

PXR and the gut-liver axis: a recent update

Moumita Dutta*, Joe Jongpyo Lim*, and Julia Yue Cui

*Co-first author/equal contributions

Department of Environmental and Occupational Health Sciences

University of Washington, Seattle, WA

Running title: PXR and gut-liver axis

Address correspondence to:

Julia Yue Cui, PhD, DABT

Associate Professor

Department of Environmental and Occupational Health Sciences,

University of Washington

Email: juliacui@uw.edu

Manuscript pages: 41

Figures: 1

Tables: 1

Total word count:

- Abstract: 207
- Introduction: 891
- Discussion: 298

Keywords: PXR, liver, microbiome, bile acids, L-tryptophan, drugs

List of Abbreviations

AhR: aryl hydrocarbon receptor; BA: bile acids; bai: bile acid inducible operon; BPA: bisphenol A; CV: conventional; Cyp: cytochrome P450; DCA: deoxycholic acid; DDI: drug-drug interactions; DHCA: dehydrocholic acid; DME: drug-metabolizing enzymes; FGF19: fibroblast growth factor 19; FKK: Felix Kopp Kortagere; FXR: farnesoid X receptor; GF: germ free; HFD: high fat diet; hPXR: human pregnane X receptor; IPA: indole-3-propionic acid; KO: knock out; LBD: ligand binding domain; LCA: lithocholic acid; LPS: lipopolysaccharides; mPXR: mouse pregnane X receptor; NAFLD: non-alcoholic fatty liver disease; NASH: nonalcoholic steatohepatitis; Oatp: organic anion transporting polypeptide; PBDE: polybrominated diphenyl ethers; PCBs: polychlorinated biphenyls; PCN: pregnenolone-16 α -carbonitrile; PXR: pregnane X receptor; rPXR: rat PXR; RXR: retinoic X receptor; Slco: solute carrier organic anion transporter family; SPA-70: specific PXR antagonist # 70; TG: transgenic; WT: wild type; zfPXR: zebrafish PXR

ABSTRACT

It is well-known that the pregnane X receptor (PXR/Nr1i2) is a critical xenobiotic-sensing nuclear receptor enriched in liver and intestine and is responsible for drug-drug interactions (DDI), due to their versatile ligand binding domain (LBD) and target genes involved in xenobiotic biotransformation. PXR can be modulated by various xenobiotics including pharmaceuticals, nutraceuticals, dietary factors, and environmental chemicals. Microbial metabolites such as certain secondary bile acids (BAs) and the tryptophan metabolite indole-3-propionic acid (IPA) are endogenous PXR activators. Gut microbiome is increasingly recognized as an important regulator for host xenobiotic biotransformation and intermediary metabolism. PXR regulates and is regulated by the gut-liver axis. This review summarizes recent research advancements leveraging pharmaco- and toxico-metagenomic approaches that have redefined the previous understanding of PXR. Key topics covered in this review include 1) genome-wide investigations on novel PXR-target genes, novel PXR-DNA interaction patterns, and novel PXR-targeted intestinal bacteria; 2) key PXR-modulating activators and suppressors of exogenous and endogenous sources; 3) novel bi-directional interactions between PXR and gut microbiome under physiological, pathophysiological, pharmacological, and toxicological conditions; and 4) modifying factors of PXR-signaling including species- and sex difference, and time (age, critical windows of exposure, and circadian rhythm). The review also discusses critical knowledge gaps and important future research topics centering around PXR.

Significance statement:

This review summarizes recent research advancements leveraging O'mics approaches that have redefined the previous understanding of the xenobiotic-sensing nuclear receptor PXR. Key topics include: 1) genome-wide investigations on novel PXR-targeted host genes and intestinal bacteria as well as novel PXR-DNA interaction patterns; 2) key PXR modulators including microbial metabolites under physiological, pathophysiological, pharmacological, and toxicological conditions; and 3) modifying factors including species, sex, and time.

INTRODUCTION

Brief historical perspective

Prior to the discovery of the pregnane X receptor (PXR), it was initially discovered that newborn rats exhibited higher sensitivity to non-metabolized cardiac glycoside ouabain in comparison to adults (Klaassen, 1972; Klaassen, 1973). This increased sensitivity was due to the lower hepatic uptake in newborns, whereas pretreatment of both newborn and adult rats with pregnenolone-16 α -carbonitrile (PCN) markedly increased the hepatic uptake and biliary excretion of ouabain (Klaassen, 1974a; Klaassen, 1974b). PCN treatment in isolated rat hepatocytes markedly enhanced the uptake of another glycoside [³H] digoxin, which was a substrate of the organic anion transporter 2 (Oatp2, now known as Slco1a4/Oatp1a4) (Eaton and Klaassen, 1979). It was later noted that PCN stimulated the enzyme activity of a single form of cytochrome P450 (P-450PCN) that was immunochemically distinguishable from another CYP inducible by phenobarbital in rat liver (P450-PB) (Newman and Guzelian, 1983). In addition, glucocorticoids such as dexamethasone could also induce P-450PCN in rat liver, and the notion of the existence of a class of microsomal enzyme inducers was proposed (Schuetz et al., 1984). These pioneer studies together supported the existence of a receptor that can be activated by chemicals such as PCN, which in turn co-regulates the xenobiotic-inducible drug metabolism and transport systems (Staudinger et al., 2001).

At the turn of the century, the nuclear receptor PXR was discovered and was initially recognized as a major receptor for xenobiotics that trans-activate many genes involved in drug metabolism and transport including *Cyp3a* (previously known as P-450PCN) and *Oatp1a4* (previously known as *Oatp2*) (Moore and Kliewer, 2000; Kliewer et al., 2002; Tirona et al., 2003; Cheng et al., 2005; Maher et al., 2005; Kurihara et al., 2007; Petrick and Klaassen, 2007; Alnouti and Klaassen, 2008; Buckley and Klaassen, 2009; Hernandez et al., 2009; Aleksunes and Klaassen, 2012). PXR is highly expressed in the liver and

intestine and can be activated by various drugs, nutraceuticals, dietary factors, and environmental chemicals (Moore et al., 2003), leading to drug-drug, food-drug, and drug-toxicant interactions. While the DNA-binding domain of PXR is highly conserved across species (Kliewer et al., 2002), its ligand-binding domain displays more variability and versatility, which contribute to the species difference in PXR activation and target gene expression. While being well-recognized as a classic drug-receptor over the years, studies using PXR-null and humanized PXR transgenic mice have demonstrated that PXR also contributes to intermediary metabolism through worsening diet-induced obesity, hepatic steatosis, and hepatic inflammation (Spruiell et al., 2014a; Spruiell et al., 2014b; Kim et al., 2021). However, pharmacological activation of PXR has anti-inflammatory effects (Cheng et al., 2012; Huang et al., 2018). The physiological functions of PXR have also included transactivating genes involved in lipid metabolism (Zhou et al., 2006; Zhou et al., 2008) and cell cycle (Guzelian et al., 2006).

Recent research advancements leveraging pharmaco- and toxico-metagenomic approaches have redefined our understanding of PXR. First, genome-wide investigations using chromatin immunoprecipitation coupled with second-generation sequencing (ChIP-Seq) have expanded the list of direct-PXR target genes and identified novel PXR-DNA interaction patterns (Cui et al., 2010; Smith et al., 2014). While it was widely recognized based on *in vitro* investigations that PXR binds to AGGTCA-like direct repeats separated by 3 or 4 base pairs (DR-3 or DR4) as well as everted repeats 6 (ER-6) and ER-8 (Kliewer et al., 2002), an *in vivo* roadmap of PXR bindings in mouse liver with extended search for longer response elements have identified that DR-4 is the most prevalent PXR-DNA binding motif *in vivo*, and more importantly, there is a novel DR-(5n+4) periodic pattern of PXR-DNA interactions (defined as an “accordion model”) (Cui et al., 2010). In addition, PXR-DNA interactions within the target gene loci are not limited to promoter regions but are also localized to regions such as distal enhancers, downstream, and introns (Cui et al., 2010).

Second, while it was previously thought that PXR interacts with its binding partner retinoic X receptor (RXR) as a dimer, computational approaches have demonstrated that PXR and RXR function as a hetero-tetramer, with the unique β -stranded interface of the PXR homodimer in the middle, and two RXR proteins on the outside (Noble et al., 2006; Teotico et al., 2008). In addition, the key amino acids forming the PXR homodimer interface are highly conserved during evolution, and disruption of these amino acids results in decreased transcription activity of PXR (Noble et al., 2006; Teotico et al., 2008). Third, novel endogenous PXR ligands and/or activators have been identified, which are microbial metabolites of BAs and tryptophan (Staudinger et al., 2001; Xie et al., 2001; Venkatesh et al., 2014). Along the same lines, novel bi-directional interactions of PXR and the gut microbiome have been discovered under physiological, pathophysiological, pharmacological, and toxicological conditions (Cheng et al., 2018; Li et al., 2018; Dempsey et al., 2019; Scoville et al., 2019; Lim et al., 2020; Kim et al., 2021; Little et al., 2021). Last but not least, modifying factors including sex (Fu et al., 2012; Barretto et al., 2021), time – including liver development (Hart et al., 2009), and early life exposures to PXR-activating xenobiotics (Li et al., 2016a; Li et al., 2016b), aging (Fu et al., 2012), and circadian rhythm, have been further elucidated. Ongoing research studies continue to expand our knowledge base regarding endogenous functions of PXR and novel target genes, novel PXR ligands/modulators, and PXR vs. gut microbiome crosstalk.

This review will therefore provide an updated summary of the recent discoveries of PXR with a primary focus on PXR and the gut-liver axis within the context of exposures and discuss important further research topics in this area from our perspective.

PXR-modulating xenobiotics

PXR activators

PXR is responsible for the regulation of various drug-metabolizing enzymes (DME) following activation by various ligands and/or activators (Kliwer et al., 1998). Due to its

promiscuous LBD, a wide variety of possible ligands can interact with PXR and modulate complex DME expression through (drug-drug interaction) DDI (Lehmann et al., 1998). Many synthetic drugs, natural products, environmental chemicals as well as microbial metabolites and diets have been recognized as regulators of PXR signaling (Table 1) (Chang and Waxman, 2006). It should be noted that chemicals can only be classified as PXR ligands if the direct binding to the LBD of PXR has been established. It should also be noted that PXR-activating xenobiotics may have off-target effects which are not related to PXR signaling.

Synthetic drugs:

The anti-tuberculosis drug, rifampicin is a well-known ligand and activator of human PXR (hPXR), which binds and activates the receptor at a relatively low concentration (Blumberg et al., 1998; Moore et al., 2000), however, rifampicin does not activate the rodent PXR (Gibson et al., 2002). In rodents, the prototypical known PXR ligand is pregnenolone-16 α -carbonitrile (PCN) (Marek et al., 2005; Ma et al., 2008). Cell-based reporter assays have shown that activation of hPXR by rifampicin up-regulates the expression of *CYP3A4* in human, rabbit, pig, dog, and rhesus monkeys (Jones et al., 2000; Moore et al., 2000; Gibson et al., 2002). *In vitro* ligand assays showed that hPXR binds rifampicin (Jones et al., 2000), which then triggers the release of co-repressors and initiates recruitment of co-activators to promote transcription of PXR-target genes (Takeshita et al., 2002; Ourlin et al., 2003).

In addition to the prototypical ligands, many other therapeutic compounds can activate PXR either as direct ligands or indirect activators (Chang and Waxman, 2006). Dexamethasone is another PXR activator in both mice and humans (Pascussi et al., 2001; Yueh et al., 2005). The barbiturates anti-convulsant drugs, such as phenobarbital, are PXR activators in a species-specific manner. For example, phenobarbital preferably activates PXR in humans, pigs, and rabbits, but has little effect in rodents including mice and rats

(Jones et al., 2000; Moore and Kliewer, 2000; Smirlis et al., 2001; Wei et al., 2002; Chang and Waxman, 2006; Pinne et al., 2016). Specifically, phenobarbital enhances the recruitment of the steroid receptor coactivator-1 (SRC-1) to hPXR but not to mPXR (Li et al., 2019). The antifungal drug clotrimazole has been reported to bind to hPXR at a concentration of 10 μ M, stimulating co-activator recruitment and enhancing the transcription of PXR target genes (Lehmann et al., 1998; Jones et al., 2000), whereas clotrimazole is a weaker activator of rat and mouse PXR (Bertilsson et al., 1998; Jones et al., 2000). The protease inhibitor and anti-HIV drug ritonavir acts as a ligand of hPXR and up-regulates PXR-target gene expression in primary human hepatocyte cultures (Chang and Waxman, 2006). Cancer chemotherapeutic drugs, such as paclitaxel, activate hPXR in cell-based reporter assays (Synold et al., 2001) where it acts as an hPXR ligand and up-regulates the expression of PXR-target genes including *CYP3A4* and the multi-drug resistance 1 (*MDR1*) transporter (Kostrubsky et al., 1998). The muscle relaxant, etomidolone, has been identified as a novel selective PXR agonist in a stable hPXR-Luc HepG2 cell line and up-regulated *CYP3A4* mRNA expression in HepaRG cells in a PXR-dependent manner (Lynch et al., 2021). The calcium channel blockers, such as nifedipine, bendipine, and lacidipine, are used in the treatment of high blood pressure. Studies have reported that these drugs are associated with hPXR activation (Bertilsson et al., 1998; Chang and Waxman, 2006; Lynch et al., 2021). Several other drugs including carbamazepine (El-Sankary et al., 2001; Luo et al., 2002), bosentan (van Giersbergen et al., 2002), mevastatin (Raucy et al., 2002), tamoxifen (Desai et al., 2002; Zhuo et al., 2014), topotecan (Schuetz et al., 2002) have all been reported to be activators of hPXR in cell-based reporter gene assays. In addition, 11 additional drugs have recently been identified as novel hPXR activators (Lynch et al., 2021).

The activation of PXR by chemotherapeutic drugs can result in drug resistance and lower efficacy, partly through up-regulating *CYP3A4* (Chen, 2010; Mani et al., 2013).

Lin et al. investigated the therapeutic value of hPXR antagonists and the expression of *CYP3A4*. They found that SPA70 is a potent antagonist of hPXR, which ablated the expression of *CYP3A4*, suggesting that SPA70 can be a potential therapeutic as an hPXR antagonist (Lin et al., 2017).

Natural products:

Several plant-derived compounds have been identified as agonists and/or activators of hPXR or mPXR in cell-based reporter assays. For example, the active ingredient of St. John's wort, namely hyperforin, has been reported as a ligand of hPXR and the direct binding of hyperforin to the PXR ligand-binding domain has been visualized by X-ray crystallography (Watkins et al., 2003). Hyperforin up-regulates the PXR-target gene *CYP3A/Cyp3a* expression of both human and mouse origins in cell-based reporter assays (Cantoni et al., 2003; Komoroski et al., 2004). Guggulsterones are plant-derived sterol compounds that activate hPXR and mPXR, as evidenced by increased expression of PXR-target genes (Brobst et al., 2004; Ding and Staudinger, 2005; Chang and Waxman, 2006). The plant-derived diterpene forskolin is used in traditional medicine to treat cardiovascular disorders (Seamon et al., 1981) and acts as a ligand for both hPXR and mPXR, as evidenced by increased PXR-target gene expression in cell-based reporter gene assays (Ding and Staudinger, 2005). Artemisinin, a naturally occurring sesquiterpene, is an hPXR ligand that promotes interactions between PXR and its co-activator DRIP205 (Burk et al., 2005). However, artemisinin is known to decrease the basal expression of mPXR (Burk et al., 2005). Other plant-derived compounds such as the kava extract desmethoxyyangonin and dihydromethysticin also activate hPXR in cell-based reporter gene assays (Raucy, 2003; Ma et al., 2004).

Environmental chemicals:

Several environmental contaminants have been reported to activate PXR signaling or increase the expression of PXR in various animal species. These environmental contaminants include pesticides, steroids, plasticizers, heavy metals, etc. Several insecticides including organochlorines and pyrethroids have been reported to activate hPXR and up-regulate *CYP3A4* mRNA expression (Milnes et al., 2008). The herbicide alachlor has been reported to activate PXR *in vitro* and up-regulates *CYP3A* mRNA expression in rat liver (Mikamo et al., 2003; Lemaire et al., 2006). Using *in vitro* gene expression assays, 15 different classes of pesticides that act as hPXR activators have been identified as summarized in Table 1 (Lemaire et al., 2006). Activation of PXR and the corresponding increase in *Cyp3a* gene expression occur after exposure to nonylphenol in juvenile fish liver (Meucci and Arukwe, 2006). The pyrethroid insecticide cis-bifenthrin (cis-bf) up-regulates the mRNA and protein expression of PXR in the HepG2 cell line (Xiang et al., 2018). The heavy metal ions Ag^+ and Pb^{2+} up-regulate the PXR mRNA in embryonic zebrafish fibroblasts (Liu et al., 2019). In *CYP3A4* transgenic (TG) mice, arsenic has been reported to increase the mRNA expression of both *PXR* and *CYP3A4*, as well as *CYP3A4* protein expression (Medina-Diaz et al., 2009). Chronic uranium exposure has been reported to increase *PXR* mRNA expression in brain, liver, and kidney of rats (Souidi et al., 2005). The non-coplanar polychlorinated biphenyls (PCBs) have been reported to activate hPXR (Jacobs et al., 2005; Al-Salman and Plant, 2012). Bisphenol A (BPA) is an agonist of hPXR but not mPXR (Sui et al., 2012). Several formerly used flame retardants, such as the polybrominated diphenyl ethers (PBDEs) are activators of PXR of both human and mouse origins (Pacyniak et al., 2007; Sueyoshi et al., 2014). Phthalate esters are used in the manufacture of plastic products and are activators of both human and rodent PXR (Chang and Waxman, 2006).

Food components:

Several dietary components have been reported as activators of PXR of either humans or of other organisms. Mycotoxins are found in food of humans and animals and have been reported as activators of PXR (Ding et al., 2006; Ayed-Boussema et al., 2012a; Ayed-Boussema et al., 2012b). Aflatoxin B1, which is a potent liver carcinogen is a PXR activator and up-regulates the expression of PXR-target genes (Ayed-Boussema et al., 2012a; Daujat-Chavanieu and Gerbal-Chaloin, 2020). Patulin, another mycotoxin associated with fruits, has been reported to activate PXR and its target genes in primary human hepatocytes (Ayed-Boussema et al., 2012b). Molecular docking showed isorhamnetin, a flavonol compound present in various fruits and vegetables, is a hPXR ligand and subsequently represses the NF- κ B pathway (Dou et al., 2014). Menaquinone-4 (MK-4), a vitamin K2 analog and medication for osteoporosis, is a hPXR ligand and up-regulates PXR-target gene expression in both *in vivo* and *in vitro* experiments (Tabb et al., 2003; Avior et al., 2015; Sultana et al., 2018). Vitamin E is a potent activator of hPXR with similar efficacy as rifampicin (Landes et al., 2003). A high fat diet (HFD) can lead to enhanced PXR signaling in mice (Saraswathi et al., 2017). The nutritional factor alpha-ketoglutarate (AKG) can enhance PXR activity and down-regulate the NF- κ B signaling pathway, although the molecular mechanism needs to be further explored (He et al., 2017).

PXR Repressors

While most studies in the literature have focused on PXR activators, PXR repressors have also been identified as summarized in Table 1. Phytoestrogens and phytochemicals including coumestrol and sulforaphane bind to the LBD of hPXR and inhibit the expression of PXR-target genes in both hepatocytes and in livers of hPXR-TG mice (Wang et al., 2008). Sulforaphane (SFN), a phytochemical present in broccoli, binds to the LBD of hPXR and antagonizes the PXR signaling (Zhou et al., 2007). The antifungal drug ketoconazole is a well-known repressor of PXR activity in HepG2 liver cancer-derived cells, human

hepatocytes, LS174T colon cancer-derived cells, as well as CV-1 cells co-transfected with plasmids expressing either pCMX-hPXR or pCMX-mPXR along with a CYP3A4 luciferase reporter plasmid (Huang et al., 2007). The anti-neoplastic chemical ET-743 suppresses hPXR signaling and down-regulates *MDR1* gene expression *in vitro* (Synold et al., 2001). The specific PXR antagonist # 70 (SPA-70) is another compound that serves as a potent and selective repressor of hPXR (Lin et al., 2017). Lipopolysaccharides (LPS), which is produced by resident gram-negative bacteria, inhibits PXR expression and down-regulates *Cyp3a11* mRNA expression in mouse liver (Xu et al., 2004; Xu et al., 2005). Ochratoxin A, a potent mycotoxin produced by *Aspergillus sp* is a repressor of PXR in the HepG2 cell line (Doricakova and Vrzal, 2015; Daujat-Chavanieu and Gerbal-Chaloin, 2020; Shen et al., 2020).

Regulation of complex gut-liver cross-talk

Previously, it is well known that the major bile acid sensor farsenoid X receptor (FXR) is the primary receptor regulating the expression of host bile acid processing genes within the gut-liver axis. The intestinal activation of FXR by bile acids activate *Fgf15* in mice and *FGF19* in humans, which provides a negative feedback regulation of the host hepatic bile acid biosynthesis through down-regulating *Cyp7a1* – the rate-limiting bile acid synthetic enzyme in liver (Wang et al., 2012; Li and Chiang 2015; Xiang et al., 2021). In addition to FXR, emerging research showed that PXR also contributes to the regulation of gut-liver crosstalk. The nuclear receptor PXR is expressed in several tissues in the body including kidney, lung, placenta, ovary but PXR is predominantly expressed in the liver and small intestine (Mohandas, 2017; Kliewer, 2002; Burk, 2004). The intestinal-derived secondary bile acid LCA activates PXR in mouse liver and thus increases the expression of the PXR-target gene *Cyp3a11* (Staudinger et al., 2001). In addition, PXR transcriptionally regulates several bile acid transporters within the gut-liver axis (*Ntcp*, *Ost alpha*, *Ost beta*, and

Oatp1a4) (Beaudoin et al., 2020; Stauginder et al., 2001). However, organ-specific PXR-signaling has been observed. For example, the microbial tryptophan metabolite IPA is a PXR activator in intestine as evidenced up-regulation of the PXR-target gene Mdr1 in mouse jejunum (Venkatesh et al., 2014), however, it does not readily activate PXR in mouse liver (Morgan et al., 2018). In contrast, other ligands such as St. John's Wort extract (with hyperforin being one of the active ingredients) increase the expression and activity of PXR-target genes in both intestine and liver (Durr et al., 2000; Moore et al., 2000; Cantoni et al., 2003). In summary, in addition to FXR, PXR also contributes to the regulation of genes involved in bile acid homeostasis and xenobiotic biotransformation within the gut-liver axis.

PXR, novel endogenous PXR activators, and the gut microbiome

The interactions between host PXR and gut microbiome are bi-directional. First, distinct microbial metabolites act as PXR activators (Table 1) under pathophysiological conditions to modulate the expression of PXR-target genes in a tissue-specific manner. Second, PXR of the host impacts the gut microbiome composition and functions under physiological, pharmacological, and toxicological conditions. This section will summarize the key findings regarding the bi-directional interactions between the host PXR signaling and the gut microbiome.

Regulation of the host PXR signaling by microbial metabolite

The most hydrophobic secondary bile acid (BA) generated from dehydroxylation reactions by the gut microbiota is lithocholic acid (LCA), which is the most toxic BA to hepatocytes at high concentrations, such as during cholestasis (Fickert et al., 2006; Woolbright et al., 2014; Xu et al., 2020). Toxic levels of LCA and other secondary BAs produce liver damage through inflammation, oxidative stress, DNA damage, and cell death, leading to both liver and colon cancers (Allen et al., 2011; Ajouz et al., 2014; Woolbright et al., 2014; Jia and Jeon, 2019). Dietary supplement with 0.5% LCA increased the mRNA of the prototypical PXR-target gene cytochrome P450 3a11 (*Cyp3a11*) in a PXR-dependent

manner in livers of adult male mice; in addition, PXR serves as a sensor of LCA to protect against hepatotoxicity (Staudinger et al., 2001). LCA trans-activates PXR of both human and mouse origins in luciferase reporter assays, and it increased *Cyp3a11* mRNA in a PXR-dependent manner (8 mg per day for 4 days via oral gavage) (Xie et al., 2001). Gastrectomy increased the endogenous LCA, LCA-producing intestinal bacteria *Bacteroides fragilis*, PXR nuclear translocation, and *Cyp3a11* mRNA expression in mouse liver (Ishii et al., 2014). Because the major BA sensor farnesoid X receptor (FXR) is not activated by LCA, it is thought that LCA-mediated activation of PXR provides an important compensatory protective mechanism to reduce LCA overload and ameliorate liver injury (Jung et al., 2006; Ishii et al., 2014). In addition to LCA, deoxycholic acid (DCA) – another microbially derived secondary BA, can also trans-activate PXR (Xie et al., 2001).

The bacteria-derived tryptophan metabolite indole 3-propionic acid (IPA) is a PXR ligand in the mouse intestine (Venkatesh et al., 2014). Produced by the intestinal bacteria *Clostridium sporogenes*, IPA down-regulates the pro-inflammatory cytokine tumor necrosis factor- α (TNF α) in enterocytes and up-regulates the mRNAs encoding tight junction proteins and protects the gut barrier integrity in a PXR-dependent manner (Venkatesh et al., 2014). While IPA activates PXR in the mouse intestine, it does not activate PXR in the mouse liver (Morgan et al., 2018). However, circulating IPA activates PXR in the vascular endothelium of mice to reduce the vasodilatory responses to nitric oxide in isolated and cultured vessels in a PXR-dependent manner. This suggests that IPA-mediated PXR activity plays a key role in endothelial function (Pulakazhi Venu et al., 2019). This also indicates that PXR activation by IPA is tissue specific.

Based on the conclusion above that IPA activates PXR, it was proposed that microbial indole metabolite mimicry might be a novel strategy for drug discovery. In fact, the functionalized indole derivatives have generated first-in-class non-cytotoxic PXR agonists. The lead compounds are Felix Kopp Kortagere (FKK) 5 and FKK6 that activate PXR to

reduce inflammation. FKK6, later called CVK003, is a direct PXR-binding ligand and induces PXR-target gene expression in cells, human organoids, and mice. Through PXR activation, FKK6 represses pro-inflammatory cytokines in humanized PXR transgenic mice (Dvorak et al., 2020a). The removal of the phenyl-sulfonyl group of FKK6 shifts the agonist activity away from PXR towards the aryl hydrocarbon receptor (AhR), while the addition of the imidazole pyridyl group preserves PXR activity *in vitro* (Li et al., 2021). Targeting the microbial indole metabolite mimicry serves as an important mechanism to understand the crosstalk between PXR and other important xenobiotic-sensing transcription factors such as the AhR (Dvorak et al., 2020b; Li et al., 2021).

Necessity of the gut microbiome in the host PXR signaling under basal and toxicological conditions

The role of the gut microbiome in modulating the basal PXR signaling was first noted by quantifying the expression and enzyme activities of the prototypical PXR-target gene *Cyp3a* in livers of germ free (GF) mice (Toda et al., 2009b) and in livers of antibiotic-treated conventional (CV) mice (Toda et al., 2009a). Ciprofloxacin, which is a quinolone antibiotic, decreases *Cyp3a11* expression and triazolam metabolism in mouse liver by reducing LCA-producing intestinal bacteria, whereas LCA treatment increased *Cyp3a11* expression in GF mice (Toda et al., 2009a). Another study showed that most of the major Cyps and the PXR gene expression were lower in the livers of GF mice (Toda et al., 2009b), suggesting that intestinal bacteria maintain constitutive PXR-signaling in mouse liver. Transcriptomic-wide investigations including microarray (Bjorkholm et al., 2009) and RNA-Seq sequencing (Selwyn et al., 2015b) in livers of CV and GF mice further demonstrated that gut microbiome is necessary for maintaining the basal expression of various xenobiotic-processing genes and PXR-signaling. In fact, xenobiotic metabolism ranked among the topmost differentially regulated networks in the entire liver transcriptome of adult male GF mice (Selwyn et al., 2015a). The GF-mediated down-regulation of *Cyp3a11* gene expression is modified by age

and blunted the ontogenic increase of *Cyp3a11* in mouse liver (Selwyn et al., 2015a). Interestingly, conventionalization through exposing the GF mice with feces from CV mice restored the constitutive expression of *Cyp3a11* mRNA and protein in the liver (Selwyn et al., 2016), and this observation further confirmed the necessity of gut microbiome in the basal expression of this PXR-target gene in the liver. Extended investigations in various sections of the intestine of CV and GF mice showed that the gut microbiome is also important in maintaining the constitutive expression of *Cyp3a* in an intestinal section-specific manner (Fu et al., 2017). Therefore, the gut microbiome is a functional modifier of PXR and *Cyp3a*-mediated drug metabolism in the liver and intestine under basal conditions (Dempsey and Cui, 2019).

Following exposure to known PXR-activating chemicals, the gut microbiome may serve as a novel modifier of PXR-target gene expression in the liver. The previously used flame retardant polybrominated diphenyl ethers (PBDEs) are bio-accumulative and thus persistent in the environment long after their use was banned. Among the 209 possible PBDE congeners, the non-coplanar BDE-47 and BDE-99 are known to activate PXR in mouse liver (Pacyniak et al., 2007). Interestingly, oral exposure to BDE-47 and BDE-99 up-regulated the mRNA of many drug-processing genes, including the PXR-target gene *Cyp3a11* in mouse liver. This up-regulation was further augmented by the lack of gut microbiome (Li et al., 2017), suggesting that the gut microbiome inactivates the PBDE parent compounds or metabolites, thus less PXR activators are available under CV conditions. In serum of CV mice, oral exposure to BDE-47 and BDE-99 decreased the tryptophan microbial metabolite indole-3-propionic acid (IPA) (Scoville et al., 2019), which is a known endogenous PXR ligand in mouse intestine (Venkatesh et al., 2014). The imbalance between endogenous vs. exogenous PXR activators following xenobiotic exposure may serve as a contributing mechanism for drug-drug and food-drug interactions.

Regulation of gut microbiome by PXR-activating xenobiotics

While the intestinal microbiome modulates the basal and inducible PXR signaling as discussed above, the host PXR gene and exposure to PXR activators can reciprocally modulate the gut microbiome composition and metabolism. Following oral exposure to PCN, which is the prototypical PXR ligand in mice, there was a distinct change in the gut microbiome as evidenced by the principal coordinate analysis of beta diversity (Dempsey et al., 2019). At the class level, PCN decreased *Actinobacteria* and a taxon in the *Firmicutes* phylum but increased *Gammaproteobacteria*. At the genus level, PCN decreased *Dorea*, *Anaeroplasma*, and a taxon in the *Peptococcaceae* family, but increased *Ruminococcus*. In addition, PCN decreased two taxa in *Bifidobacterium*, which is known to have bile acid (BA) deconjugation activities (Foley et al., 2019), which corresponds to decreased gene abundance of the bile salt hydrolase (*bsh*) in the microbial DNA from PCN-exposed mice and reduced expression (Dempsey et al., 2019). PCN also tended to decrease the microbial DNA encoding the bile acid-inducible operon (*bai*) *CD* and *baiJ*, although a statistical significance was not achieved. In livers of PCN-exposed CV mice, there was a decrease in the total secondary BAs, and this was due to a significant reduction in deoxycholic acid (DCA), 3-dehydrocholic acid (3-DHCA), 12-dehydrocholic acid (12-DHCA), and a trend of a decrease (not significant) in tauro-lithocholic acid (T-LCA) and tauro-hyodeoxycholic acid (T-HDCA) (Dempsey et al., 2019). In summary, pharmacological activation of PXR by its prototypical ligand PCN down-regulates distinct BA-metabolizing intestinal bacteria and alters BA homeostasis.

Following exposure to PXR-activating therapeutic drugs, the gut microbiome can also be modulated. The cholesterol-lowering statins are known PXR activators (Howe et al., 2011; Hoffart et al., 2012). Two PXR-activating statins, namely atorvastatin and pravastatin, produce gut dysbiosis and reduced butyrate production in a PXR-dependent manner in mice (Caparros-Martin et al., 2017).

Following exposure to PXR-activating environmental chemicals, gut dysbiosis has been observed (Cheng et al., 2018; Li et al., 2018; Scoville et al., 2019; Cruz et al., 2020; Lim et al., 2020; Gomez et al., 2021), although caution is needed in interpreting the results, as these environmental toxicants may also have off-target effects on the gut microbiome independent from PXR activation. Oral exposure to BDE-47 and BDE-99, which are known to activate PXR and CAR in mouse liver (Pacyniak et al., 2007), produced profound gut dysbiosis as evidenced by decreased alpha diversity and 45 differentially regulated bacteria in the large intestinal content of adult male mice (Li et al., 2018). Most notably, there was an increase in *Akkermansia muciniphila* and *Erysipelotrichaceae Allobaculum spp.*, which have been reported to have anti-inflammatory and anti-obesity functions (Png et al., 2010; Ravussin et al., 2012; Derrien et al., 2017). BDE-99 increased many unconjugated BAs in multiple bio-compartments in a gut microbiome-dependent manner, which correlates with an increase in microbial 7 α -dehydroxylation enzymes for secondary BA synthesis and increased expression of host intestinal transporters for BA absorption. PBDEs also down-regulated host BA-synthetic enzymes and transporters in mouse livers in a gut microbiome-dependent manner (Li et al., 2018). In addition to BAs, BDE-47 and BDE-99 also modulated other intermediary metabolites such as those from amino acid and carbohydrate metabolism (Scoville et al., 2019). Among serum, liver, as well as small and large intestinal content (SIC and LIC, respectively), the LIC is the bio-compartment where PBDEs altered the largest number of aqueous metabolites, and most of the differential regulation was observed in the GF mice. For example, the gut microbiome was necessary for PBDE-mediated down-regulation in branched-chain and aromatic amino acid metabolites, whereas gene-metabolite networks revealed a positive association between the mRNA expression of the hepatic glycan synthesis gene α -1,6-mannosyltransferase (*Alg12*) and mannose, which are important for protein glycosylation (Scoville et al., 2019). Thus, the lack of gut microbiota augmented the PBDE-mediated effects on intermediary metabolism in adult male

mice. Maternal exposure to BDE-47 produced persistent gut dysbiosis in adult male mouse pups, and persistently increased fecal and hepatic BAs within the 12 α hydroxylation pathway, which corresponds to the up-regulation of the hepatic rate-limiting BA-synthetic enzyme *Cyp7a1* (Gomez et al., 2021). For all the studies described above, follow-up investigations using PXR-null mice are necessary to determine whether the observed effects by PBDEs are PXR-dependent. The PBDE-mediated dysbiosis in human microbiome was further investigated in a fermentation system using fresh human stool, and it was shown that PBDEs produced an imbalance in sulfur, short-chain fatty acids, and aromatic organic compounds, and altered the microbial volatolome in a dose- and time-dependent manner (Cruz et al., 2020).

Several non-coplaner congeners of the Fox River polychlorinated biphenyls (PCBs) mixture are known PXR activators, and oral exposure to the Fox River mixture (6 mg/kg) produced gut dysbiosis including an up-regulation in *A. muciniphila*, *Clostridium scindens*, and *Enterococcus* in large intestinal pellet of adult female CV mice. There was also an increase in multiple BAs in serum and small intestinal pellets in a gut microbiota-dependent manner, and Pearson's correlation analysis identified a positive correlation between 5 taxa and most secondary BAs (Cheng et al., 2018). At the dose of 30 mg/kg of PCBs, NADP and arginine are predicted to interact with the drug-metabolizing enzymes within the Cyp1-3 family, and this was also highly correlated with the presence of *Ruminiclostridium* and *Roseburia*. This suggests a novel role of the gut-liver axis in PCB-mediated effect on intermediary metabolism (Lim et al., 2020). In addition to the Fox River PCB mixture, the Aroclor1260 PCB mixture also altered the composition of the gut microbiome in a diet-induced obese mouse model and altered beta diversity in a PXR-dependent manner (Wahlang et al., 2021).

Regulation of gut microbiome by the host PXR gene under basal and pathophysiological conditions

While PXR has been extensively studied and well-recognized as a xenobiotic-sensing receptor for drugs, environmental chemicals, and nutraceuticals, very recent studies showed that this classic drug receptor also plays important biological functions under basal conditions (Little et al., 2021) and pathophysiological conditions, such as diet-induced nonalcoholic steatohepatitis (NASH) and toxicant-induced nonalcoholic steatohepatitis (TASH) (Spruiell et al., 2014a; Spruiell et al., 2014b; Wahlang et al., 2021). Pharmacological activation of PXR also has anti-inflammatory functions (Cheng et al., 2012). Interestingly, such novel PXR-mediated functions are associated with PXR-dependent modulation of the gut microbiome and microbial metabolites.

Under physiological conditions, studies using PXR-null mice have shown that the absence of PXR increased the microbial richness and the pro-inflammatory bacteria (*Helicobacteraceae* and *Helicobacter*), and decreased the fecal levels of many abundant taurine-conjugated BAs. Thus PXR may function to maintain gut flora and immune surveillance under basal conditions (Little et al., 2021). The suppression of microbial abundance by PXR under basal conditions was also independently observed in another study (Wahlang et al., 2021). Interestingly, the basal effect of PXR on the gut microbiome was distinct from pharmacological and toxicological activation of PXR (Cheng et al., 2018; Li et al., 2018; Dempsey et al., 2019; Lim et al., 2020; Little et al., 2021). *Bona fide* PXR-targeted intestinal bacteria *Dorea*, *Mogibacteriaceae*, *Ruminococcaceae*, *Streptococcus*, and *Anaeroplasma* were consistently suppressed by PXR under both basal and PXR-activated conditions (Cheng et al., 2018; Li et al., 2018; Dempsey and Cui, 2019; Lim et al., 2020; Little et al., 2021). Interestingly, hPXR-TG mice had a distinct microbial profile and a general trend of reduced fecal DCA compared to wild-type mice, suggesting that the species difference in the PXR protein may lead to different gut microbiome configurations and functions (Little et al., 2021).

The PXR gene can produce adverse effects in pathophysiological conditions. In a high fat diet (HFD)-induced obese mouse model, it was demonstrated that the presence of PXR worsens obesity as evidenced by protection from weight gain and liver steatosis in PXR-null mice (Spruiell et al., 2014a; Spruiell et al., 2014b). PXR activation is also detrimental in the regulation of glucose metabolism (Hukkanen et al., 2014). Specifically, in mice, PXR-deficiency improves HFD induced obesity and genetically induced obesity (i.e. the ob/ob mice), which are models for type-II diabetes (He et al., 2013; Zhao et al., 2017). Interestingly, humanized PXR-transgenic mice (hPXR-TG) also carry a genetic predisposition for type-II diabetes (Spruiell et al., 2014a). PXR-TG mice have impaired glucose utilization, elevated fasting glucose levels, and severely impaired glucose tolerance during high fat diet treatment, suggesting there is a species difference of PXR in regulating obesity and glucose signaling (Spruiell et al., 2014a; Spruiell et al., 2014b). Interestingly, the HFD-mediated increase in intestinal *Firmicutes/Bacteroidetes* ratio, which is a hallmark for obesity and increased energy harvest from diet (Turnbaugh et al., 2006), was completely abolished in male PXR-null mice. There was also a PXR-dependent, HFD-mediated decrease in the anti-obese *Allobaculum* and the anti-inflammatory *Bifidobacterium* (Kim et al., 2021). The PXR-dependent gut dysbiosis following a HFD-induced obesity was associated with PXR-enhanced weight gain, hepatic steatosis, inflammation, as well as PXR-dependent up-regulation in hepatic genes involved in microbial response, inflammation, oxidative stress, and cancer (Kim et al., 2021). In contrast to the higher susceptibility to HFD-induced non-alcoholic fatty liver disease (NAFLD) phenotype in male mice, the resistance to NAFLD in females may be explained by a PXR-dependent decrease in pro-inflammatory bacteria (*Ruminococcus gnavus* and *Peptococcaceae*) (Kim et al., 2021). In a TASH mouse model exposed to both HFD and the Aroclor1260 PCB mixture, PXR-dependent gut dysbiosis was also noted including increased *Actinobacteria* and *Verrucomicrobia* abundance in PXR-null mice (Wahlang et al., 2021). In conclusion,

PXR exacerbates hepatic steatosis and inflammation accompanied by obesity- and inflammation-prone gut microbiome signature, suggesting that gut microbiome may contribute to PXR-mediated exacerbation of obesity.

Modifying factors of PXR-signaling

Species difference

Overview

It's well-known that there are species differences in PXR activity (Jones et al., 2000; LeCluyse, 2001; Xie and Evans, 2001). Activation and repression of PXR among various species contribute to species-specific efficacy and toxicity of pharmaceuticals and other xenobiotics in humans, animals, and the ecosystem (Pinne et al., 2016; Creusot et al., 2021). As discussed earlier, rifampicin, an anti-tuberculosis drug, and SR12813, selectively activates PXR in humans and rabbits, whereas PCN is a classical synthetic compound that activates PXR in rodents (Li and Chiang, 2005; Marek et al., 2005; Li and Chiang, 2006; Ma et al., 2008). Using PXR and its prototypical target gene *CYP3A/Cyp3a* expression as a read-out, previous investigations revealed similarities and differences in activation of PXR and toxicities from using endogenous and exogenous compounds in hPXR, mPXR, rat PXR (rPXR), and zebrafish PXR (zfPXR) (Reschly and Krasowski, 2006; Reschly et al., 2007; Krasowski et al., 2011). For example, tropanyl 3,5-dimethylbenzoate, a 5-HT₃ receptor agonist, activates hPXR but not rPXR (Shukla et al., 2011). The gut microbiota is capable of producing vitamin K₂ (Conly and Stein, 1992). Interestingly, vitamin K₂ acts as a PXR activator in humans but not in zebrafish (Creusot et al., 2021).

Although many chemicals activate PXR in a species-specific manner, common PXR-activating compounds across multiple species have also been identified. Using human cell lines including LS174T, LoVo, HCT116, and a mouse xenograft model, activation of PXR has been observed in both humans and mice using rifampicin and PCN, respectively, along with enhanced colon cancer cell growth, proliferation, invasion, and metastasis through

activation of fibroblast growth factor 19 (FGF19) signaling (Wang et al., 2011). The microbial-derived secondary BAs LCA and DCA, as well as the primary BA CDCA activates PXR in humans and rodents (Xie et al., 2001). Benzo[a]pyrene, a polycyclic aromatic hydrocarbon, activates hPXR and mPXR, but not zfPXR (Cui et al., 2017; Creusot et al., 2021). Pharmaceuticals, such as hyperforin, mevastatin, and n-butyl-4-aminobenzoate, activates hPXR but not zfPXR (Creusot et al., 2021). Other chemicals within classes of pharmaceuticals, phytochemicals, environmental chemicals, endogenous compounds, and pesticides, and their examples of PXR activation are listed in Table 1.

Evolutionary differences of PXR across species

The vitamin D receptor (VDR) and PXR are suggested to have been separated from a duplication process of a common ancestral gene (Reschly and Krasowski, 2006). Due to speciation and evolutionary pressure, a distinction of functional differences in PXR and its LBD have likely arisen (Reschly and Krasowski, 2006; Reschly et al., 2007; Krasowski et al., 2011). It was shown that hPXR, mPXR, rPXR, and zfPXR share approximately 95% identity in their DNA binding domain (DBD) but share only 75-80% in their amino acid sequences in the LBD (LeCluyse, 2001). This indicates that species-specific PXR activation is likely a result of adaptation to different environmental exposures (LeCluyse, 2001; Ekins et al., 2008), and it is supported by structural analysis of the PXR LBD (Watkins et al., 2001; Ngan et al., 2009). The amino acid substitution may result in changes in the LBD. In the PXR LBD, five out of nine amino acids in mPXR are conserved in hPXR, and the remaining four residues, Phe184, Arg203, Lys334, and Ser414, are substituted by Ser187, Leu203, Glu334, and Ile414, respectively (Zhang et al., 1999; LeCluyse, 2001). Conservation of the five amino acids between mPXR and hPXR supports activation of PXR by certain chemicals, such as LCA in humans and mice. Therefore, substitutions in amino acid residues are likely responsible for the differential response of PXR in various species.

Sex difference in PXR activity

The expression and activity of PXR and its targets differ between males and females. In mouse models, the expression of *Cyp3a11* and *Cyp3a25*, the prototypical targets of PXR in mice, is higher in females than males (Cui et al., 2012; Lu et al., 2013). Likewise, expression levels of PXR targets are slightly different between male and females in humans (Wolbold et al., 2003; Scandlyn et al., 2008). Because PXR is a critical component in xenobiotic biotransformation, characterizing and investigating the sex difference of PXR from xenobiotic exposures and diseases remain an important task. Correspondingly, drugs that are substrates of CYP3A4, such as cyclosporine, erythromycin, nifedipine, and ifosfamide have higher clearance rates in women (Austin et al., 1980; Kahan et al., 1986; Krecic-Shepard et al., 2000; Schmidt et al., 2001), which may lead to gender-dependent dosage regimens and toxicological effects because of species difference in the PXR-CYP3A4 pathway (Tanaka, 1999; Parkinson et al., 2004; Scandlyn et al., 2008).

Regarding the sex difference in toxicological responses related to PXR signaling, the hepatic *Cyp3a11* gene expression from exposure to PCBs is higher in female mice than in male mice (Wahlang et al., 2019). Exposure to LCA to PXR-null mice produced cholestasis more prominently in males than in females (Uppal et al., 2005). When wild type (WT) and *Cyp3a* null mice were fed 60% high fat diet, female *Cyp3a* knockout (KO) mice gained 50% less weight than WT mice, but male *Cyp3a* KO mice gained more weight than the WT control. Interestingly, HFD increased the expression of PXR in *Cyp3a* KO female mice whereas PXR was downregulated in male *Cyp3a* KO mice (Kumar et al., 2018). In humans, the risk of drug-induced hepatotoxicity during anti-tuberculosis treatment from rifampicin is higher in women than in men (Shehu et al., 2016). Wang et al. hypothesized that sex-specific single nucleotide polymorphisms genotype and the resulting haplotype of PXR differentially regulate the expression of CYP3A4, leading to the sex-specific influence of hepatotoxicity (Wang et al., 2015). Significant associations were found between two PXR haplotypes, h001101 and h000110, and increased hepatotoxicity during antituberculosis

treatment with rifampicin, isoniazid, or pyrazinamide (Wang et al., 2015). Single nucleotide polymorphism (SNP) and haplotype differences of PXR may be a significant reason for differences in PXR activity.

The gut microbiome is suggested to be a critical driver of gender differences of PXR. GF mice had attenuated sex differences in PXR and its target genes in the liver, compared to CV mice (Weger et al., 2019). In addition, PXR also reciprocally drives sexually dimorphic hepatic changes in lipid and xenobiotic metabolism in response to gut microbiota in mice, in that the expression of most microbiota-sensitive hepatic genes in response to antibiotic-mediated depletion of microbiota in mice were PXR-dependent in males, but not in females (Barretto et al., 2021). Pathway enrichment analysis suggest that that microbiota-PXR interaction regulates fatty acid and xenobiotic metabolism, and data on liver triglyceride content indicates that antibiotic treatment reduced liver triglyceride content and hampered xenobiotic metabolism in male mice in a PXR-dependent manner (Barretto et al., 2021).

PXR and critical time-windows

Using the prototypical PXR-target gene *Cyp3a11* as a read-out for PXR-activation, it was shown that *Cyp3a11* mRNA undergoes extensive regulation at critical time windows. For example, during mouse liver development, *Cyp3a11* mRNA is low before birth, peaks around postnatal day 5, and maintains at high levels till young adulthood (Hart et al., 2009). It should be noted that the ontogenic regulation of *Cyp3a11* may not be solely PXR dependent, as it has been shown that distinct chromatin epigenetic signatures, such as increased permissive mark histone H3 lysine 4 di-methylation (H3K4me2) positively associates with the ontogenic increase of *Cyp3a11* mRNA in developing mouse liver (Li et al., 2009). During human liver development, the *Cyp3a11* ortholog *CYP3A4* gene locus is also extensively modified by the histone code, and the postnatal liver has reduced occupancy of repressive histone marks and higher occupancy of active histone marks compared to the fetal liver (Giebel et al., 2016). During aging, *Cyp3a11* mRNA displays a

consistent male-predominant pattern and decreases earlier in the livers of female mice than age-matched male mice (Fu et al., 2012). *Cyp3a11* mRNA also exhibits diurnal variations between day and night, with higher expression around 4 am followed by a decrease to relatively steady levels during the daytime (Zhang et al., 2009). Activation of PXR using its prototypical ligand PCN at various developmental ages showed that the highest fold-induction occurred in the 60-day-old young adult, followed by postnatal day 5 (Li et al., 2016b). Neonatal activation of PXR also persistently down-regulated the mRNA and protein expression of multiple *CYP4A* isoforms, which are prototypical target genes of the lipid-sensing nuclear receptor peroxisome proliferator-activated receptor alpha (PPAR α), in livers of adult male mouse pups in a PXR-dependent manner (Li et al., 2016a).

Pharmacological and mathematical models of PXR

There have been a few mathematical models developed to describe PXR activation and CYP3A up-regulation in humans and rodents. These mathematical models implemented a system of ordinary differential equations to organize and simulate the molecular activation of PXR, signaling pathway, and induction of its targets. One of the first to depict a compartmental model of *CYP3A4* gene regulation by PXR considered the standard cyclical process of PXR activation: rifampicin enters the cell and binds to PXR, which forms a heterodimer with RXR α ; the PXR/RXR α binds to DNA and induces *CYP3A4* mRNA, which is translated into protein; *CYP3A4* mRNA is degraded and translated protein metabolizes the rifampicin in the cell (Luke et al., 2010). This model included a feedback loop and used zero- and first-order kinetics to model the steady-state mRNA levels of *CYP3A4* and incorporated plasma and liver in their compartmental model (Luke et al., 2010).

Similar to the model by Luke et al., the rPXR pharmacokinetic/pharmacodynamic (PK/PD) model developed by Li et al. describes the change in levels of CYP3A1 and CYP3A2 with respect to time following the activation of rPXR from dexamethasone

exposure at various drug concentrations (Li et al., 2012). The model formulated by Li et al. comprised of *in vivo* exposure to dexamethasone by intraperitoneal injection (100 mg/kg), measurement of total 6 β -testosterone hydroxylation formation enzyme activity, and *CYP3A1* and *CYP3A2* mRNAs and protein levels, and contained two compartments, i.e. blood and liver (Li et al., 2012). The resulting model, upon estimation and calculation of parameters, such as clearance, apparent volume of distribution, and duration of zero-order absorption, and using transit compartments, was able to show the concentration-induction response relationship between dexamethasone and the prototypical rPXR targets (Li et al., 2012).

Data for the conceptual models for PXR can be obtained relatively easily by RT-qPCR and western blots (Luke et al., 2010; Bailey et al., 2011; Kolodkin et al., 2013). Additional parameters, such as cytoplasm-to-nucleus mRNA translocation and RNA processing, can be performed to further improve physiological and molecular relevance. These improved models can serve as important first-pass detection of CYP3A induction and PXR activation in novel compounds and can be used as a start of investigating a systems approach of PXR and other receptors and their targets, i.e. PXR and the glucocorticoid receptor (GR) (Kolodkin et al., 2013). However, no mathematical or statistical model describing the PXR-CYP3A relationship across species has been developed. Therefore, the development and expansion of such models will allow future research to investigate *CYP3A* and *PXR* mRNA or protein induction from cellular experiments in newly studied organisms have a good potential in assessing toxicity and drug-drug interactions across species.

Current challenges and knowledge gaps

With the expansion of research on PXR recently, several challenges and knowledge gaps are noted in this area. First, prototypical PXR-target genes (e.g. *Cyp3a*) may be regulated by other mechanisms such as epigenetic modifiers and other transcription factors, thus they may not be a *bona fide* biomarker for PXR activation. Second, species

differences in both PXR and the PXR-modulating endogenous molecules between humans and animal models continue to be a challenge in translational research. In addition, while several novel endogenous PXR ligands are being resolved, there is a knowledge gap regarding how other endogenous small molecules among the vast majority of poorly characterized “dark matter” within the metabolome modulate PXR. Certain endogenous molecules may serve as potent PXR ligands in animal models but only weak PXR ligands or display no activity towards PXR in humans; in addition, these molecules may serve as potent PXR ligands in certain tissues (e.g. intestine) but only have weak PXR-modulating activities in other tissues (liver). Last but not least, the consequence of PXR activation may be context specific and can be either beneficial (e.g. chemical detoxification and anti-inflammation) or harmful (e.g. drug-drug interactions and promoting NASH). Therefore, it is very important to understand the complexity of the species difference, tissue specificity, and context-specificity duality of PXR modulation, and additional research efforts are needed to further characterize PXR target genes in different organs and species as well as novel PXR-modulating molecules.

Within the challenges discussed above we may find new opportunities to address them: strategies in derivatizing endogenous PXR ligands to improve their activities towards human PXR and tissues other than their original functional sites may provide novel therapeutic options to treat complex human disease while bypassing the adverse drug reactions. In addition, with the availability of single cell transcriptomics and single cell metabolomics, it is possible to improve the precision and resolution of understanding the PXR functions and modulating factors.

Overall conclusion and closing remarks

Taken together, as summarized in Figure 1, PXR is a versatile xenobiotic-sensing nuclear receptor that has been re-discovered as a critical modulator for intermediary metabolism and metabolic diseases. Notably, PXR plays critical roles within the gut-liver

axis within the context of exposures and disease outcomes. PXR activity can be modulated under basal, physiological, pathophysiological, pharmacological, and toxicological conditions by a variety of environmental factors. These factors include drugs, environmental chemicals, dietary constituents, microbial metabolites, etc. Modifying factors such as sex, age, exposure time windows, and species specificity, may interact the PXR signaling within the gut liver axis. While the well-known outcomes of PXR activation with are drug-drug interactions through transcriptional regulation of various host genes involved in xenobiotic biotransformation, studies in the literature have also discovered *bona fide* PXR-targeted intestinal bacteria that are suppressed by PXR, as well as the importance of PXR in modulating inflammation and intermediary metabolism. Additional research using targeted and untargeted metabolomics is needed to further expand our knowledge regarding novel PXR-modulating small molecules from the diet, microbiome, and other exposures. It is also important to discover individual microbes and complex microbial symbiotic/competitive interactions that are involved in the production or consumption of PXR-modulating molecules. The discovery of novel endogenous PXR modulators is expected to aid in developing novel therapeutic modalities for drug-drug interactions, food-drug interactions, drug-toxicant interactions, as well as PXR-mediated metabolic diseases. We think it is also important to study the modulating of PXR-signaling by context-specific chemical mixtures, involving co-exposures of both exogenous and endogenous PXR-modulating molecules. Due to the known species difference of PXR, it is important to continue utilizing humanized PXR transgenic models and in vitro systems to cross-reference findings in various animal species. Development and expansion of mathematical models will allow future research to investigate PXR-CYP3A induction to investigate species and gender differences. Characterizing the sex difference in PXR-signaling continues to be a research priority and incorporating additional metagenomics and metabolomics approaches will facilitate the identification of novel regulators that drive the gender-divergent PXR modulation and

response. It is also important to further investigate the interactions between PXR and other transcription factors as well as co-factors. Additionally, relatively less is known about PXR and the gut-liver axis among the pediatric population. Further understanding the role of PXR in pediatric pharmaco-metagenomics is important to improve the safety and efficacy of using drugs in this vulnerable population.

ACKNOWLEDGEMENTS:

The authors would like to thank Dr. Curtis D. Klaassen for editing the manuscript.

AUTHORSHIP CONTRIBUTIONS:

Participated in research design: Dutta, Lim, and Cui.

Conducted experiments: N/A.

Contributed new reagents or analytic tools: N/A.

Performed data analysis: Dutta, Lim, and Cui.

Wrote or contributed to the writing of the manuscript: Dutta, Lim, and Cui.

REFERENCES

- Ajouz H, Mukherji D, and Shamseddine A (2014) Secondary bile acids: an underrecognized cause of colon cancer. *World J Surg Oncol* **12**:164.
- Al-Salman F and Plant N (2012) Non-coplanar polychlorinated biphenyls (PCBs) are direct agonists for the human pregnane-X receptor and constitutive androstane receptor, and activate target gene expression in a tissue-specific manner. *Toxicol Appl Pharmacol* **263**:7-13.
- Aleksunes LM and Klaassen CD (2012) Coordinated regulation of hepatic phase I and II drug-metabolizing genes and transporters using AhR-, CAR-, PXR-, PPARalpha-, and Nrf2-null mice. *Drug Metab Dispos* **40**:1366-1379.
- Allen K, Jaeschke H, and Copple BL (2011) Bile acids induce inflammatory genes in hepatocytes: a novel mechanism of inflammation during obstructive cholestasis. *Am J Pathol* **178**:175-186.
- Alnouti Y and Klaassen CD (2008) Regulation of sulfotransferase enzymes by prototypical microsomal enzyme inducers in mice. *J Pharmacol Exp Ther* **324**:612-621.
- Austin KL, Mather LE, Philpot CR, and McDonald PJ (1980) Intersubject and dose-related variability after intravenous administration of erythromycin. *Br J Clin Pharmacol* **10**:273-279.
- Avior Y, Levy G, Zimerman M, Kitsberg D, Schwartz R, Sadeh R, Moussaieff A, Cohen M, Itskovitz-Eldor J, and Nahmias Y (2015) Microbial-derived lithocholic acid and vitamin K2 drive the metabolic maturation of pluripotent stem cells-derived and fetal hepatocytes. *Hepatology* **62**:265-278.
- Ayed-Boussema I, Pascussi JM, Maurel P, Bacha H, and Hassen W (2012a) Effect of aflatoxin B1 on nuclear receptors PXR, CAR, and AhR and their target cytochromes P450 mRNA expression in primary cultures of human hepatocytes. *Int J Toxicol* **31**:86-93.

- Ayed-Boussema I, Pascussi JM, Rjiba K, Maurel P, Bacha H, and Hassen W (2012b) The mycotoxin, patulin, increases the expression of PXR and AhR and their target cytochrome P450s in primary cultured human hepatocytes. *Drug Chem Toxicol* **35**:241-250.
- Bailey I, Gibson GG, Plant K, Graham M, and Plant N (2011) A PXR-mediated negative feedback loop attenuates the expression of CYP3A in response to the PXR agonist pregnenolone-16 α -carbonitrile. *PLoS One* **6**:e16703.
- Barretto SA, Lasserre F, Huillet M, Regnier M, Polizzi A, Lippi Y, Fougerat A, Person E, Bruel S, Betoulieres C, Naylies C, Lukowicz C, Smati S, Guzylack L, Olier M, Theodorou V, Mselli-Lakhal L, Zalko D, Wahli W, Loiseau N, Gamet-Payraastre L, Guillou H, and Ellero-Simatos S (2021) The pregnane X receptor drives sexually dimorphic hepatic changes in lipid and xenobiotic metabolism in response to gut microbiota in mice. *Microbiome* **9**:93. doi: 10.1186/s40168-021-01050-9
- Beaudoin JJ, Brouwer KLR, and Malinen MM (2020) Novel insights into the organic solute transporter alpha/beta, OSTalpha/beta: From the bench to the bedside. *Pharmacol Ther* **211**:107542.
- Bertilsson G, Heidrich J, Svensson K, Asman M, Jendeberg L, Sydow-Backman M, Ohlsson R, Postlind H, Blomquist P, and Berkenstam A (1998) Identification of a human nuclear receptor defines a new signaling pathway for CYP3A induction. *Proc Natl Acad Sci U S A* **95**:12208-12213.
- Bjorkholm B, Bok CM, Lundin A, Rafter J, Hibberd ML, and Pettersson S (2009) Intestinal microbiota regulate xenobiotic metabolism in the liver. *PLoS One* **4**:e6958.
- Blumberg B, Sabbagh W, Jr., Juguilon H, Bolado J, Jr., van Meter CM, Ong ES, and Evans RM (1998) SXR, a novel steroid and xenobiotic-sensing nuclear receptor. *Genes Dev* **12**:3195-3205.
- Brobst DE, Ding X, Creech KL, Goodwin B, Kelley B, and Staudinger JL (2004) Guggulsterone activates multiple nuclear receptors and induces CYP3A gene expression through the pregnane X receptor. *J Pharmacol Exp Ther* **310**:528-535.
- Buckley DB and Klaassen CD (2009) Induction of mouse UDP-glucuronosyltransferase mRNA expression in liver and intestine by activators of aryl-hydrocarbon receptor, constitutive androstane receptor, pregnane X receptor, peroxisome proliferator-activated receptor alpha, and nuclear factor erythroid 2-related factor 2. *Drug Metab Dispos* **37**:847-856.
- Burk O, Arnold KA, Nussler AK, Schaeffeler E, Efimova E, Avery BA, Avery MA, Fromm MF, and Eichelbaum M (2005) Antimalarial artemisinin drugs induce cytochrome P450 and MDR1 expression by activation of xenosensors pregnane X receptor and constitutive androstane receptor. *Mol Pharmacol* **67**:1954-1965.
- Cantoni L, Rozio M, Mangolini A, Hauri L, and Caccia S (2003) Hyperforin contributes to the hepatic CYP3A-inducing effect of *Hypericum perforatum* extract in the mouse. *Toxicol Sci* **75**:25-30.
- Caparros-Martin JA, Lareu RR, Ramsay JP, Peplies J, Reen FJ, Headlam HA, Ward NC, Croft KD, Newsholme P, Hughes JD, and O'Gara F (2017) Statin therapy causes gut dysbiosis in mice through a PXR-dependent mechanism. *Microbiome* **5**:95.
- Chang TK and Waxman DJ (2006) Synthetic drugs and natural products as modulators of constitutive androstane receptor (CAR) and pregnane X receptor (PXR). *Drug Metab Rev* **38**:51-73.
- Cheng J, Shah YM, and Gonzalez FJ (2012) Pregnane X receptor as a target for treatment of inflammatory bowel disorders. *Trends Pharmacol Sci* **33**:323-330.
- Cheng SL, Li X, Lehmler HJ, Phillips B, Shen D, and Cui JY (2018) Gut microbiota modulates interactions between polychlorinated biphenyls and bile acid homeostasis. *Toxicol Sci* **166**:269-287.

- Cheng X, Maher J, Dieter MZ, and Klaassen CD (2005) Regulation of mouse organic anion-transporting polypeptides (Oatps) in liver by prototypical microsomal enzyme inducers that activate distinct transcription factor pathways. *Drug Metab Dispos* **33**:1276-1282.
- Conly JM and Stein K (1992) The production of menaquinones (vitamin K₂) by intestinal bacteria and their role in maintaining coagulation homeostasis. *Prog Food Nutr Sci* **16**:307-343.
- Creusot N, Garoche C, Grimaldi M, Boulahtouf A, Chiavarina B, Bourguet W, and Balaguer P (2021) A comparative study of human and zebrafish pregnane X receptor activities of pesticides and steroids using in vitro reporter gene assays. *Front Endocrinol (Lausanne)* **12**:665521.
- Cruz R, Palmeira JD, Martins ZE, Faria MA, Ferreira H, Marques A, Casal S, and Cunha SC (2020) Multidisciplinary approach to determine the effect of polybrominated diphenyl ethers on gut microbiota. *Environ Pollut* **260**:113920.
- Cui H, Gu X, Chen J, Xie Y, Ke S, Wu J, Golovko A, Morpurgo B, Yan C, Phillips TD, Xie W, Luo J, Zhou Z, and Tian Y (2017) Pregnane X receptor regulates the AhR/Cyp1A1 pathway and protects liver cells from benzo-[alpha]-pyrene-induced DNA damage. *Toxicol Lett* **275**:67-76.
- Cui JY, Gunewardena SS, Rockwell CE, and Klaassen CD (2010) ChIPing the cistrome of PXR in mouse liver. *Nucleic Acids Res* **38**:7943-7963.
- Cui JY, Renaud HJ, and Klaassen CD (2012) Ontogeny of novel cytochrome P450 gene isoforms during postnatal liver maturation in mice. *Drug Metab Dispos* **40**:1226-1237.
- Daujat-Chavanieu M and Gerbal-Chaloin S (2020) Regulation of CAR and PXR expression in health and disease. *Cells* **9**. (11):2395
- Dempsey JL and Cui JY (2019) Microbiome is a functional modifier of P450 drug metabolism. *Curr Pharmacol Rep* **5**:481-490.
- Dempsey JL, Wang D, Siginir G, Fei Q, Raftery D, Gu H, and Yue Cui J (2019) Pharmacological activation of PXR and CAR downregulates distinct bile acid-metabolizing intestinal bacteria and alters bile acid homeostasis. *Toxicol Sci* **168**:40-60.
- Desai PB, Nallani SC, Sane RS, Moore LB, Goodwin BJ, Buckley DJ, and Buckley AR (2002) Induction of cytochrome P450 3A4 in primary human hepatocytes and activation of the human pregnane X receptor by tamoxifen and 4-hydroxytamoxifen. *Drug Metab Dispos* **30**:608-612.
- Derrien M, Belzer C, and de Vos WM (2017) Akkermansia muciniphila and its role in regulating host functions. *Microb Pathog* **106**:171-181.
- Ding X and Staudinger JL (2005) Induction of drug metabolism by forskolin: the role of the pregnane X receptor and the protein kinase a signal transduction pathway. *J Pharmacol Exp Ther* **312**:849-856.
- Doricakova A and Vrzal R (2015) A food contaminant ochratoxin A suppresses pregnane X receptor (PXR)-mediated CYP3A4 induction in primary cultures of human hepatocytes. *Toxicology* **337**:72-78.
- Dou W, Zhang J, Li H, Kortagere S, Sun K, Ding L, Ren G, Wang Z, and Mani S (2014) Plant flavonol isorhamnetin attenuates chemically induced inflammatory bowel disease via a PXR-dependent pathway. *J Nutr Biochem* **25**:923-933.
- Durr D, Stieger B, Kullak-Ublick GA, Rentsch KM, Steinert HC, Meier PJ, and Fattinger K (2000) St John's Wort induces intestinal P-glycoprotein/MDR1 and intestinal and hepatic CYP3A4. *Clin Pharmacol Ther* **68**:598-604.
- Dvorak Z, Kopp F, Costello CM, Kemp JS, Li H, Vrzalova A, Stepankova M, Bartonkova I, Jiskrova E, Poulikova K, Vyhldalova B, Nordstroem LU, Karunaratne CV, Ranhotra HS, Mun KS, Naren AP, Murray IA, Perdew GH, Brtko J, Toporova L, Schon A,

- Wallace BD, Walton WG, Redinbo MR, Sun K, Beck A, Kortagere S, Neary MC, Chandran A, Vishveshwara S, Cavalluzzi MM, Lentini G, Cui JY, Gu H, March JC, Chatterjee S, Matson A, Wright D, Flannigan KL, Hirota SA, Sartor RB, and Mani S (2020a) Targeting the pregnane X receptor using microbial metabolite mimicry. *EMBO Mol Med* **12**:e11621.
- Dvorak Z, Sokol H, and Mani S (2020b) Drug Mimicry: Promiscuous receptors PXR and AhR, and microbial metabolite interactions in the intestine. *Trends Pharmacol Sci* **41**:900-908.
- Eaton DL and Klaassen CD (1979) Effects of microsomal enzyme inducers on carrier-mediated transport systems in isolated rat hepatocytes. *J Pharmacol Exp Ther* **208**:381-385.
- Ekins S, Reschly EJ, Hagey LR, and Krasowski MD (2008) Evolution of pharmacologic specificity in the pregnane X receptor. *BMC Evol Biol* **8**:103.
- El-Sankary W, Gibson GG, Ayrton A, and Plant N (2001) Use of a reporter gene assay to predict and rank the potency and efficacy of CYP3A4 inducers. *Drug Metab Dispos* **29**:1499-1504.
- Fickert P, Fuchsbichler A, Marschall HU, Wagner M, Zollner G, Krause R, Zatloukal K, Jaeschke H, Denk H, and Trauner M (2006) Lithocholic acid feeding induces segmental bile duct obstruction and destructive cholangitis in mice. *Am J Pathol* **168**:410-422.
- Foley MH, O'Flaherty S, Barrangou R, and Theriot CM (2019) Bile salt hydrolases: Gatekeepers of bile acid metabolism and host-microbiome crosstalk in the gastrointestinal tract. *PLoS Pathog* **15**:e1007581.
- Fu ZD, Csanaky IL, and Klaassen CD (2012) Effects of aging on mRNA profiles for drug-metabolizing enzymes and transporters in livers of male and female mice. *Drug Metab Dispos* **40**:1216-1225.
- Fu ZD, Selwyn FP, Cui JY, and Klaassen CD (2017) RNA-Seq profiling of intestinal expression of xenobiotic processing genes in germ-free mice. *Drug Metab Dispos* **45**:1225-1238.
- Gibson GG, Plant NJ, Swales KE, Ayrton A, and El-Sankary W (2002) Receptor-dependent transcriptional activation of cytochrome P4503A genes: induction mechanisms, species differences and interindividual variation in man. *Xenobiotica* **32**:165-206.
- Giebel NL, Shadley JD, McCarver DG, Dorko K, Gramignoli R, Strom SC, Yan K, Simpson PM, and Hines RN (2016) Role of chromatin structural changes in regulating human CYP3A ontogeny. *Drug Metab Dispos* **44**:1027-1037.
- Gomez MV, Dutta M, Suvorov A, Shi X, Gu H, Mani S, and Cui, J.Y. (2021) Early life exposure to environmental contaminants (BDE-47, TBBPA, and BPS) produced persistent alterations in fecal microbiome in adult male mice. *Toxicol Sci* **179**:14-30.
- Guzelian J, Barwick JL, Hunter L, Phang TL, Quattrochi LC, and Guzelian PS (2006) Identification of genes controlled by the pregnane X receptor by microarray analysis of mRNAs from pregnenolone 16alpha-carbonitrile-treated rats. *Toxicol Sci* **94**:379-387.
- Hart SN, Cui Y, Klaassen CD, and Zhong XB (2009) Three patterns of cytochrome P450 gene expression during liver maturation in mice. *Drug Metab Dispos* **37**:116-121.
- He J, Gao J, Xu M, Ren S, Stefanovic-Racic M, O'Doherty RM, and Xie W (2013) PXR ablation alleviates diet-induced and genetic obesity and insulin resistance in mice. *Diabetes* **62**:1876-1887.
- He L, Li H, Huang N, Zhou X, Tian J, Li T, Wu J, Tian Y, Yin Y, and Yao K (2017) Alpha-ketoglutarate suppresses the NF-kappaB-mediated inflammatory pathway and enhances the PXR-regulated detoxification pathway. *Oncotarget* **8**:102974-102988.

- Hernandez JP, Mota LC, and Baldwin WS (2009) Activation of CAR and PXR by dietary, environmental and occupational chemicals alters drug metabolism, intermediary metabolism, and cell proliferation. *Curr Pharmacogenomics Person Med* **7**:81-105.
- Hoffart E, Ghebregiorghis L, Nussler AK, Thasler WE, Weiss TS, Schwab M, and Burk O (2012) Effects of atorvastatin metabolites on induction of drug-metabolizing enzymes and membrane transporters through human pregnane X receptor. *Br J Pharmacol* **165**:1595-1608.
- Howe K, Sanat F, Thumser AE, Coleman T, and Plant N (2011) The statin class of HMG-CoA reductase inhibitors demonstrate differential activation of the nuclear receptors PXR, CAR and FXR, as well as their downstream target genes. *Xenobiotica* **41**:519-529.
- Huang H, Wang H, Sinz M, Zoekler M, Staudinger J, Redinbo MR, Teotico DG, Locker J, Kalpana GV, and Mani S (2007) Inhibition of drug metabolism by blocking the activation of nuclear receptors by ketoconazole. *Oncogene* **26**:258-268.
- Huang K, Mukherjee S, DesMarais V, Albanese JM, Rafti E, Draghi li A, Maher LA, Khanna KM, Mani S, and Matson AP (2018) Targeting the PXR-TLR4 signaling pathway to reduce intestinal inflammation in an experimental model of necrotizing enterocolitis. *Pediatr Res* **83**:1031-1040.
- Hukkanen J, Hakkola J, and Rysa J (2014) Pregnane X receptor (PXR)--a contributor to the diabetes epidemic? *Drug Metabol Drug Interact* **29**:3-15.
- Ishii M, Toda T, Ikarashi N, Kusunoki Y, Kon R, Ochiai W, Machida Y, and Sugiyama K (2014) Gastrectomy increases the expression of hepatic cytochrome P450 3A by increasing lithocholic acid-producing enteric bacteria in mice. *Biol Pharm Bull* **37**:298-305.
- Jacobs MN, Nolan GT, and Hood SR (2005) Lignans, bacteriocides and organochlorine compounds activate the human pregnane X receptor (PXR). *Toxicol Appl Pharmacol* **209**:123-133.
- Jia B and Jeon CO (2019) Promotion and induction of liver cancer by gut microbiome-mediated modulation of bile acids. *PLoS Pathog* **15**:e1007954.
- Jones SA, Moore LB, Shenk JL, Wisely GB, Hamilton GA, McKee DD, Tomkinson NC, LeCluyse EL, Lambert MH, Willson TM, Kliever SA, and Moore JT (2000) The pregnane X receptor: a promiscuous xenobiotic receptor that has diverged during evolution. *Mol Endocrinol* **14**:27-39.
- Jung D, Mangelsdorf DJ, and Meyer UA (2006) Pregnane X receptor is a target of farnesoid X receptor. *J Biol Chem* **281**:19081-19091.
- Kahan BD, Kramer WG, Wideman C, Flechner SM, Lorber MI, and Van Buren CT (1986) Demographic factors affecting the pharmacokinetics of cyclosporine estimated by radioimmunoassay. *Transplantation* **41**:459-464.
- Kim S, Choi S, Dutta M, Asubonteng JO, Polunas M, Goedken M, Gonzalez FJ, Cui JY, and Gyamfi MA (2021) Pregnane X receptor exacerbates nonalcoholic fatty liver disease accompanied by obesity- and inflammation-prone gut microbiome signature. *Biochem Pharmacol*:114698. doi: 10.1016/j.bcp.2021.114698.
- Klaassen CD (1972) Immaturity of the newborn rat's hepatic excretory function for ouabain. *J Pharmacol Exp Ther* **183**:520-526.
- Klaassen CD (1973) Comparison of the toxicity of chemicals in newborn rats to bile duct-ligated and sham-operated rats and mice. *Toxicol Appl Pharmacol* **24**:37-44.
- Klaassen CD (1974a) Effect of microsomal enzyme inducers on the biliary excretion of cardiac glycosides. *J Pharmacol Exp Ther* **191**:201-211.
- Klaassen CD (1974b) Stimulation of the development of the hepatic excretory mechanism for ouabain in newborn rats with microsomal enzyme inducers. *J Pharmacol Exp Ther* **191**:212-218.

- Kliwer SA, Goodwin B, and Willson TM (2002) The nuclear pregnane X receptor: a key regulator of xenobiotic metabolism. *Endocr Rev* **23**:687-702.
- Kliwer SA, Moore JT, Wade L, Staudinger JL, Watson MA, Jones SA, McKee DD, Oliver BB, Willson TM, Zetterstrom RH, Perlmann T, and Lehmann JM (1998) An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. *Cell* **92**:73-82.
- Kolodkin A, Sahin N, Phillips A, Hood SR, Bruggeman FJ, Westerhoff HV, and Plant N (2013) Optimization of stress response through the nuclear receptor-mediated cortisol signaling network. *Nat Commun* **4**:1792. doi: 10.1038/ncomms2799.
- Komoroski BJ, Zhang S, Cai H, Hutzler JM, Frye R, Tracy TS, Strom SC, Lehmann T, Ang CY, Cui YY, and Venkataramanan R (2004) Induction and inhibition of cytochromes P450 by the St. John's wort constituent hyperforin in human hepatocyte cultures. *Drug Metab Dispos* **32**:512-518.
- Kostrubsky VE, Lewis LD, Strom SC, Wood SG, Schuetz EG, Schuetz JD, Sinclair PR, Wrighton SA, and Sinclair JF (1998) Induction of cytochrome P4503A by taxol in primary cultures of human hepatocytes. *Arch Biochem Biophys* **355**:131-136.
- Krasowski MD, Ni A, Hagey LR, and Ekins S (2011) Evolution of promiscuous nuclear hormone receptors: LXR, FXR, VDR, PXR, and CAR. *Mol Cell Endocrinol* **334**:39-48.
- Krecic-Shepard ME, Park K, Barnas C, Slimko J, Kerwin DR, and Schwartz JB (2000) Race and sex influence clearance of nifedipine: results of a population study. *Clin Pharmacol Ther* **68**:130-142.
- Kumar R, Litoff EJ, Boswell WT, and Baldwin WS (2018) High fat diet induced obesity is mitigated in Cyp3a-null female mice. *Chem Biol Interact* **289**:129-140.
- Kurihara I, Lee DK, Petit FG, Jeong J, Lee K, Lydon JP, DeMayo FJ, Tsai MJ, and Tsai SY (2007) COUP-TFII mediates progesterone regulation of uterine implantation by controlling ER activity. *PLoS Genet* **3**:e102.
- Landes N, Pfluger P, Kluth D, Birringer M, Ruhl R, Bol GF, Glatt H, and Brigelius-Flohe R (2003) Vitamin E activates gene expression via the pregnane X receptor. *Biochem Pharmacol* **65**:269-273.
- LeCluyse EL (2001) Pregnane X receptor: molecular basis for species differences in CYP3A induction by xenobiotics. *Chem Biol Interact* **134**:283-289.
- Lehmann JM, McKee DD, Watson MA, Willson TM, Moore JT, and Kliwer SA (1998) The human orphan nuclear receptor PXR is activated by compounds that regulate CYP3A4 gene expression and cause drug interactions. *J Clin Invest* **102**:1016-1023.
- Lemaire G, Mnif W, Pascussi JM, Pillon A, Rabenoelina F, Fenet H, Gomez E, Casellas C, Nicolas JC, Cavailles V, Duchesne MJ, and Balaguer P (2006) Identification of new human pregnane X receptor ligands among pesticides using a stable reporter cell system. *Toxicol Sci* **91**:501-509.
- Li CY, Cheng SL, Bammler TK, and Cui JY (2016a) Editor's Highlight: Neonatal activation of the xenobiotic-sensors PXR and CAR results in acute and persistent down-regulation of PPARalpha-signaling in mouse liver. *Toxicol Sci* **153**:282-302.
- Li CY, Dempsey JL, Wang D, Lee S, Weigel KM, Fei Q, Bhatt DK, Prasad B, Raftery D, Gu H, and Cui JY (2018) PBDEs altered gut microbiome and bile acid homeostasis in male C57BL/6 mice. *Drug Metab Dispos* **46**:1226-1240.
- Li CY, Lee S, Cade S, Kuo LJ, Schultz IR, Bhatt DK, Prasad B, Bammler TK, and Cui JY (2017) Novel interactions between gut microbiome and host drug-processing genes modify the hepatic metabolism of the environmental chemicals polybrominated diphenyl ethers. *Drug Metab Dispos* **45**:1197-1214.
- Li CY, Renaud HJ, Klaassen CD, and Cui JY (2016b) Age-specific regulation of drug-processing genes in mouse liver by ligands of xenobiotic-sensing transcription factors. *Drug Metab Dispos* **44**:1038-1049.

- Li H, Illes P, Karunaratne CV, Nordstrom LU, Luo X, Yang A, Qiu Y, Kurland IJ, Lukin DJ, Chen W, Jiskrova E, Krasulova K, Pecinkova P, DesMarais VM, Liu Q, Albanese JM, Akki A, Longo M, Coffin B, Dou W, Mani S, and Dvorak Z (2021) Deciphering structural bases of intestinal and hepatic selectivity in targeting pregnane X receptor with indole-based microbial mimics. *Bioorg Chem* **109**:104661. doi: 10.1016/j.bioorg.2021.104661.
- Li L, Li ZQ, Deng CH, Ning MR, Li HQ, Bi SS, Zhou TY, and Lu W (2012) A mechanism-based pharmacokinetic/pharmacodynamic model for CYP3A1/2 induction by dexamethasone in rats. *Acta Pharmacol Sin* **33**:127-136.
- Li T and Chiang JY (2005) Mechanism of rifampicin and pregnane X receptor inhibition of human cholesterol 7 alpha-hydroxylase gene transcription. *Am J Physiol Gastrointest Liver Physiol* **288**:G74-84.
- Li T and Chiang JY (2006) Rifampicin induction of CYP3A4 requires pregnane X receptor cross talk with hepatocyte nuclear factor 4alpha and coactivators, and suppression of small heterodimer partner gene expression. *Drug Metab Dispos* **34**:756-764.
- Li T and Chiang JY (2015) Bile acids as metabolic regulators. *Curr Opin Gastroenterol* **31**:159-165.
- Li Y, Cui Y, Hart SN, Klaassen CD, and Zhong XB (2009) Dynamic patterns of histone methylation are associated with ontogenic expression of the Cyp3a genes during mouse liver maturation. *Mol Pharmacol* **75**:1171-1179.
- Lim JJ, Li X, Lehmler HJ, Wang D, Gu H, and Cui JY (2020) Gut microbiome critically impacts PCB-induced changes in metabolic fingerprints and the hepatic transcriptome in mice. *Toxicol Sci* **177**:168-187.
- Lin W, Wang YM, Chai SC, Lv L, Zheng J, Wu J, Zhang Q, Wang YD, Griffin PR, and Chen T (2017) SPA70 is a potent antagonist of human pregnane X receptor. *Nat Commun* **8**:741.
- Little M, Dutta M, Li H, Matson A, Shi X, Mascarinas G, Molla B, Weigel K, Gu H, Mani S, and Cui JY (2021) Understanding the physiological functions of the host xenobiotic-sensing nuclear receptors PXR and CAR on the gut microbiome using genetically modified mice. *Acta Pharmaceutica Sinica B* (2021) doi: 10.1016/j.aspb.2021.07.022
- Liu G, Tian J, Yin H, Yin J, and Tang Y (2019) Self-protective transcriptional alterations in ZF4 cells exposed to Pb(NO₃)₂ and AgNO₃. *J Biochem Mol Toxicol* **33**:e22408.
- Lu YF, Jin T, Xu Y, Zhang D, Wu Q, Zhang YK, and Liu J (2013) Sex differences in the circadian variation of cytochrome p450 genes and corresponding nuclear receptors in mouse liver. *Chronobiol Int* **30**:1135-1143.
- Luke NS, DeVito MJ, Shah I, and El-Masri HA (2010) Development of a quantitative model of pregnane X receptor (PXR) mediated xenobiotic metabolizing enzyme induction. *Bull Math Biol* **72**:1799-1819.
- Luo G, Cunningham M, Kim S, Burn T, Lin J, Sinz M, Hamilton G, Rizzo C, Jolley S, Gilbert D, Downey A, Mudra D, Graham R, Carroll K, Xie J, Madan A, Parkinson A, Christ D, Selling B, LeCluyse E, and Gan LS (2002) CYP3A4 induction by drugs: correlation between a pregnane X receptor reporter gene assay and CYP3A4 expression in human hepatocytes. *Drug Metab Dispos* **30**:795-804.
- Lynch C, Sakamuru S, Huang R, Niebler J, Ferguson SS, and Xia M (2021) Characterization of human pregnane X receptor activators identified from a screening of the Tox21 compound library. *Biochem Pharmacol* **184**:114368. doi: 10.1016/j.bcp.2020.114368.
- Ma X, Idle JR, and Gonzalez FJ (2008) The pregnane X receptor: from bench to bedside. *Expert Opin Drug Metab Toxicol* **4**:895-908.
- Ma Y, Sachdeva K, Liu J, Ford M, Yang D, Khan IA, Chichester CO, and Yan B (2004) Desmethoxyyangonin and dihydromethysticin are two major pharmacological

- kavalactones with marked activity on the induction of CYP3A23. *Drug Metab Dispos* **32**:1317-1324.
- Maher JM, Cheng X, Slitt AL, Dieter MZ, and Klaassen CD (2005) Induction of the multidrug resistance-associated protein family of transporters by chemical activators of receptor-mediated pathways in mouse liver. *Drug Metab Dispos* **33**:956-962.
- Marek CJ, Tucker SJ, Konstantinou DK, Elrick LJ, Haefner D, Sigalas C, Murray GI, Goodwin B, and Wright MC (2005) Pregnenolone-16 α -carbonitrile inhibits rodent liver fibrogenesis via PXR (pregnane X receptor)-dependent and PXR-independent mechanisms. *Biochem J* **387**:601-608.
- Medina-Diaz IM, Estrada-Muniz E, Reyes-Hernandez OD, Ramirez P, Vega L, and Elizondo G (2009) Arsenite and its metabolites, MMA(III) and DMA(III), modify CYP3A4, PXR and RXR α expression in the small intestine of CYP3A4 transgenic mice. *Toxicol Appl Pharmacol* **239**:162-168.
- Meucci V and Arukwe A (2006) The xenoestrogen 4-nonylphenol modulates hepatic gene expression of pregnane X receptor, aryl hydrocarbon receptor, CYP3A and CYP1A1 in juvenile Atlantic salmon (*Salmo salar*). *Comp Biochem Physiol C Toxicol Pharmacol* **142**:142-150.
- Mikamo E, Harada S, Nishikawa J, and Nishihara T (2003) Endocrine disruptors induce cytochrome P450 by affecting transcriptional regulation via pregnane X receptor. *Toxicol Appl Pharmacol* **193**:66-72.
- Milnes MR, Garcia A, Grossman E, Grun F, Shiotsugu J, Tabb MM, Kawashima Y, Katsu Y, Watanabe H, Iguchi T, and Blumberg B (2008) Activation of steroid and xenobiotic receptor (SXR, NR1I2) and its orthologs in laboratory, toxicologic, and genome model species. *Environ Health Perspect* **116**:880-885.
- Moore LB, Goodwin B, Jones SA, Wisely GB, Serabjit-Singh CJ, Willson TM, Collins JL, and Kliewer SA (2000) St. John's wort induces hepatic drug metabolism through activation of the pregnane X receptor. *Proc Natl Acad Sci U S A* **97**:7500-7502.
- Morgan ET, Dempsey JL, Mimche SM, Lamb TJ, Kulkarni S, Cui JY, Jeong H, and Slitt AL (2018) Physiological Regulation of Drug Metabolism and Transport: Pregnancy, Microbiome, Inflammation, Infection, and Fasting. *Drug Metab Dispos* **46**:503-513.
- Moore JT and Kliewer SA (2000) Use of the nuclear receptor PXR to predict drug interactions. *Toxicology* **153**:1-10.
- Moore JT, Moore LB, Maglich JM, and Kliewer SA (2003) Functional and structural comparison of PXR and CAR. *Biochim Biophys Acta* **1619**:235-238.
- Moore LB, Parks DJ, Jones SA, Bledsoe RK, Consler TG, Stimmel JB, Goodwin B, Liddle C, Blanchard SG, Willson TM, Collins JL, and Kliewer SA (2000) Orphan nuclear receptors constitutive androstane receptor and pregnane X receptor share xenobiotic and steroid ligands. *J Biol Chem* **275**:15122-15127.
- Morgan ET, Dempsey JL, Mimche SM, Lamb TJ, Kulkarni S, Cui JY, Jeong H, and Slitt AL (2018) Physiological regulation of drug metabolism and transport: pregnancy, microbiome, inflammation, infection, and fasting. *Drug Metab Dispos* **46**:503-513.
- Newman SL and Guzelian PS (1983) Identification of the cyanopregnenolone-inducible form of hepatic cytochrome P-450 as a catalyst of aldrin epoxidation. *Biochem Pharmacol* **32**:1529-1531.
- Ngan CH, Beglov D, Rudnitskaya AN, Kozakov D, Waxman DJ, and Vajda S (2009) The structural basis of pregnane X receptor binding promiscuity. *Biochemistry* **48**:11572-11581.
- Noble SM, Carnahan VE, Moore LB, Luntz T, Wang H, Ittoop OR, Stimmel JB, Davis-Searles PR, Watkins RE, Wisely GB, LeCluyse E, Tripathy A, McDonnell DP, and Redinbo MR (2006) Human PXR forms a tryptophan zipper-mediated homodimer. *Biochemistry* **45**:8579-8589.

- Ourlin JC, Lasserre F, Pineau T, Fabre JM, Sa-Cunha A, Maurel P, Vilarem MJ, and Pascussi JM (2003) The small heterodimer partner interacts with the pregnane X receptor and represses its transcriptional activity. *Mol Endocrinol* **17**:1693-1703.
- Pacyniak EK, Cheng X, Cunningham ML, Crofton K, Klaassen CD, and Guo GL (2007) The flame retardants, polybrominated diphenyl ethers, are pregnane X receptor activators. *Toxicol Sci* **97**:94-102.
- Pascussi JM, Drocourt L, Gerbal-Chaloin S, Fabre JM, Maurel P, and Vilarem MJ (2001) Dual effect of dexamethasone on CYP3A4 gene expression in human hepatocytes. Sequential role of glucocorticoid receptor and pregnane X receptor. *Eur J Biochem* **268**:6346-6358.
- Parkinson A, Mudra DR, Johnson C, Dwyer A, and Carroll KM (2004) The effects of gender, age, ethnicity, and liver cirrhosis on cytochrome P450 enzyme activity in human liver microsomes and inducibility in cultured human hepatocytes. *Toxicol Appl Pharmacol* **199**:193-209.
- Petrick JS and Klaassen CD (2007) Importance of hepatic induction of constitutive androstane receptor and other transcription factors that regulate xenobiotic metabolism and transport. *Drug Metab Dispos* **35**:1806-1815.
- Pinne M, Ponce E, and Raucy JL (2016) Transactivation assays to assess canine and rodent pregnane X receptor (PXR) and constitutive androstane receptor (CAR) activation. *PLoS One* **11**:e0164642.
- Png CW, Linden SK, Gilshenan KS, Zoetendal EG, McSweeney CS, Sly LI, McGuckin MA, and Florin TH (2010) Mucolytic bacteria with increased prevalence in IBD mucosa augment in vitro utilization of mucin by other bacteria. *Am J Gastroenterol* **105**:2420-2428.
- Pulakazhi Venu VK, Saifeddine M, Mihara K, Tsai YC, Nieves K, Alston L, Mani S, McCoy KD, Hollenberg MD, and Hirota SA (2019) The pregnane X receptor and its microbiota-derived ligand indole 3-propionic acid regulate endothelium-dependent vasodilation. *Am J Physiol Endocrinol Metab* **317**:E350-E361.
- RL (2012) Responses of gut microbiota to diet composition and weight loss in lean and obese mice. *Obesity (Silver Spring)* **20**:738-747.
- Raucy J, Warfe L, Yueh MF, and Allen SW (2002) A cell-based reporter gene assay for determining induction of CYP3A4 in a high-volume system. *J Pharmacol Exp Ther* **303**:412-423.
- Raucy JL (2003) Regulation of CYP3A4 expression in human hepatocytes by pharmaceuticals and natural products. *Drug Metab Dispos* **31**:533-539.
- Reschly EJ, Bainy AC, Mattos JJ, Hagey LR, Bahary N, Mada SR, Ou J, Venkataramanan R, and Krasowski MD (2007) Functional evolution of the vitamin D and pregnane X receptors. *BMC Evol Biol* **7**:222.
- Reschly EJ and Krasowski MD (2006) Evolution and function of the NR1I nuclear hormone receptor subfamily (VDR, PXR, and CAR) with respect to metabolism of xenobiotics and endogenous compounds. *Curr Drug Metab* **7**:349-365.
- Saraswathi V, Perriotte-Olson C, Ganesan M, Desouza CV, Alnouti Y, Duryee MJ, Thiele GM, Nordgren TM, and Clemens DL (2017) A combination of dietary N-3 fatty acids and a cyclooxygenase-1 inhibitor attenuates nonalcoholic fatty liver disease in mice. *J Nutr Biochem* **42**:149-159.
- Scandlyn MJ, Stuart EC, and Rosengren RJ (2008) Sex-specific differences in CYP450 isoforms in humans. *Expert Opin Drug Metab Toxicol* **4**:413-424.
- Schmidt R, Baumann F, Hanschmann H, Geissler F, and Preiss R (2001) Gender difference in ifosfamide metabolism by human liver microsomes. *Eur J Drug Metab Pharmacokinet* **26**:193-200.

- Schuetz EG, Wrighton SA, Barwick JL, and Guzelian PS (1984) Induction of cytochrome P-450 by glucocorticoids in rat liver. I. Evidence that glucocorticoids and pregnenolone 16 alpha-carbonitrile regulate de novo synthesis of a common form of cytochrome P-450 in cultures of adult rat hepatocytes and in the liver in vivo. *J Biol Chem* **259**:1999-2006.
- Schuetz E, Lan L, Yasuda K, Kim R, Kocarek TA, Schuetz J, and Strom S (2002) Development of a real-time in vivo transcription assay: application reveals pregnane X receptor-mediated induction of CYP3A4 by cancer chemotherapeutic agents. *Mol Pharmacol* **62**:439-445.
- Scoville DK, Li CY, Wang D, Dempsey JL, Raftery D, Mani S, Gu H, and Cui JY (2019) Polybrominated diphenyl ethers and gut microbiome modulate metabolic syndrome-related aqueous metabolites in mice. *Drug Metab Dispos* **47**:928-940.
- Seamon KB, Padgett W, and Daly JW (1981) Forskolin: unique diterpene activator of adenylate cyclase in membranes and in intact cells. *Proc Natl Acad Sci U S A* **78**:3363-3367.
- Selwyn FP, Cheng SL, Bammler TK, Prasad B, Vrana M, Klaassen C, and Cui JY (2015a) Developmental regulation of drug-processing genes in livers of germ-free mice. *Toxicol Sci* **147**:84-103.
- Selwyn FP, Cheng SL, Klaassen CD, and Cui JY (2016) Regulation of hepatic drug-metabolizing enzymes in germ-free mice by conventionalization and probiotics. *Drug Metab Dispos* **44**:262-274.
- Selwyn FP, Cui JY, and Klaassen CD (2015b) RNA-Seq quantification of hepatic drug processing genes in germ-free mice. *Drug Metab Dispos* **43**:1572-1580.
- Shehu AI, Li G, Xie W, and Ma X (2016) The pregnane X receptor in tuberculosis therapeutics. *Expert Opin Drug Metab Toxicol* **12**:21-30.
- Shukla SJ, Sakamuru S, Huang R, Moeller TA, Shinn P, Vanleer D, Auld DS, Austin CP, and Xia M (2011) Identification of clinically used drugs that activate pregnane X receptors. *Drug Metab Dispos* **39**:151-159.
- Smirlis D, Muangmoonchai R, Edwards M, Phillips IR, and Shephard EA (2001) Orphan receptor promiscuity in the induction of cytochromes p450 by xenobiotics. *J Biol Chem* **276**:12822-12826.
- Smith RP, Eckalbar WL, Morrissey KM, Luizon MR, Hoffmann TJ, Sun X, Jones SL, Force Aldred S, Ramamoorthy A, Desta Z, Liu Y, Skaar TC, Trinklein ND, Giacomini KM, and Ahituv N (2014) Genome-wide discovery of drug-dependent human liver regulatory elements. *PLoS Genet* **10**:e1004648.
- Soudi M, Gueguen Y, Linard C, Dudoignon N, Grison S, Baudelin C, Marquette C, Gourmelon P, Aigueperse J, and Dublineau I (2005) In vivo effects of chronic contamination with depleted uranium on CYP3A and associated nuclear receptors PXR and CAR in the rat. *Toxicology* **214**:113-122.
- Spruiell K, Jones DZ, Cullen JM, Awumey EM, Gonzalez FJ, and Gyamfi MA (2014a) Role of human pregnane X receptor in high fat diet-induced obesity in pre-menopausal female mice. *Biochem Pharmacol* **89**:399-412.
- Spruiell K, Richardson RM, Cullen JM, Awumey EM, Gonzalez FJ, and Gyamfi MA (2014b) Role of pregnane X receptor in obesity and glucose homeostasis in male mice. *J Biol Chem* **289**:3244-3261.
- Staudinger JL, Goodwin B, Jones SA, Hawkins-Brown D, MacKenzie KI, LaTour A, Liu Y, Klaassen CD, Brown KK, Reinhard J, Willson TM, Koller BH, and Kliewer SA (2001) The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity. *Proc Natl Acad Sci U S A* **98**:3369-3374.

- Sueyoshi T, Li L, Wang H, Moore R, Kodavanti PR, Lehmler HJ, Negishi M, and Birnbaum LS (2014) Flame retardant BDE-47 effectively activates nuclear receptor CAR in human primary hepatocytes. *Toxicol Sci* **137**:292-302.
- Sui Y, Ai N, Park SH, Rios-Pilier J, Perkins JT, Welsh WJ, and Zhou C (2012) Bisphenol A and its analogues activate human pregnane X receptor. *Environ Health Perspect* **120**:399-405.
- Sultana H, Watanabe K, Rana MM, Takashima R, Ohashi A, Komai M, and Shirakawa H (2018) Effects of vitamin K(2) on the expression of genes involved in bile acid synthesis and glucose homeostasis in mice with humanized PXR. *Nutrients* **10**.
- Synold TW, Dussault I, and Forman BM (2001) The orphan nuclear receptor SXR coordinately regulates drug metabolism and efflux. *Nat Med* **7**:584-590.
- Tabb MM, Sun A, Zhou C, Grun F, Errandi J, Romero K, Pham H, Inoue S, Mallick S, Lin M, Forman BM, and Blumberg B (2003) Vitamin K2 regulation of bone homeostasis is mediated by the steroid and xenobiotic receptor SXR. *J Biol Chem* **278**:43919-43927.
- Takeshita A, Taguchi M, Koibuchi N, and Ozawa Y (2002) Putative role of the orphan nuclear receptor SXR (steroid and xenobiotic receptor) in the mechanism of CYP3A4 inhibition by xenobiotics. *J Biol Chem* **277**:32453-32458.
- Tanaka E (1999) Gender-related differences in pharmacokinetics and their clinical significance. *J Clin Pharm Ther* **24**:339-346.
- Teotico DG, Frazier ML, Ding F, Dokholyan NV, Temple BR, and Redinbo MR (2008) Active nuclear receptors exhibit highly correlated AF-2 domain motions. *PLoS Comput Biol* **4**:e1000111.
- Tirona RG, Lee W, Leake BF, Lan LB, Cline CB, Lamba V, Parviz F, Duncan SA, Inoue Y, Gonzalez FJ, Schuetz EG, and Kim RB (2003) The orphan nuclear receptor HNF4alpha determines PXR- and CAR-mediated xenobiotic induction of CYP3A4. *Nat Med* **9**:220-224.
- Toda T, Ohi K, Kudo T, Yoshida T, Ikarashi N, Ito K, and Sugiyama K (2009a) Ciprofloxacin suppresses Cyp3a in mouse liver by reducing lithocholic acid-producing intestinal flora. *Drug Metab Pharmacokinet* **24**:201-208.
- Toda T, Saito N, Ikarashi N, Ito K, Yamamoto M, Ishige A, Watanabe K, and Sugiyama K (2009b) Intestinal flora induces the expression of Cyp3a in the mouse liver. *Xenobiotica* **39**:323-334.
- Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, and Gordon JI (2006) An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* **444**:1027-1031.
- Uppal H, Toma D, Saini SP, Ren S, Jones TJ, and Xie W (2005) Combined loss of orphan receptors PXR and CAR heightens sensitivity to toxic bile acids in mice. *Hepatology* **41**:168-176.
- van Giersbergen PL, Gnerre C, Treiber A, Dingemans J, and Meyer UA (2002) Bosentan, a dual endothelin receptor antagonist, activates the pregnane X nuclear receptor. *Eur J Pharmacol* **450**:115-121.
- Venkatesh M, Mukherjee S, Wang H, Li H, Sun K, Benechet AP, Qiu Z, Maher L, Redinbo MR, Phillips RS, Fleet JC, Kortagere S, Mukherjee P, Fasano A, Le Ven J, Nicholson JK, Dumas ME, Khanna KM, and Mani S (2014) Symbiotic bacterial metabolites regulate gastrointestinal barrier function via the xenobiotic sensor PXR and Toll-like receptor 4. *Immunity* **41**:296-310.
- Wahlang B, Alexander NC, 2nd, Li X, Rouchka EC, Kirpich IA, and Cave MC (2021) Polychlorinated biphenyls altered gut microbiome in CAR and PXR knockout mice exhibiting toxicant-associated steatohepatitis. *Toxicol Rep* **8**:536-547.

- Wahlang B, Jin J, Hardesty JE, Head KZ, Shi H, Falkner KC, Prough RA, Klinge CM, and Cave MC (2019) Identifying sex differences arising from polychlorinated biphenyl exposures in toxicant-associated liver disease. *Food Chem Toxicol* **129**:64-76.
- Wang DQ, Neuschwander-Tetri BA, Portincasa P, and Pandak WM (2012) Interactions between Bile Acids and Nuclear Receptors and Their Effects on Lipid Metabolism and Liver Diseases. *J Lipids* **2012**:560715.
- Wang H, Li H, Moore LB, Johnson MD, Maglich JM, Goodwin B, Ittoop OR, Wisely B, Creech K, Parks DJ, Collins JL, Willson TM, Kalpana GV, Venkatesh M, Xie W, Cho SY, Roboz J, Redinbo M, Moore JT, and Mani S (2008) The phytoestrogen coumestrol is a naturally occurring antagonist of the human pregnane X receptor. *Mol Endocrinol* **22**:838-857.
- Wang H, Venkatesh M, Li H, Goetz R, Mukherjee S, Biswas A, Zhu L, Kaubisch A, Wang L, Pullman J, Whitney K, Kuro-o M, Roig AI, Shay JW, Mohammadi M, and Mani S (2011) Pregnane X receptor activation induces FGF19-dependent tumor aggressiveness in humans and mice. *J Clin Invest* **121**:3220-3232.
- Wang JY, Tsai CH, Lee YL, Lee LN, Hsu CL, Chang HC, Chen JM, Hsu CA, Yu CJ, and Yang PC (2015) Gender-Dimorphic Impact of PXR Genotype and Haplotype on Hepatotoxicity During Antituberculosis Treatment. *Medicine (Baltimore)* **94**:e982.
- Watkins RE, Maglich JM, Moore LB, Wisely GB, Noble SM, Davis-Searles PR, Lambert MH, Kliewer SA, and Redinbo MR (2003) 2.1 A crystal structure of human PXR in complex with the St. John's wort compound hyperforin. *Biochemistry* **42**:1430-1438.
- Watkins RE, Wisely GB, Moore LB, Collins JL, Lambert MH, Williams SP, Willson TM, Kliewer SA, and Redinbo MR (2001) The human nuclear xenobiotic receptor PXR: structural determinants of directed promiscuity. *Science* **292**:2329-2333.
- Weger BD, Gobet C, Yeung J, Martin E, Jimenez S, Betrisey B, Foata F, Berger B, Balvay A, Foussier A, Charpagne A, Boizet-Bonhoure B, Chou CJ, Naef F, and Gachon F (2019) The mouse microbiome is required for sex-specific diurnal rhythms of gene expression and Metabolism. *Cell Metab* **29**:362-382 e368. doi: 10.1016/j.cmet.2018.09.023.
- Wei P, Zhang J, Dowhan DH, Han Y, and Moore DD (2002) Specific and overlapping functions of the nuclear hormone receptors CAR and PXR in xenobiotic response. *Pharmacogenomics J* **2**:117-126.
- Wolbold R, Klein K, Burk O, Nussler AK, Neuhaus P, Eichelbaum M, Schwab M, and Zanger UM (2003) Sex is a major determinant of CYP3A4 expression in human liver. *Hepatology* **38**:978-988.
- Woolbright BL, Li F, Xie Y, Farhood A, Fickert P, Trauner M, and Jaeschke H (2014) Lithocholic acid feeding results in direct hepato-toxicity independent of neutrophil function in mice. *Toxicol Lett* **228**:56-66.
- Xiang D, Chu T, Li M, Wang Q, and Zhu G (2018) Effects of pyrethroid pesticide cis-bifenthrin on lipogenesis in hepatic cell line. *Chemosphere* **201**:840-849.
- Xiang J, Zhang Z, Xie H, Zhang C, Bai Y, Cao H, Che Q, Guo J, and Su Z (2021) Effect of different bile acids on the intestine through enterohepatic circulation based on FXR. *Gut Microbes* **13**:1949095.
- Xie W and Evans RM (2001) Orphan nuclear receptors: the exotics of xenobiotics. *J Biol Chem* **276**:37739-37742.
- Xie W, Radomska-Pandya A, Shi Y, Simon CM, Nelson MC, Ong ES, Waxman DJ, and Evans RM (2001) An essential role for nuclear receptors SXR/PXR in detoxification of cholestatic bile acids. *Proc Natl Acad Sci U S A* **98**:3375-3380.
- Xu DX, Chen YH, Wang JP, Sun MF, Wang H, Wei LZ, and Wei W (2005) Perinatal lipopolysaccharide exposure downregulates pregnane X receptor and Cyp3a11 expression in fetal mouse liver. *Toxicol Sci* **87**:38-45.

- Xu DX, Wei W, Sun MF, Wu CY, Wang JP, Wei LZ, and Zhou CF (2004) Kupffer cells and reactive oxygen species partially mediate lipopolysaccharide-induced downregulation of nuclear receptor pregnane x receptor and its target gene CYP3a in mouse liver. *Free Radic Biol Med* **37**:10-22.
- Xu G, Dai M, Zheng X, Lin H, Liu A, and Yang J (2020) Cholestatic models induced by lithocholic acid and alphanaphthylisothiocyanate: Different etiological mechanisms for liver injury but shared JNK/STAT3 signaling. *Mol Med Rep* **22**:1583-1593.
- Yueh MF, Kawahara M, and Raucy J (2005) High volume bioassays to assess CYP3A4-mediated drug interactions: induction and inhibition in a single cell line. *Drug Metab Dispos* **33**:38-48.
- Zhang YK, Yeager RL, and Klaassen CD (2009) Circadian expression profiles of drug-processing genes and transcription factors in mouse liver. *Drug Metab Dispos* **37**:106-115.
- Zhao LY, Xu JY, Shi Z, Englert NA, and Zhang SY (2017) Pregnane X receptor (PXR) deficiency improves high fat diet-induced obesity via induction of fibroblast growth factor 15 (FGF15) expression. *Biochem Pharmacol* **142**:194-203.
- Zhou C, Poulton EJ, Grun F, Bammler TK, Blumberg B, Thummel KE, and Eaton DL (2007) The dietary isothiocyanate sulforaphane is an antagonist of the human steroid and xenobiotic nuclear receptor. *Mol Pharmacol* **71**:220-229.
- Zhou J, Febbraio M, Wada T, Zhai Y, Kuruba R, He J, Lee JH, Khadem S, Ren S, Li S, Silverstein RL, and Xie W (2008) Hepatic fatty acid transporter Cd36 is a common target of LXR, PXR, and PPARgamma in promoting steatosis. *Gastroenterology* **134**:556-567.
- Zhou J, Zhai Y, Mu Y, Gong H, Uppal H, Toma D, Ren S, Evans RM, and Xie W (2006) A novel pregnane X receptor-mediated and sterol regulatory element-binding protein-independent lipogenic pathway. *J Biol Chem* **281**:15013-15020.
- Zhuo W, Hu L, Lv J, Wang H, Zhou H, and Fan L (2014) Role of pregnane X receptor in chemotherapeutic treatment. *Cancer Chemother Pharmacol* **74**:217-227.

FOOTNOTES:

Supported by National Institutes of Health (NIH) grants [R01ES025708, R01ES030197, R01GM111381, R01ES031098], the University of Washington Center for Exposures, Diseases, Genomics, and Environment [P30ES007033], Environmental Pathology/Toxicology Training Program [T32ES007032], the University of Washington Sheldon Murphy Endowment, and the University of Washington Environmental Health and Microbiome Research (EHMiR) Center (DEOHS). No author has an actual or perceived conflict of interest with the contents of this article.

FIGURE LEGEND

Figure 1. Overall summary of PXR and the gut-liver axis within the context of exposures and outcomes. Briefly, PXR activity can be modulated under basal, physiological, pathophysiological, pharmacological, and toxicological conditions by a variety of environmental factors. These factors include drugs, environmental chemicals, dietary constituents, microbial metabolites, etc. Modifying factors such as sex, age, exposure time windows, and species specificity, may interact the PXR signaling within the gut liver axis. While the well-known outcomes of PXR activation with are drug-drug interactions through transcriptional regulation of various host genes involved in xenobiotic biotransformation, studies in the literature have also discovered *bona fide* PXR-targeted intestinal bacteria that are suppressed by PXR, as well as the importance of PXR in modulating inflammation and intermediary metabolism.

Table 1. PXR-modulating chemicals					
		Chemical IDs	Activator/repressor	Species	Reference
Exogenous molecules	Drugs	Rifampicin	Activator and ligand	Human	(Blumberg et al., 1998; Li and Chiang, 2006; Li and Chiang, 2005)
		RU486	Activator	Human	(Banerjee et al., 2015; Kliewer, 2015;
		Etomidoline	Activator	Human	Lynch et al., 2021)
		Clotrimazole	Activator	Human, weak activator in rodent	(Jones et al., 2000; Lehmann et al., 1998; Banerjee et al., 2015; Kliewer, 2015)
		Colfibrate	Activator and ligand	Rat	(Daujat-Chavanieu and Gerbal-Chaloin, 2020)
		Phenobarbital	Activator	Human	(Li et al., 2019; Tamasi et al., 2009)
		Dexamethasone	Activator	Human and rodent	(Pascussi et al., 2001; Yueh et al., 2005; Pascussi et al., 2000; Shi et al., 2010)
		Cyclophosphamide (CPA)	Activator	Human	(Lindley et al., 2002; Zhuo et al., 2014)
		Ritanovir	Activator	Human	(Chang and Waxman, 2006; Shehu et al., 2019; Brewer and Chen, 2016)
		Paclitaxel	Activator	Human	(Kostrubsky et al., 1998)
		Felodipine	Activator	Human	(Dybdahl et al., 2012; Pinne et al., 2016)
		Nifedipine	Activator	Human	(Bertilsson et al., 1998; Chang and Waxman, 2006; Lynch et al., 2021)
		Bendipine	Activator	Human	(Bertilsson et al., 1998; Chang and Waxman, 2006; Lynch et al., 2021)
		carbamazepine	Activator	Human	(El-Sankary et al., 2001; Luo et al., 2002)
		Bosentan	Activator	Human	(van Giersbergen et al., 2002)
		Mevastatin	Activator	Human	(Raucy et al., 2002)
		Tamoxifen	Activator	Human	(Desai et al., 2002; Zhuo et al., 2014)
		Topotecan	Activator	Human	(Schuetz et al., 2002)
		Lacidipine	Activator	Human	(Bertilsson et al., 1998; Chang and Waxman, 2006; Lynch et al., 2021)
		Resveratrol	Inhibitor	Human	(Smutny and Pavek, 2014), (Deng et al., 2014)
	Ketoconazole	Inhibitor	Human	(Mani et al., 2013; Huang et al., 2007)	
Exogenous molecules	Drugs	ET-743	Inhibitor	Human	(Synold et al., 2001)
		SPA70	Inhibitor	Human	(Lin et al., 2017)

	Phytochemicals	St. John's wort (hyperforin)	Activator	Human	(Watkins et al., 2003; Kliewer, 2015)	
		Forskolin	Activator	Human, Mouse	(Seamon et al., 1981; Ding and Staudinger, 2005a)	
		Guggulsterones	Activator	Human, Mouse	(Chang and Waxman, 2006; Brobst et al., 2004; Ding and Staudinger, 2005b)	
		Artemisinin	Activator	Human	(Burk et al., 2005)	
		Kava extract	Activator	Human	(Raucy, 2003), (Ma et al., 2004)	
		Coumestrol	Repressor	Human and humanized mouse	(Wang et al., 2008)	
		Sulforaphane	Repressor	Human	(Zhou et al., 2007)	
		Schiandrol B (SolB)	Activator	Human	(Zeng et al., 2017)	
	Environmental chemicals	BPA	Activator	Human	(Sui et al., 2012; Barrett, 2012)	
		PBDEs	Activator	Human, Rodents	(Pacyniak et al., 2007; Sueyoshi et al., 2014)	
		PCBs	Activator	Human, Rodents	(Egusquiza et al., 2020; Wahlang et al., 2014; Jacobs et al., 2005)	
		PFOA	Activator	Rodents	(Bjork et al., 2011; Ren et al., 2009)	
		PFOS	Activator	Human, Rodents	(Bjork et al., 2011; Ren et al., 2009)	
		Phthalate monoesters	Activator	Human, Rodents	(Hurst and Waxman, 2004; Cooper et al., 2008)	
		Metal ions (Pb+, Ag+), As	Activator	Zebrafish, humanized mouse	(Liu et al., 2019; Medina-Diaz et al., 2009)	
		Uranium	Activator	Rat	(Souidi et al., 2005)	
	Exogenous molecules	Pesticides	Chlordane (trans-nonachlor)	Activator	Human	(Jacobs et al., 2005)
			Alachlor	Activator	Human, Rat	(Mikamo et al., 2003; Lemaire et al., 2006)
			Nonylphenol	Activator	Fish	(Meucci and Arukwe, 2006)
			Cis-bifenthrin	Activator	Human	(Xiang et al., 2018)
			DDT	Activator	Human, Mouse	(Lemaire et al., 2004)
Dieldrin			Activator	Human	(Milnes et al., 2008)	
Cholpyrifos			Activator	Mouse	(Lemaire et al., 2004)	
Cypermethrine			Activator	Human	(Lemaire et al., 2004; Abass et al., 2012)	
Endosulfan		Activator	Human	(Lemaire et al., 2004; Coumoul et al., 2002)		
Miscellaneous		dane	Activator	Human, Rat	(Lemaire et al., 2004; Mikamo et al., 2003)	
		Methoxychlor	Activator	Human, Rat	(Lemaire et al., 2004; Mikamo et al., 2003; Lemaire et al., 2006)	
		CITCO	Activator	Humans	(Lin et al., 2020)	
		SR12813	Activator	Human, Rabbit,	(Jones et al., 2000; Pinne et al., 2016)	

				Canine	
		Pregnenolone 16Chlorpyrifosile (PCN)	Activator and ligand	Rodents	(Ma et al., 2008; Marek et al., 2005)
		FKK6	Activator and ligand	Human, mouse	(Dvorak et al., 2020)
	Food components	Aflatoxin B1	Activator	Human	(Ayed-Boussema et al., 2012a; Daujat-Chavanieu and Gerbal-Chaloin, 2020)
		Patulin	Activator	Human	(Ayed-Boussema et al., 2012b)
		Isorhamnetin	Activator and ligand	Mouse	(Dou et al., 2014)
		High fat diet	Activator	Mouse	(Saraswathi et al., 2017)
		Alpha-ketoglutarate	Activator	Pig	(He et al., 2017)
		Ochratoxin A	Inhibitor	Human	(Doricakova and Vrzal, 2015; Daujat-Chavanieu and Gerbal-Chaloin, 2020)
		Vitamins	Vit K	Activator and ligand	Mouse
	Vit E		Activator	Human	(Landes et al., 2003)
	Endogenous Molecules	Microbial metabolites	Lithocholic acid (LCA)	Activator	Human, Rodents
3-keto LCA			Activator	Human, Rodents	(Xie et al., 2001; Staudinger et al., 2001)
Deoxycholic acid (DCA)			Activator	Mouse	(Xie et al., 2001)
Indole-3-propionic acid			Ligand and activator	Human, Mouse	(Venkatesh et al., 2014)
Skatole (3-methyl-indole)			Partial agonist and low affinity ligand	Human	(Vyhldalova et al., 2020)
LPS			Inhibitor	Mouse	(Xu et al., 2004; Xu et al., 2005)

Figure 1

