A1 (ab170858), mouse anti-GAPDH (sc-32233)] at 4°C overnight. Membranes were washed and exposed to HRP-conjugated secondary antibodies (anti-mouse IgG or anti-rabbit IgG) for 1 hour at room temperature. Protein was detected by Clarity Western ECL Substrate system (Bio-Rad) and was visualized by the Bio-Rad gel documentation system. All Western blots are cropped from the full-length blots that have been included in the Supplemental Material.

Glucuronidation Assays. Microsomal proteins were purified from tissues (50 mg), and 100 μ g of liver homogenate was incubated in a previously reported glucuronidation assay buffer (Verreault et al., 2006) in the presence of bilirubin (20 μ M), SN-38 (50 μ M) for 30 minutes, or imipramine (500 μ M) for 2 hours at 37°C. The formation of bilirubin monoglucuronide, SN-38 glucuronide, and imipramine glucuronide was quantified by liquid chromatography coupled to electrospray ionization - tandem mass spectrometry (LC/ESI-MS/MS) as previously reported (Lu et al., 2017). The formation of glucuronide conjugates is expressed as area under the curve or peak area ratio.

Plasmid Cloning and Transient Transfection Assays. The various TK-pGL3 plasmids were obtained by cloning three copies of the corresponding dimerized oligonucleotides (Table 2) in the thymidine kinase promoter-driven luciferase reporter (TK-pGL3) vector. For transfection experiments, HepG2 cells (75×10^3 /well) were seeded into 24-well plates and transfected with 100 ng of luciferase reporter, 30 ng of the pRL-NULL expression vector, and 10 ng of expression plasmid. All samples were complemented with a pBS-SK+ plasmid to ensure 500 ng DNA/well. Cells were transfected with the ExGen 500 reagent for 6 hours at 37°C, and subsequently incubated for 24 hours with either ethanol or LXR ligands at the indicated concentrations. Luciferase and renilla activities were determined as reported (Lu et al., 2017).

Electrophoretic Mobility Shift Assays. Electrophoretic mobility shift assays (EMSAs) using in vitro-produced LXR α and RXR α were performed as described (Johansson et al., 2003) using the radiolabeled probes (100,000 cpm) as indicated in Supplemental Fig. 1. Oligonucleotides used in these studies are shown in Table 2.

Chromatin Immunoprecipitation Assays. Chromatin immunoprecipitation (ChIP) assays were performed according to the method of Forsberg et al. (2000) in a previously modified manner (Verreault et al., 2006). Briefly, 20×10^6 HepG2 cells were treated with either vehicle or T0901317 (1 μ M) for 4 hours. Protein-DNA cross-linking, nuclear extract preparation, and sonication were performed as reported (Forsberg et al., 2000). A volume of lysate equivalent to 40×10^5 cells was immunoprecipitated using 4 μ g of anti-LXR (sc1202; Santa-Cruz), anti-PXR (sc9690) antibodies, or with 4 μ g of an anti-UGT2B (sc23479) antibody as a negative control. A separate volume of lysate was kept without immunoprecipitation for subsequent purification of input genomic DNA or was incubated in the presence of protein A-sepharose alone. One-tenth of the immunoprecipitated DNA was PCR amplified as described in Supplemental Fig. 1.

Statistical Analysis. Statistically significant differences between vehicle- and T0901317-treated animals were evaluated using unpaired *t* test ($*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $****P < 0.0001$), and between different genotypes we performed Tukey's multiple comparison test ($^{\#}P < 0.05$; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$; $^{\#\#\#\#}P < 0.0001$).

Results

Induction of the UGT1A1 Gene in Neonatal hUGT1 Mice. The LXR agonist T090137 has an affinity for both LXR α and LXR β (Schultz et al., 2000), with a binding affinity that displayed an EC₅₀ of approximately 20 nM. It is also capable of activating the PXR (Shenoy et al., 2004) and farnesoid X receptor (Houck et al., 2004) but at a higher concentration than for LXR (EC₅₀ = 4–7 μ M). There are five human UGT1 isoforms expressed in the liver (Strassburg et al., 1997) (UGT1A1, UGT1A3, UGT1A4, UGT1A6, and UGT1A9), with UGT1A1 controlling the metabolism and clearance of serum bilirubin.

TABLE 2

Sequence of oligonucleotides used in LXR binding element analysis
Bold nucleotides represent potential LXR/RXR binding sequences and underlined nucleotides correspond to mutated bases.

EMSA	Sequence (5'-3')
DR4 –9903 wt	ATAGAGAGG TGACCACAGGAGACCTAAG CACT
DR4 –9855 wt	TAAAGAG GTGACTCAGTTCAGTGGAAAG
DR4 –7632 wt	GAGGAGGA AGGATCACTTGAGCCCAGAA ATTCAT
DR4 –4076 wt	TCTGAAGGG ATTAGTTTAGGACAACCCTC
DR4 –888 wt	TAACCG TGTACCTGTAGTAAGCAAAG GGCC
DR4 –9855 mt	TAAAGAG GTGACTCATTTT AGTGGAAAG
LXRE consensus	CCCAGG TTTAAATAAGTT CATCACA
ChIP	
UGT1A3 LXRE	Forward 5'-TCAGCTGCTGCCTGATAAAACA TG Reverse 5'-GACCGATCATGACTATCTTGA AA
UGT1A1 –9903 bp	Forward 5'-CATTGAGACTTGACCCATCTGG Reverse 5'-TTAACACTTCTACTTCTCTGC
UGT1A1 –9855 bp	Forward 5'-CACAGGAGACCTAAGCACTCGC Reverse 5'-CTGGAACACTGAACTCAGTGTG
UGT1A1 –7632 bp	Forward 5'-GACAACATAGTGGGTCAACAT CAT Reverse 5'-GGTCTCTCTCTGTAGCTGTTGC
UGT1A1 –4076 bp	Forward 5'-ATTACGGAATAGTTTGGAC Reverse 5'-GTAATCAAGAGATGACTAGAG GT
UGT1A1 –888 bp LXRE	Forward 5'-GAGCCCTGAGTGGCTGAGGTG Reverse 5'-GAACCTGAAAGACCGAGTCTCT GT
DR4 multicopy	
LXRE consensus (x3)	5'-TGATGA ACTTATTTAAACCCTGGGGATC TG
DR4 –9903 (x3)	5'-ATAGAGAGG TGACCACAGGAGACCTAA GCACT
DR4 –9855 (x3)	5'-TAAAGAG GTGACTCAGTTCAGTGGAA G
DR4 –4076 (x3)	5'-TCTGAAGGG ATTAGTTTAGGACAACC CTC
DR4 –888 (x3)	5'-ACCG TGTACCTGTAGTAAGCAAAG GGCCTA

To investigate the potential of LXR to induce the *UGT1A1* gene, 10-day-old neonatal *hUGT1* mice were treated once with vehicle (propylene glycol/tween 80, 40:1) or with the LXR agonist T0901317 (50 mg/kg) by oral gavage. After 48 hours, serum was collected for TSB analysis and the livers collected for RNA extraction. Neonatal *hUGT1* mice develop severe levels of serum bilirubin. T0901317 treatment of 48 hours reduced TSB levels to normal adult levels (Fig. 1A). This drop in TSB levels resulted from a significant induction of liver *UGT1A1* gene expression (300-fold) as quantitated by qPCR (Fig. 1B). Also, analysis of RNA expression demonstrated there was substantial induction of liver *UGT1A3* (200-fold) and *UGT1A4* (400-fold), with *UGT1A6* and *UGT1A9* genes being unaffected (Fig. 1C). We have shown previously that T0901317 treatment to transgenic *UGT1* mice induced the *UGT1A3* gene in liver and intestinal tissue, along with induction of *UGT1A3* directed glucuronidation activity toward chemodeoxycholic acid (CDCA) and lithocholic acid (LCA) (Verreault et al., 2006).

The LXR Receptors Display Gene Specificity. To examine the specificity of the LXR receptors toward induction of liver *UGT1A1*, *hUGT1*, *hUGT1/Lxr α ^{-/-}*, *hUGT1/Lxr β ^{-/-}*, and *hUGT1/Lxr α ^{-/-}/Lxr β ^{-/-}* (*hUGT1/Lxr $\alpha\beta$ ^{-/-}*) mice were developed. To confirm the knockout conditions in these mice, qPCR analysis was performed to verify the elimination of LXR α mRNA expression in *hUGT1/Lxr α ^{-/-}* and *hUGT1/Lxr $\alpha\beta$ ^{-/-}* mice and the elimination of LXR β mRNA

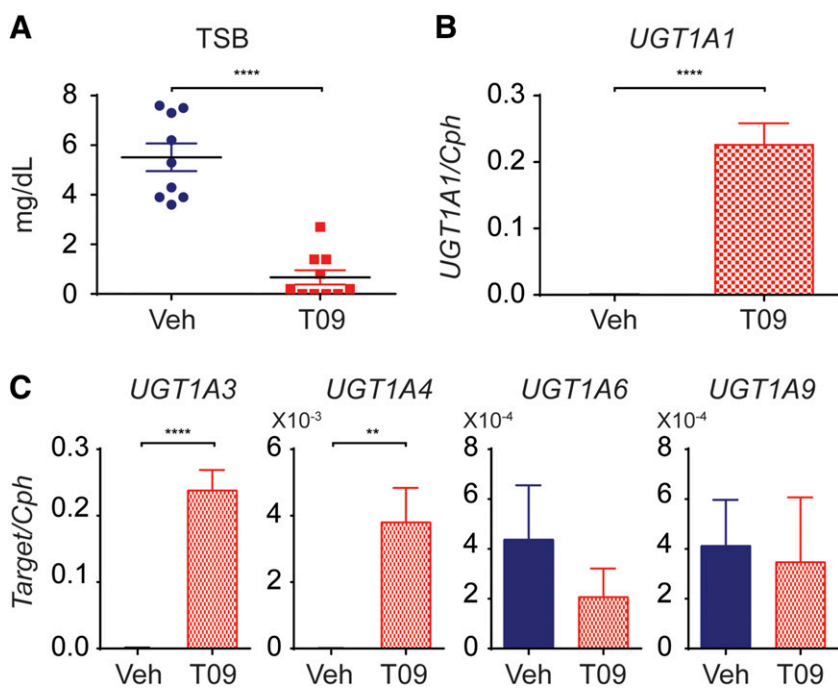


Fig. 1. Induction of UGT1A mRNA expression by T0901317 in neonatal *hUGT1*. Ten-day-old neonatal *hUGT1* mice were treated with 50 mg/kg T0901317 or vehicle (propylene glycol/tween 80 4:1) by oral gavage. After 48 hours, blood was collected for TSB analysis (A) and the mice sacrificed to collect the livers. Tissues were pulverized under liquid nitrogen and used for the preparation of total RNA using the TRIzol protocol. Gene expression of *UGT1A1* (B), *UGT1A3*, *UGT1A4*, *UGT1A6*, and *UGT1A9* (C) transcript levels were determined by qPCR and expressed relative to *mCpH*. Values are the means \pm S.E.M. ($n > 4$). Statistically significant differences are outlined in *Materials and Methods*.

expression in *hUGT1/Lxr β ^{-/-}* and *hUGT1/Lxr α ^{-/-}* mice (Fig. 2, A and B). Interestingly, there was a consistent reduction in *Lxr α* gene expression in *Lxr β ^{-/-}* mice treated with T0901317, yet the values were not statistically significant. Knockout of the *Lxr* genes leads to a loss of function, as demonstrated in gene expression patterns of the *LXR α* target genes stearoyl CoA desaturase-1 (*Scd1*) and *Scd2* (Chu et al., 2006; Caputo et al., 2014; Zhang et al., 2014). These proteins catalyze the conversion of saturated fatty acids into monounsaturated fatty acids and have previously been described to be activated by *LXR* agonists in either an sterol regulatory element-binding-1c (SREBP-1c) dependent or independent manner. The oral treatment of neonatal *hUGT1* and *hUGT1/Lxr β ^{-/-}* mice with T0901317 resulted in transcriptional activation of *Scd1* and *Scd2* genes (Fig. 2, C and D). Mice lacking the *Lxr α* gene did not show increases in *SCD1* or *SCD2* RNA (Fig. 2, C and D).

Induction of UGT1A1 by LXR. Using these same tissues, the induction profile of the *UGT1A1* gene was like that of *Scd1*, with greater induction in *hUGT1* and *hUGT1/Lxr β ^{-/-}* mice and significantly reduced induction in *hUGT1/Lxr α ^{-/-}* and *hUGT1/Lxr β ^{-/-}* mice (Fig. 3B). However, the induction of the *UGT1A1* gene in *hUGT1/Lxr α ^{-/-}* and *hUGT1/Lxr β ^{-/-}* was still considerable with approximately 30% of those values detected in the liver from *hUGT1* mice. Surprisingly, the TSB values in the *hUGT1* mice and the other *LXR* variants were all greatly reduced when compared with vehicle-treated mice (Fig. 3A). The phenotype changes in serum TSB values in *hUGT1*, *hUGT1/Lxr α ^{-/-}*, *hUGT1/Lxr β ^{-/-}*, and *hUGT1/Lxr α ^{-/-}* mice suggests that UGT1A1 was induced in each of these strains following T0901317 treatment. This was confirmed by Western blot analysis showing that induction of liver UGT1A1 occurs in all strains treated, with liver abundance reflecting that of gene expression (Fig. 3C). Also, glucuronidation analysis using liver extracts has confirmed that bilirubin (Fig. 3D) and SN-38 (Fig. 3E), substrates that are conjugated by UGT1A1, show a functional induction pattern like *UGT1A1* gene expression. Because the induction levels of UGT1A1 in *hUGT1/Lxr β ^{-/-}* mice, which do not express functional *LXR*, are like those observed in *hUGT1/Lxr β ^{-/-}* mice, this finding indicates that *LXR β* plays a negligible role in the induction of UGT1A1 in neonatal mice.

T0901317 and LXR Elicit Cross-Talk with Other Nuclear Receptors. The dramatic induction of UGT1A1 by T0901317 in all *LXR* mouse lines led us to examine in greater detail this unusual property of T0901317. In the liver, the *Cyp2b10*, *Cyp3a11*, and *Cyp4a10* genes are significantly induced in *hUGT1* mice (Fig. 4, A–C) by T0901317. It is interesting to note that in the absence of *LXR α* , T0901317 elicits a highly synergistic *Cyp3a11* induction response. This observation indicates that activation of PXR by T0901317, which can induce *Cyp3a11*, may be repressed by activated *LXR α* . In contrast, induction of *Cyp4a10*, which can be induced by *PPAR α* , is not induced above control values in *LXR α* deficient mice, indicating that induction of *Cyp4a10* by *PPAR α* requires functional *LXR α* . In both of these examples, activation of either PXR or *PPAR α* by T0901317 is interconnected with *LXR α* and not *LXR β* . Different from these examples, however, is the induction pattern by CAR activation of the *Cyp2b10* gene in *hUGT1*, *hUGT1/Lxr α ^{-/-}*, *hUGT1/Lxr β ^{-/-}*, *hUGT1/Lxr α ^{-/-}*, and *hUGT1/Car^{-/-}* mice (Fig. 4C). Western blot analysis of liver extracts from these treated mice demonstrates that the induction of CYP2B10 by T0901317 is CAR dependent because induction is significantly reduced in *hUGT1/Car^{-/-}* mice (Fig. 4D). Thus, the human *UGT1A1* gene can be induced by T0901317 through activation of PXR, *PPAR α* , and CAR, in addition to the more dominant action of *LXR α* .

Induction of Liver UGT1A1 by GW3965. In addition to T0901317, the synthetic *LXR* agonist GW3965 has been used as an alternative agent to examine *LXR*-activated target genes. We treated 10-day-old neonatal *hUGT1*, *hUGT1/Lxr β ^{-/-}*, and *hUGT1/Lxr α ^{-/-}* mice with GW3965 (75 mg/kg) and examined TSB values and *UGT1A1* gene expression patterns after 2 days of exposure (Fig. 5A). TSB values were dramatically reduced in *hUGT1* and *hUGT1/Lxr β ^{-/-}* mice, but the values did not change in *hUGT1/Lxr α ^{-/-}* mice, confirming that induction of UGT1A1 follows activation of *LXR α* and not *LXR β* . This was confirmed by two additional sets of data. First, the induction of liver *UGT1A1* gene expression did not occur in *hUGT1/Lxr α ^{-/-}* mice (Fig. 5B), and second, Western blot analysis shows induction of UGT1A1 in *hUGT1* and *hUGT1/Lxr β ^{-/-}* mice only (Fig. 5C). Based upon current knowledge of the specificity of T0901317 and GW3965

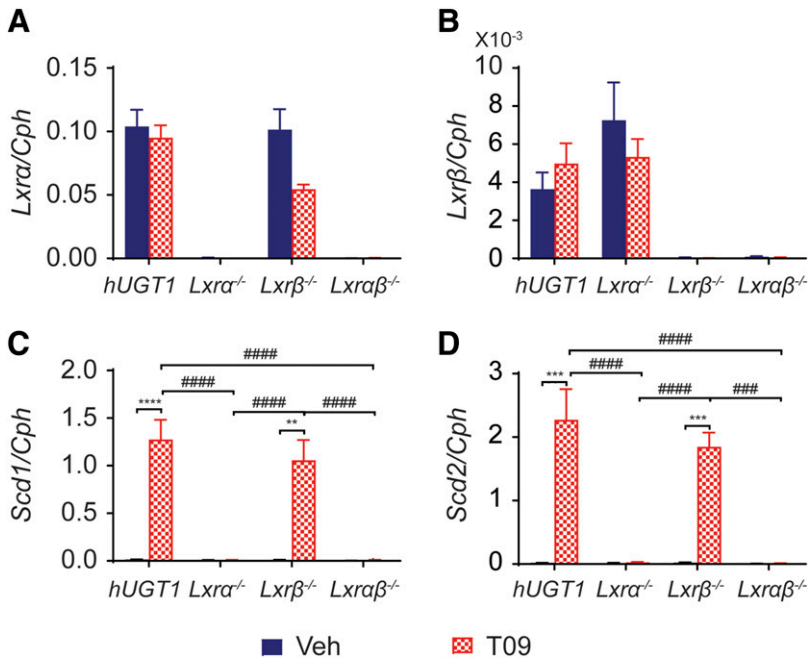


Fig. 2. Gene expression of *Lxrα* and *Lxrβ* and LXR downstream target genes *Scd1* and *Scd2* in LXR-deficient mice. Following the treatment of neonatal *hUGT1*, *hUGT1/Lxrα^{-/-}* (*Lxrα^{-/-}*), *hUGT1/Lxrβ^{-/-}* (*Lxrβ^{-/-}*), and *hUGT1/Lxrαβ^{-/-}* (*Lxrαβ^{-/-}*) mice with 50 mg/kg T0901317 for 48 hours (see Fig. 1), *Lxrα* (A), *Lxrβ* (B), *Scd1* (C), and *Scd2* (D) gene expression was determined by real-time PCR and expressed relative to mouse *Cph* gene expression. Values are the means \pm S.E.M. ($n > 4$). Statistically significant differences are outlined in *Materials and Methods*.

to activate both LXR α and LXR β , the induction of liver UGT1A1 is driven selectively by activated LXR α . It can be noted that the TSB levels following oral GW3965 treatment are not as low as noted for T0901317 treatment (Fig. 1). It is possible that T0901317, which is able to activate not only LXR but also PXR and CAR, is also inducing UGT1A1 in

intestinal tissue, a site that contributes toward bilirubin clearance (Chen et al., 2017).

Identification of a Functional LXR Response Elements within the UGT1A1 Gene Promoter. A computer-assisted analysis (Quandt et al., 1995) of the *UGT1A1* promoter gene sequence revealed the

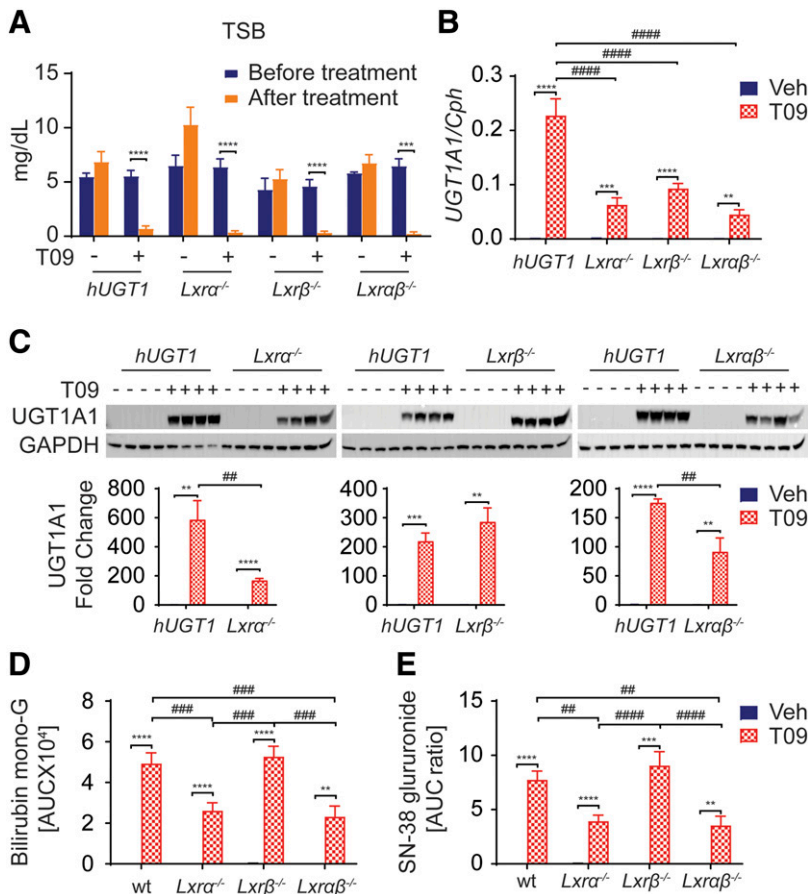


Fig. 3. The loss of LXR α and/or LXR β impacts T0901317-dependent modulation of UGT1A1 expression and activity. Ten-day-old neonatal *hUGT1*, *hUGT1/Lxrα^{-/-}* (*Lxrα^{-/-}*), *hUGT1/Lxrβ^{-/-}* (*Lxrβ^{-/-}*), and *hUGT1/Lxrαβ^{-/-}* (*Lxrαβ^{-/-}*) mice were treated with 50 mg/kg T0901317 or vehicle (propylene glycol/Tween 80 4:1) by oral gavage. After 48 hours, serum was collected, and the mice were sacrificed to collect liver tissue. The liver was pulverized under liquid nitrogen and used for the preparation of total RNA, whole-cell extract, and glucuronidation assays. (A) Before and 48 hours after the treatment TSB levels were taken. (B) *UGT1A1* gene expression was determined by real-time PCR and expressed relative to *Cph* gene expression. (C) Whole-liver tissue extracts were used to perform Western blot analysis to examine UGT1A1 expression. The bands have been cropped from the full-length blots but not enhanced in any way. Densitometric values of UGT1A1 protein bands obtained using the GAPDH signal as normalizing control are shown. 100 μ g of liver homogenate was incubated in the presence of bilirubin (20 μ M) (D) or SN-38 (50 μ M) (E) for 30 minutes at 37°C. The formation of bilirubin monoglucuronide and SN-38 glucuronide was quantified by liquid chromatography coupled to electrospray ionization-tandem mass spectrometry (LC/ESI-MS/MS). The formation of glucuronide conjugates is expressed as area under the curve and peak area ratio. Values are the means \pm S.E.M. ($n > 4$). Statistically significant differences were performed as outlined in *Materials and Methods*.

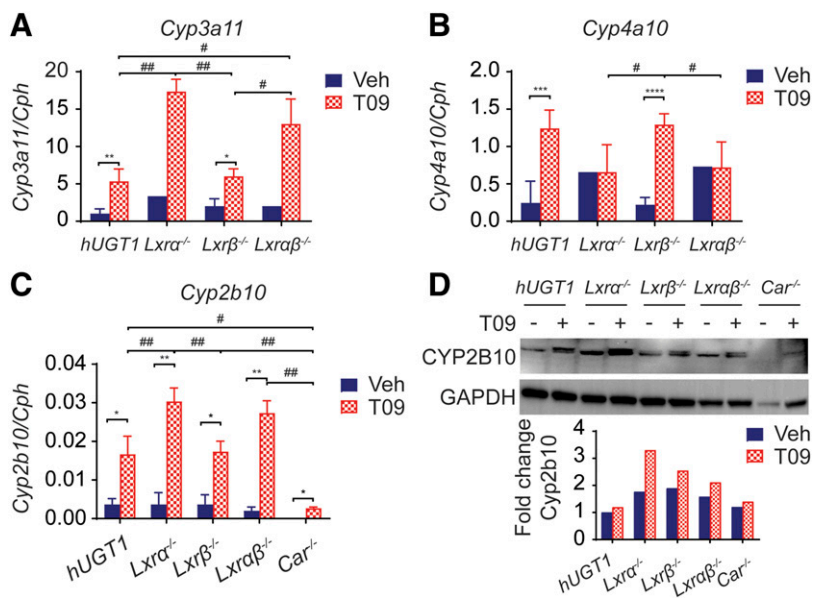


Fig. 4. The impact of the LXR agonist T0901317 and activation of additional nuclear receptors. Ten-day-old neonatal *hUGT1*, *hUGT1/Lxra*^{-/-} (*Lxra*^{-/-}), *hUGT1/Lxrβ*^{-/-} (*Lxrβ*^{-/-}), and *hUGT1/Lxraβ*^{-/-} (*Lxraβ*^{-/-}) mice were treated with 50 mg/kg T0901317 or vehicle (propylene glycol/Tween 80 4:1) by oral gavage. RNA was isolated from liver tissue after 48 hours and gene expression was evaluated by real-time PCR for *Cyp3a11* (A), *Cyp4a10* (B), and *Cyp2b10* (C). Samples of liver tissue were used for analysis of CYP2B10 expression by Western blot analysis (D). Statistically significant differences were performed as outlined in *Materials and Methods*.

presence of five degenerated LXR response element (LXRE) sequences (Willy et al., 1995) localized at positions -9903, -9855, -7632, -4076, and -888 bp of the promoter (Supplemental Fig. 1). Occupancy of these response elements by LXR α in living cells was analyzed through a ChIP assay performed on DNA from vehicle- or T0901317-treated HepG2 cells (Fig. 6). Only the DNA sequences encompassing the -9855 bp LXRE and an LXRE previously identified within the human UGT1A3 promoter (positive control) (Verreault et al., 2006) were precipitated by the anti-LXR α antibody in T0901317-treated cells (Fig. 6, A and C, Lane 8) but not in untreated cells (Lane 3). None of the other LXRE-like sequences found in the *UGT1A1* gene promoter were PCR amplified in any of the precipitated DNA preparations, suggesting that they are not occupied by LXR α / β . Because T0901317 was also reported as an activator of PXR (Mitro et al., 2007), DNA was also immunoprecipitated with an anti-PXR antibody (Lanes 4 and 9). No amplifications were observed.

Transient transfection with a -9855 bp LXRE-TK-pGL3 construct revealed that coexpression of LXR α and RXR α increased activity of the LXRE in human hepatoma HepG2 cells, an effect that was dose-dependently enhanced in the presence of T0901317 and GW3965

(0.1–10 μ M) (Fig. 6B). In EMSA, a clear binding was observed when a radiolabeled probe encompassing the -9855 bp LXRE sequence was incubated in the presence of both LXR α and RXR α (Fig. 6C, Lane 4). Introducing mutated bases within the 3'-half-site of the LXRE (Table 1) completely abolished the formation of this binding (Fig. 6, lane 5). In competition experiments, LXR α /RXR α binding to the WT probe was efficiently competed by itself and the consensus LXRE (Fig. 6, Lanes 6–8 and 12–14, respectively). In contrast, the mutated response elements failed to significantly compete for LXR α binding (lanes 9–11). Overall, these data identify the LXRE motif at position -9855 bp in the human *UGT1A1* gene promoter as a functionally active LXR response element.

The Role of LXR α and Expression of UGT1A1 in Adult *hUGT1* Liver. There is a limited expression of human UGT1A1 in the liver tissue of neonatal *hUGT1* mice, but in adult *hUGT1* mice there is adequate expression. When we examined the constitutive expression pattern of UGT1A1 by qPCR and Western blot analysis, there was an excellent agreement with gene expression and protein detection (Fig. 7). However, it is clear from this analysis that LXR α plays a key role in basal or constitutive expression of liver UGT1A1, with greatly reduced expression in *hUGT1/Lxra*^{-/-} and *hUGT1/Lxraβ*^{-/-} mice.

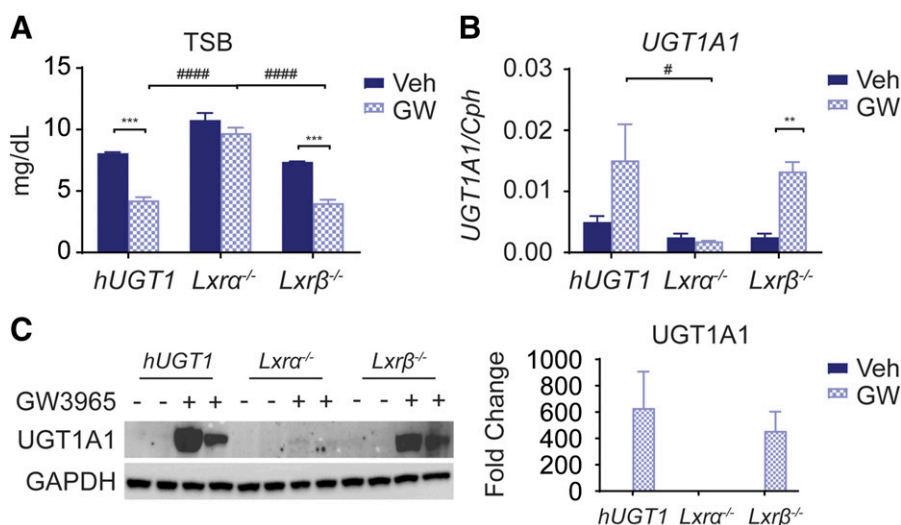


Fig. 5. Induction of UGT1A1 by the LXR agonist GW3965. Ten-day-old neonatal *hUGT1*, *hUGT1/Lxra*^{-/-} (*Lxra*^{-/-}), *hUGT1/Lxrβ*^{-/-} (*Lxrβ*^{-/-}), and *hUGT1/Lxraβ*^{-/-} (*Lxraβ*^{-/-}) mice were treated with 75 mg/kg T0901317 or vehicle (propylene glycol/Tween 80 4:1) by oral gavage. After 48 hours, serum was collected along with liver tissue. TSB values (A) were determined along with UGT1A1 gene expression (B). Liver tissue was prepared as outlined and used in Western Blot analysis to examine UGT1A1 expression (C).

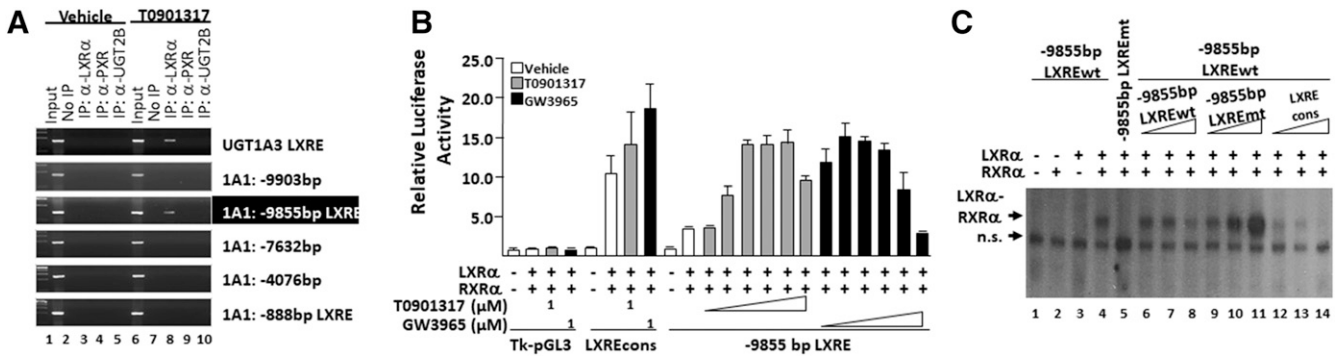


Fig. 6. Ligand-activated LXR α activates a positive LXRE at position -9855 in the UGT1A1 promoter. (A) Soluble chromatin was prepared from HepG2 cells treated with vehicle (ethanol-lanes 1–5), T0901317 (1 μ M-lanes 6–10) for 4 hours and immunoprecipitated with antibodies directed against LXR α (lanes 3 and 8), PXR (lanes 4 and 9), or with an anti-UGT2B antibody as a negative control (lanes 5 and 10). The final DNA extracts were amplified using pairs of primers covering the five degenerated DR4 motifs identified within the *UGT1A1* gene promoter or a previously reported LXRE from the human *UGT1A3* gene promoter (Verreault et al., 2006) as a positive control (a). 1/50 (input) or 1/10 (precipitated DNA) of PCR products were separated on an ethidium-bromide–stained 2% agarose gel. (B) HepG2 cells were transfected with the indicated LXRE-driven luciferase (Luc) reporter plasmids (TK-pGL3 constructs; 100 ng) in the absence or presence of LXR α and RXR α plasmids (10 ng) and a pRL-NUL expression plasmid (30 ng). Cells were treated for 24 hours with vehicle (ethanol) or increasing concentrations of T0901317 or GW3965 (0.1; 0.5; 1; 2.5; 5; and 10 μ M) as indicated. Values are expressed as fold induction over the control (TKpGL3, set at 1), normalized to internal renilla activity. (C) EMSA were performed with the end-labeled -9855 bp LXRE wild-type (wt) or mutated (mt) probes, in the presence of in vitro produced RXR α , LXR α , or both, as indicated. Competition of the wild type probe was performed by adding 5-, 25-, or 50-fold molar excess of the indicated cold competitor probe in EMSA with RXR α and LXR α . n.s., nonspecific binding.

When the *Lxr α* allele is either null or exists in the heterozygous state (*hUGT1/Lxr α ^{+/-}*), the expression of liver UGT1A1 is greatly reduced. This indicates that both *Lxr α* alleles are necessary to maintain the full expression of UGT1A1.

Discussion

Liver X receptors α and β have been shown to play pivotal roles in the transcriptional control of lipid metabolism (Edwards et al., 2002; Wagner et al., 2003; Wang and Tontonoz, 2018). Activated LXRs regulate the expression of genes that are linked to functional control of cholesterol absorption, transport, efflux, excretion, and conversion to bile acids (Peet et al., 1998a; Zhang et al., 2012). Under these conditions, the LXRs bind directly to these genes at LXR enhancer sequences to

induce transcription (Sabol et al., 2005). Also, LXRs regulate fatty acid metabolism by controlling the lipogenic transcriptional factor sterol regulatory element-binding protein 1c (Repa et al., 2000; Wagner et al., 2003) that in turn regulates genes that encode proteins involved in fatty acid elongation and desaturation (Schultz et al., 2000). Along with these important regulatory events, LXRs also drive the incorporation of polyunsaturated fatty acids into phospholipids. Each of these regulatory events leads to the proper homeostasis of the membrane environment and lipid composition, which if disrupted can impact diseases such as atherosclerosis, diabetes, fatty liver disease, and cancer.

Although there is approximately 78% amino acid similarity between LXR α and LXR β , the genes encoding these proteins in mice exist on different chromosomes, with *Lxr α* residing on chromosome 2 and *Lxr β* on chromosome 7. The evolutionary split of these genes indicates that their expression may differ in selective tissues coupled with changes in receptor function. The LXRs are activated by naturally occurring oxysterols and by synthetic nonsteroidal compounds such as T0901317 and GW3965. However, LXR α and LXR β are expressed differently with regards to abundance and tissue specificity. Annicotte et al. (2004) employed selectively in situ hybridization in embryonic and adult tissue to demonstrate that LXR α is highly expressed in metabolically active tissues such as the liver, intestine, and adipose tissue while LXR β is ubiquitously expressed. Although there is a commonality in the ability of ligands to activate the LXRs, there are several key examples that the LXRs elicit unique functional roles. For example, mice lacking LXR α accumulate massive amounts of cholesterol in the liver when fed a high-cholesterol diet (Peet et al., 1998b). This results from the inability of cytochrome P450 7A1, a key LXR α target gene, to metabolize cholesterol to bile acids. This does not occur when cholesterol is fed to LXR β -deficient mice. Numerous other examples have been reported favoring a selective advantage in controlling physiologic functions or gene regulation for one of the receptors over the other (Alberti et al., 2001; Jakobsson et al., 2014; Zhang et al., 2014; Whitfield et al., 2016; Endo-Umeda et al., 2018).

In the absence of ligand, the LXR/RXR complexes bind to transcriptional corepressors, such as the nuclear corepressor 1 (NCoR1), and repress target gene expression (Chen and Evans, 1995; Horlein et al., 1995; Li et al., 2013). Upon ligand binding, LXRs dissociate from NCoR1 and assemble with coactivators, leading to modulation of histone acetylation or chromatin remodeling, facilitating transcriptional

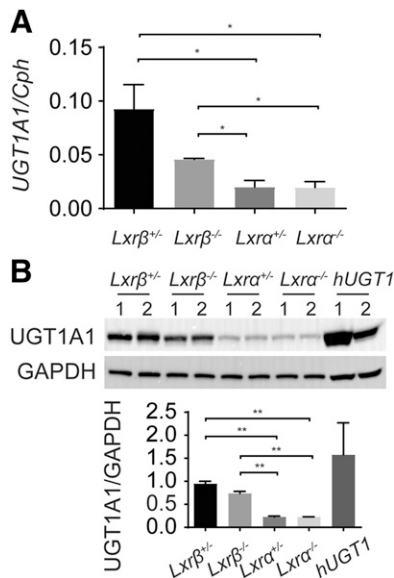


Fig. 7. Contribution of LXR toward the expression of UGT1A1 in adult liver. Adult livers from untreated *hUGT1*, *hUGT1/Lxr α ^{-/-}* (*Lxr α ^{-/-}*), *hUGT1/Lxr α ^{+/-}* (*Lxr α ^{+/-}*), *hUGT1/Lxr β ^{-/-}* (*Lxr β ^{-/-}*), and *hUGT1/Lxr β ^{+/-}* (*Lxr β ^{+/-}*) mice were collected to examine *UGT1A1* gene expression by qPCR (A) and protein expression by Western blot analysis (B).

activation. When NCoR1 is selectively deleted in target tissues, LXR becomes activated in the absence of ligand and stimulates transcription of target genes (Li et al., 2013). In neonatal *hUGT1* mice that are deficient in intestinal NCoR1, the *UGT1A1* gene was derepressed leading to the metabolism of serum bilirubin (Chen et al., 2017). Gene expression data indicated that LXR target genes were in part activated in *hUGT1/Ncor1*^{-/-} mice, leading us to examine in greater detail the role of LXR α and LXR β in the potential induction of the *UGT1A1* gene. Using neonatal *hUGT1* mice deficient in LXR α , LXR β , or both LXR α and LXR β that were orally treated with synthetic LXR agonists T0901317 or GW3695, induction of hepatic UGT1A1 was dominated by LXR α . However, the induction patterns of UGT1A1 and TSB values in these mice were quite different when we compared the impact of the two agents. The oral administration of GW3695 led to hepatic induction of UGT1A1 in *hUGT1* and *hUGT1/Lxr β* ^{-/-} mice only, which matched the reduction in TSB values. The absence of UGT1A1 induction in *hUGT1/Lxr α* ^{-/-} and *hUGT1/Lxr $\alpha\beta$* ^{-/-} mice establishes that activated LXR α underlies the induction process.

The most well-characterized synthetic LXR ligand to date is T0901317, but this agent elicited a different pattern of induction. Although the general pattern of UGT1A1 induction in these mice by T0901317 was like that of GW3695, a reduction in TSB was observed in all treatment groups. Also, there was considerable *UGT1A1* gene and protein expression detected in *hUGT1/Lxr α* ^{-/-} and *hUGT1/Lxr $\alpha\beta$* ^{-/-} mice, which showed little induction when these mice were treated with GW3695. We now believe that the induction of hepatic *UGT1A1* in *hUGT1/Lxr α* ^{-/-} and *hUGT1/Lxr $\alpha\beta$* ^{-/-} mice by T0901317 results from activation of additional nuclear receptors, such as PXR and CAR, and not LXR. It has been reported previously that T0901317 can activate PXR and effectively induce PXR target genes such as *Cyp3a11* in mice (Shenoy et al., 2004). PXR activation in neonatal *hUGT1* mice leads to the induction of UGT1A1 and a reduction in TSB levels (Chen et al., 2012; Fujiwara et al., 2012) and, in this study, dramatically induces liver CYP3A11 RNA in all four mouse lines. It is interesting to note that T0901317 induced *Cyp3a11* gene induction in *hUGT1/Lxr α* ^{-/-} and *hUGT1/Lxr $\alpha\beta$* ^{-/-} mice two- to threefold greater than in *hUGT1* and *hUGT1/Lxr β* ^{-/-} mice. This superinduction of the *Cyp3a11* gene by T0901317 in *hUGT1/Lxr α* ^{-/-} and *hUGT1/Lxr $\alpha\beta$* ^{-/-} mice indicates that LXR α expression serves to inhibit full activation of the PXR receptor or has inhibitory action associated with the *Cyp3a11* gene. Also, T0901317 induced hepatic CYP2B10 in a fashion that was independent of the expression of LXR α and LXR β . The induction of CYP2B10 was driven by activated CAR because no induction of UGT1A1 occurred in *hUGT1/Car*^{-/-} mice. It has been suggested that there is functional cross-talk between LXR α and CAR with activation of CAR leading to the inhibition of LXR target genes (Zhai et al., 2010). If this mechanism is in play following T0901317 exposure, which activates both LXR α and CAR, induction of LXR α target genes may be attenuated as a result of the inhibitory actions of CAR. The use of T0901317 as an LXR agonist to examine the physiologic impacts and gene expression patterns following LXR activation can be complicated knowing that it serves as an agonist for PXR, farnesoid X receptor (Houck et al., 2004), and CAR while inducing cross-talk between LXR α , CAR, and PXR.

With the treatment of *hUGT1* neonatal mice with T0901317 or GW3695 we present convincing evidence the LXR α is directly activating the *UGT1A1* gene in the liver, an event that leads to the metabolism and clearance of serum bilirubin. This analysis is based upon the induction patterns of UGT1A1 in *hUGT1* mice that are deficient either LXR α or LXR β in hepatic tissue. However, it does not account for the relative abundance of LXR α and LXR β in this tissue or the relative binding affinities of the ligands to the receptors. Few examples

document the abundance of these receptors in different tissues. Using LXR α and LXR β cDNAs to generate antisense RNA for in situ hybridization experiments, Annicotte (Annicotte et al., 2004) examined the developmental expression of the receptors in postcoitum at different days as well as in adult tissue. The conclusions from this experiment indicated that LXR α and LXR β were both expressed in liver tissue but the abundance of LXR β RNA dropped as the pregnancy came closer to term, with LXR β RNA being reduced in adult liver. However, LXR β is expressed in liver tissue. Because GW3695 has a greater binding affinity toward LXR β , we can be confident that both LXR α and LXR β are being activated in liver tissue following oral administration. With the identification of the LXRE binding sequence flanking the human *UGT1A1* gene and the complete absence of UGT1A1 induction by GW3695 in *hUGT1/Lxr α* ^{-/-} mice, our findings strongly implicate LXR α as a regulator of liver UGT1A1 gene expression.

The initial discovery that LXRs could be activated by oxysterols was the first of many clues suggesting that LXRs controlled cholesterol homeostasis, eventually implicating the production of bile acids and controlling lipogenesis (Wang and Tontonoz, 2018). Our findings have confirmed that LXR α is a potent regulator of the *UGT1A1* gene during development and could serve as a therapeutic target in events that require the regulation of accumulating serum bilirubin. It remains unclear the physiologic advantage of regulating the *UGT1A1* gene along with other genes of the *UGT1* locus by LXR α because there is no perceived link between oxysterols and the function of UGT1A1. One might consider, however, that oxysterols play an important role in maintaining adequate LXR-driven constitutive expression of the *UGT1A1* gene, which is essential in facilitating metabolism and elimination of the major heme metabolic product, bilirubin. Regardless, these findings suggest that events linking abnormal cholesterol and lipid homeostasis to the onset of lipogenic diseases will have an impact on the control and regulation of UGT1A1 and the functional properties of this important protein in endogenous and exogenous drug metabolism.

Authorship Contributions

Participated in research design: Hansmann, Mennillo, Yoda, Verreault, Barbier, Chen, Tukey.

Conducted experiments: Hansmann, Menillo, Yoda, Verreault, Chen.

Performed data analysis: Hansmann, Menillo, Yoda, Tukey.

Wrote or contributed to the writing of the manuscript: Hansmann, Barbier, Tukey.

References

- Alberti S, Schuster G, Parini P, Feltkamp D, Diczfalusy U, Rudling M, Angelin B, Björkhem I, Pettersson S, Gustafsson JA (2001) Hepatic cholesterol metabolism and resistance to dietary cholesterol in LXRbeta-deficient mice. *J Clin Invest* **107**:565–573.
- Alberti S, Steffensen KR, and Gustafsson JA (2000) Structural characterisation of the mouse nuclear oxysterol receptor genes LXRalpha and LXRbeta. *Gene* **243**:93–103.
- Annicotte JS, Schoonjans K, and Auwerx J (2004) Expression of the liver X receptor alpha and beta in embryonic and adult mice. *Anat Rec A Discov Mol Cell Evol Biol* **277**:312–316.
- Bosma PJ, Seppen J, Goldhoorn B, Bakker C, Oude Elferink RP, Chowdhury JR, Chowdhury NR, and Jansen PL (1994) Bilirubin UDP-glucuronosyltransferase 1 is the only relevant bilirubin glucuronidating isoform in man [published correction appears in *J Biol Chem* (1994) **269**:2542]. *J Biol Chem* **269**:17960–17964.
- Cai H, Nguyen N, Peterkin V, Yang YS, Hotz K, La Placa DB, Chen S, Tukey RH, and Stevens JC (2010) A humanized *UGT1* mouse model expressing the *UGT1A1**28 allele for assessing drug clearance by UGT1A1-dependent glucuronidation. *Drug Metab Dispos* **38**:879–886.
- Caputo M, De Rosa MC, Rescigno T, Zirpoli H, Vassallo A, De Tommasi N, Torino G, and Tecce MF (2014) Binding of polyunsaturated fatty acids to LXR α and modulation of SREBP-1 interaction with a specific SCD1 promoter element. *Cell Biochem Funct* **32**:637–646.
- Cha JY and Repa JJ (2007) The liver X receptor (LXR) and hepatic lipogenesis. The carbohydrate-response element-binding protein is a target gene of LXR. *J Biol Chem* **282**:743–751.
- Chen JD and Evans RM (1995) A transcriptional co-repressor that interacts with nuclear hormone receptors. *Nature* **377**:454–457.
- Chen S, Beaton D, Nguyen N, Senekeo-Effenberger K, Brace-Sinnokrak E, Argikar U, Rimmel RP, Trotter J, Barbier O, Ritter JK, et al. (2005) Tissue-specific, inducible, and hormonal control of the human UDP-glucuronosyltransferase-1 (*UGT1*) locus. *J Biol Chem* **280**:37547–37557.
- Chen S, Lu W, Yueh MF, Rettenmeier E, Liu M, Paszek M, Auwerx J, Yu RT, Evans RM, Wang K, et al. (2017) Intestinal NCoR1, a regulator of epithelial cell maturation, controls neonatal hyperbilirubinemia [published correction appears in *Proc Natl Acad Sci USA* (2017) **114**:E4115]. *Proc Natl Acad Sci USA* **114**:E1432–E1440.

- Chen S and Tukey RH (2018) Humanized *UGT1* mice, regulation of *UGT1A1*, and the role of the intestinal tract in neonatal hyperbilirubinemia and breast milk-induced jaundice. *Drug Metab Dispos* **46**:1745–1755.
- Chen S, Yueh MF, Evans RM, and Tukey RH (2012) Pregnane-x-receptor controls hepatic glucuronidation during pregnancy and neonatal development in humanized UGT1 mice. *Hepatology* **56**:658–667.
- Chu K, Miyazaki M, Man WC, and Ntambi JM (2006) Stearoyl-coenzyme A desaturase 1 deficiency protects against hypertriglyceridemia and increases plasma high-density lipoprotein cholesterol induced by liver X receptor activation. *Mol Cell Biol* **26**:6786–6798.
- Edwards PA, Kennedy MA, and Mak PA (2002) LXRs: oxysterol-activated nuclear receptors that regulate genes controlling lipid homeostasis. *Vascul Pharmacol* **38**:249–256.
- Endo-Umeda K, Nakashima H, Umeda N, Seki S, and Makishima M (2018) Dysregulation of kupffer cells/macrophages and natural killer T cells in steatohepatitis in LXR α knockout male mice. *Endocrinology* **159**:1419–1432.
- Forsberg EC, Downs KM, and Bresnick EH (2000) Direct interaction of NF-E2 with hypersensitive site 2 of the beta-globin locus control region in living cells. *Blood* **96**:334–339.
- Fujiwara R, Chen S, Karin M, and Tukey RH (2012) Reduced expression of UGT1A1 in intestines of humanized UGT1 mice via inactivation of NF- κ B leads to hyperbilirubinemia. *Gastroenterology* **142**:109–118.e2.
- Fujiwara R, Nguyen N, Chen S, and Tukey RH (2010) Developmental hyperbilirubinemia and CNS toxicity in mice humanized with the UDP glucuronosyltransferase 1 (*UGT1*) locus. *Proc Natl Acad Sci USA* **107**:5024–5029.
- Grefhorst A, van Dijk TH, Hammer A, van der Sluijs FH, Havinga R, Havekes LM, Romijn JA, Groot PH, Reijngoud DJ, and Kuipers F (2005) Differential effects of pharmacological liver X receptor activation on hepatic and peripheral insulin sensitivity in lean and ob/ob mice. *Am J Physiol Endocrinol Metab* **289**:E829–E838.
- Hong C and Tontonoz P (2008) Coordination of inflammation and metabolism by PPAR and LXR nuclear receptors. *Curr Opin Genet Dev* **18**:461–467.
- Hörlein AJ, Näär AM, Heinzl T, Torchia J, Gloss B, Kurokawa R, Ryan A, Kamei Y, Söderström M, Glass CK, et al. (1995) Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. *Nature* **377**:397–404.
- Houck KA, Borchert KM, Hepler CD, Thomas JS, Bramlett KS, Michael LF, and Burris TP (2004) T0901317 is a dual LXR/FXR agonist. *Mol Genet Metab* **83**:184–187.
- Hu DG, Meech R, McKinnon RA, and Mackenzie PI (2014) Transcriptional regulation of human UDP-glucuronosyltransferase genes. *Drug Metab Rev* **46**:421–458.
- Jakobsson T, Vedin LL, Hassan T, Venteclé N, Greco D, D'Amato M, Treuter E, Gustafsson JA, and Steffensen KR (2014) The oxysterol receptor LXR β protects against DSS- and TNBS-induced colitis in mice. *Mucosal Immunol* **7**:1416–1428.
- Johansson M, Bocher V, Lehto M, Chinetti G, Kuismanen E, Ehnholm C, Staels B, and Olkkonen VM (2003) The two variants of oxysterol binding protein-related protein-1 display different tissue expression patterns, have different intracellular localization, and are functionally distinct. *Mol Biol Cell* **14**:903–915.
- Li P, Spann NJ, Kaikkonen MU, Lu M, Oh DY, Fox JN, Bandyopadhyay G, Talukdar S, Xu J, Lagakos WS, et al. (2013) NCoR repression of LXRs restricts macrophage biosynthesis of insulin-sensitizing omega 3 fatty acids. *Cell* **155**:200–214.
- Lu W, Rettenmeier E, Paszek M, Yueh MF, Tukey RH, Trotter J, Barbier O, and Chen S (2017) Crypt organoid culture as an in vitro model in drug metabolism and cytotoxicity studies. *Drug Metab Dispos* **45**:748–754.
- Mangelsdorf DJ and Evans RM (1995) The RXR heterodimers and orphan receptors. *Cell* **83**:841–850.
- Millatt LJ, Bocher V, Fruchart JC, and Staels B (2003) Liver X receptors and the control of cholesterol homeostasis: potential therapeutic targets for the treatment of atherosclerosis. *Biochim Biophys Acta* **1631**:107–118.
- Mitro N, Vargas L, Romeo R, Koder A, and Saez E (2007) T0901317 is a potent PXR ligand: implications for the biology ascribed to LXR. *FEBS Lett* **581**:1721–1726.
- Nguyen N, Bonzo JA, Chen S, Chouinard S, Kelner MJ, Hardiman G, Bélanger A, and Tukey RH (2008) Disruption of the *ugt1* locus in mice resembles human Crigler-Najjar type I disease. *J Biol Chem* **283**:7901–7911.
- Oosterveer MH, Grefhorst A, Groen AK, and Kuipers F (2010) The liver X receptor: control of cellular lipid homeostasis and beyond Implications for drug design. *Prog Lipid Res* **49**:343–352.
- Peet DJ, Janowski BA, and Mangelsdorf DJ (1998a) The LXRs: a new class of oxysterol receptors. *Curr Opin Genet Dev* **8**:571–575.
- Peet DJ, Turley SD, Ma W, Janowski BA, Lobaccaro JM, Hammer RE, and Mangelsdorf DJ (1998b) Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXR alpha. *Cell* **93**:693–704.
- Quandt K, Frech K, Karas H, Wingender E, and Werner T (1995) MatInd and MatInspector: new fast and versatile tools for detection of consensus matches in nucleotide sequence data. *Nucleic Acids Res* **23**:4878–4884.
- Repa JJ, Liang G, Ou J, Bashmakov Y, Lobaccaro JM, Shimomura I, Shan B, Brown MS, Goldstein JL, and Mangelsdorf DJ (2000) Regulation of mouse sterol regulatory element-binding protein-1c gene (*SREBP-1c*) by oxysterol receptors, LXRalpha and LXRbeta. *Genes Dev* **14**:2819–2830.
- Ritter JK, Chen F, Sheen YY, Tran HM, Kimura S, Yeatman MT, and Owens IS (1992) A novel complex locus *UGT1* encodes human bilirubin, phenol, and other UDP-glucuronosyltransferase isozymes with identical carboxyl termini. *J Biol Chem* **267**:3257–3261.
- Sabol SL, Brewer HB Jr., and Santamarina-Fojo S (2005) The human ABCG1 gene: identification of LXR response elements that modulate expression in macrophages and liver. *J Lipid Res* **46**:2151–2167.
- Schultz JR, Tu H, Luk A, Repa JJ, Medina JC, Li L, Schwendner S, Wang S, Thoolen M, Mangelsdorf DJ, et al. (2000) Role of LXRs in control of lipogenesis. *Genes Dev* **14**:2831–2838.
- Senekeo-Effenberger K, Chen S, Brace-Sinnokrak E, Bonzo JA, Yueh MF, Argikar U, Kaeding J, Trotter J, Rimmel RP, Ritter JK, et al. (2007) Expression of the human UGT1 locus in transgenic mice by 4-chloro-6-(2,3-xylylidino)-2-pyrimidinylthioacetic acid (WY-14643) and implications on drug metabolism through peroxisome proliferator-activated receptor alpha activation. *Drug Metab Dispos* **35**:419–427.
- Shenoy SD, Spencer TA, Mercer-Haines NA, Alipour M, Gargano MD, Runge-Morris M, and Kocarek TA (2004) CYP3A induction by liver x receptor ligands in primary cultured rat and mouse hepatocytes is mediated by the pregnane X receptor. *Drug Metab Dispos* **32**:66–71.
- Shinar DM, Endo N, Rutledge SJ, Vogel R, Rodan GA, and Schmidt A (1994) NER, a new member of the gene family encoding the human steroid hormone nuclear receptor. *Gene* **147**:273–276.
- Song C, Kokontis JM, Hiipakka RA, and Liao S (1994) Ubiquitous receptor: a receptor that modulates gene activation by retinoic acid and thyroid hormone receptors. *Proc Natl Acad Sci USA* **91**:10809–10813.
- Strassburg CP, Manns MP, and Tukey RH (1997) Differential down-regulation of the *UDP-glucuronosyltransferase 1A* locus is an early event in human liver and biliary cancer. *Cancer Res* **57**:2979–2985.
- Tukey RH and Strassburg CP (2000) Human UDP-glucuronosyltransferases: metabolism, expression, and disease. *Annu Rev Pharmacol Toxicol* **40**:581–616.
- Ulven SM, Dalen KT, Gustafsson JA, and Nebb HI (2005) LXR is crucial in lipid metabolism. *Prostaglandins Leukot Essent Fatty Acids* **73**:59–63.
- Verreault M, Senekeo-Effenberger K, Trotter J, Bonzo JA, Bélanger J, Kaeding J, Staels B, Caron P, Tukey RH, 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.
- Wagner BL, Valledor AF, Shao G, Daige CL, Bischoff ED, Petrowski M, Jepsen K, Baek SH, Heyman RA, Rosenfeld MG, et al. (2003) Promoter-specific roles for liver X receptor/co-repressor complexes in the regulation of ABCA1 and SREBP1 gene expression. *Mol Cell Biol* **23**:5780–5789.
- Wang B and Tontonoz P (2018) Liver X receptors in lipid signalling and membrane homeostasis. *Nat Rev Endocrinol* **14**:452–463.
- Whitfield M, Ouvrier A, Cadet R, Damon-Soubeyrand C, Guiton R, Janny L, Kocer A, Marceau G, Pons-Rejraji H, Trousson A, et al. (2016) Liver X receptors (LXRs) alpha and beta play distinct roles in the mouse epididymis. *Biol Reprod* **94**:55.
- Willy PJ, Umesono K, Ong ES, Evans RM, Heyman RA, and Mangelsdorf DJ (1995) LXR, a nuclear receptor that defines a distinct retinoid response pathway. *Genes Dev* **9**:1033–1045.
- Yang N, Sun R, Liao X, Aa J, and Wang G (2017) UDP-glucuronosyltransferases (UGTs) and their related metabolic cross-talk with internal homeostasis: a systematic review of UGT isoforms for precision medicine. *Pharmacol Res* **121**:169–183.
- Yueh MF, Chen S, Nguyen N, and Tukey RH (2014) Developmental onset of bilirubin-induced neurotoxicity involves Toll-like receptor 2-dependent signaling in humanized UDP-glucuronosyltransferase1 mice. *J Biol Chem* **289**:4699–4709.
- Zelcer N and Tontonoz P (2006) Liver X receptors as integrators of metabolic and inflammatory signaling. *J Clin Invest* **116**:607–614.
- Zhai Y, Wada T, Zhang B, Khadem S, Ren S, Kuruba R, Li S, and Xie W (2010) A functional cross-talk between liver X receptor- α and constitutive androstane receptor links lipogenesis and xenobiotic responses. *Mol Pharmacol* **78**:666–674.
- Zhang X, Liu J, Su W, Wu J, Wang C, Kong X, Gustafsson JA, Ding J, Ma X, and Guan Y (2014) Liver X receptor activation increases hepatic fatty acid desaturation by the induction of SCD1 expression through an LXR α -SREBP1c-dependent mechanism. *J Diabetes* **6**:212–220.
- Zhang Y, Breevoort SR, Angdisen J, Fu M, Schmidt DR, Holmstrom SR, Kliever SA, Mangelsdorf DJ, and Schulman IG (2012) Liver LXR α expression is crucial for whole body cholesterol homeostasis and reverse cholesterol transport in mice. *J Clin Invest* **122**:1688–1699.

Address correspondence to: Robert H. Tukey, Department of Pharmacology, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0722. E-mail: rtukey@ucsd.edu