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PXR: a xenobiotic receptor of diverse function implicated in pharmacogenetics

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Abstract

The pregnane X receptor (PXR; NR1I2), a member of the nuclear receptor superfamily, regulates the expression of drug-metabolic enzymes and transporters involved in the responses of mammals to their chemical environment. The same enzyme and transporter systems are also involved in the homeostasis of numerous endogenous chemicals. The regulatory function of PXR is implicated in normal physiology and diseases, such as drug–drug interactions, hepatic steatosis, vitamin D homeostasis, bile acids homeostasis, steroid hormones homeostasis and inflammatory bowel diseases. As such, any genetic variations of this receptor could potentially have widespread effects on the disposition of xenobiotics and endobiotics. Knowledge concerning the genetic polymorphisms of PXR may help to understand the variations in human drug response and ensure safe drug use. The correlation of PXR genetic polymorphisms with several disease conditions also suggests that this receptor may represent a valid therapeutic for hepato-intestinal disorders such as inflammatory bowel disease and primary sclerosing cholangitis.

Keywords

drug metabolism; genetic variants; pharmacogenetics; pregnane X receptor; splice variants

The pregnane X receptor (PXR; NR1I2) of mouse was first identified in 1998 as a member of the nuclear receptor (NR) superfamily on the basis of its sequence homology with other NRs [1]. Human PXR (hPXR) was found subsequently and named steroid and xenobiotic receptor (SXR) or pregnane-activated receptor (PAR) [2,3]. PXR shares a common NR modular structure with a conserved N-terminal DNA-binding domain (DBD) and a C-terminal ligand-binding domain (LBD). The DBD contains two zinc-finger motifs, which mediate interaction with specific DNA sequences known as hormone response elements (HREs). The LBD, in addition to determining ligand-binding specificity, contains a ligand-inducible transactivation function (AF-2) and a motif that directs binding to the common heterodimerization partner retinoid X receptor (RXR) [4,5]. Although DBDs of the mammalian PXRs are highly conserved, sharing more than 95% amino acid identity, the LBDs of PXRs are much more divergent across species than those of other NRs [6,7].

Unlike other nuclear receptors, such as the steroid receptors (e.g., estrogen receptors- α and - β), which interact selectively with their physiological ligands, PXR ligands are structurally diverse and include prescription drugs, herbal medicines, dietary supplements, environmental

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pollutants and endobiotics [6,8]. Indeed, elucidation of the 3D structure of the PXR LBD revealed that it has a large, spherical ligand-binding cavity that allows it to interact with a wide range of hydrophobic chemicals [5,9]. Many PXR ligands have been identified among prescription drugs, and include the antibiotics rifampicin (RIF), clotrimazole and ritonavir; the antineoplastic drugs cyclophosphamide, taxol and tamoxifen; the endocrine drugs cyproterone acetate and RU486; the anti-inflammatory agent dexamethasone; the anti-Type 2 diabetes drug troglitazone; the antihypertensive drugs nifedipine and spironolactone; and the sedatives glutethimide and phenobarbital [10,11]. Commonly used herbal medicines can also activate PXR, such as St John's Wort (SJW) [12], guggulipid®, and kava kava [13]. Among dietary supplements, vitamins K2 and E have been established as weak PXR activators [14,15]. Several groups also reported that a number of environmental pollutants are PXR ligands, such as organochlorine pesticides and polybrominated diphenyl ether flame retardants [16,17]. In addition, some endobiotics were identified as PXR ligands, including certain bile acids, bile acid precursors and estrogens [18–22].

Another important feature of PXR is its species ligand specificity. For example, RIF is a potent hPXR specific activator and efficient human CYP3A4 inducer, whereas pregnenolone-16 α -carbonitrile (PCN), a synthetic anti-glucocorticoid, is a rodent-specific PXR activator. Based on the structural and functional analysis of the human and rodent PXRs, it was hypothesized that the species origin of the receptor is the determining factor for the species specificity of the ligand response. This hypothesis has led to the creation of the 'humanized' mice in which the mouse PXR (mPXR) was deleted via homologous recombination, and hPXR cDNA was introduced into the mouse liver through a liver-specific transgene [23]. These mice exhibited a 'humanized' hepatic xenobiotic response profile, readily responding to the human-specific inducer RIF in a concentration range equivalent to the standard oral dosing regimen in humans [23]. More recently, new generations of humanized mice have been reported by Ma *et al.* These include the transgenic mice carrying the genomic sequences of hPXR [24] and mice humanized with both PXR and CYP3A4 genomic sequences [25]. The creation of humanized mice represents a major step toward generating a humanized rodent toxicological model that is continuously renewable and completely standardized.

Following ligand binding, PXR forms a heterodimer with the RXR that binds to PXR response elements (PXREs), located in the 5'-flanking regions of PXR target genes, resulting in their transcriptional activation. PXR is mainly associated with the cellular response to xenobiotics, including induction of enzymes involved in drug oxidation and conjugation, as well as induction of xenobiotic and endobiotic transporters [26]. These include the phase I enzymes cytochrome P450 (CYP) 2B6, CYP2B9, CYP2C8, CYP2C9, CYP3A4 and CYP3A7, the phase II enzymes the glutathione-S-transferases (GSTs), UDP-glucuronosyltransferases (UGTs) and sulfotransferases (SULTs); and the transporters, multidrug resistance protein 1 (MDR1), MDR2, multidrug resistance-associated protein 2 (MRP2) and the organic anion transporter polypeptide 2 (OATP2) [6,27–30]. Metabolic enzymes and transporters induced by PXR activation affect the pharmacokinetics of both xenobiotics and endobiotics. Genetic variation in PXR might explain the interindividual variations in induction of phase I and phase II drug-detoxification enzymes and drug transporters. In addition, PXR defines a novel endocrine signaling pathway important for regulation of the steroid hormone, sterol, and bile acid homeostasis, so variability at the PXR genetic locus may therefore be associated with physiological and perhaps pathophysiological, changes in steroid, cholesterol or bile acid levels.

Implications of PXR-mediated gene regulation in drug metabolism, physiology & pathophysiology

The implications of PXR-mediated gene regulation in drug metabolism and drug interaction have been recognized since the initial cloning and characterization of this xenobiotic receptor. Accumulating evidence has also pointed to a role of PXR in normal physiology and diseases (Figure 1), which is consistent with the notion that xenobiotic enzymes and transporters are also involved in the biotransformation and homeostasis of many endogenous chemicals.

PXR in drug metabolism & drug–drug interactions

Our body encounters numerous xenobiotic chemicals, including prescription drugs, over-the-counter medications and herbal medicines. Many of them, especially when accumulated in excess, may exert toxic effects through various mechanisms. The process of drug metabolism is known to largely depend upon a concerted action of phase I and II enzymes, as well as drug transporters. Expressed mainly in the liver and intestine, these enzymes and transporters are capable of recognizing an amazing diversity of xenobiotics to promote their clearance.

PXR can be activated by a variety of xenobiotics and activation of PXR leads to the regulation of phase I, phase II enzymes and drug transporters [6,27,28]. The identification of RIF as a potent hPXR agonist has provided an explanation as to why this drug is an efficient inducer of drug-metabolizing enzymes, and thus prone to drug–drug interaction, a phenomenon that has long been recognized in the clinic. Another example of PXR-activating clinical drug is paclitaxel (Taxol®), one of the most commonly used antineoplastic agents. Paclitaxel is subjected to metabolic inactivation by hepatic CYP3A4 and CYP2C8. In addition to being inactivated by hepatic P450 enzymes, paclitaxel is excreted from the intestine via P-glycoprotein (p-gp, or ABCB1), a broad-specificity efflux pump protein encoded by the gene *MDR1* (also known as *ABCB1*). Synold and colleagues showed that paclitaxel activated SXR/hPXR can induce hepatic expression of CYP3A4 and CYP2C8, as well as MDR1 expression in intestinal tumor cells. This hPXR-mediated drug clearance pathway may enhance metabolism and efflux of paclitaxel, which may lead to increased intestinal drug excretion and drug resistance [31]. By contrast, docetaxel (Taxotere®), a closely related antineoplastic agent, did not activate SXR/hPXR, and thus displayed superior pharmacokinetic properties [31].

Other than RIF and paclitaxel, many other commonly used clinical drugs have also been shown to activate PXR. These include the peptide mimetic HIV-protease inhibitors [32], the cholesterol-lowering lovastatin [33] and the anti-inflammatory dexamethasone [34]. A more comprehensive analysis of the effect of commonly used clinical drugs on PXR activation has recently been published by Sinz and colleagues [35].

The regulation of drug-metabolizing enzymes by PXR is involved in clinical drug–drug interactions, in which one drug accelerates the metabolism of a second medication and may change or cause adverse results. As CYP enzymes can recognize a large spectrum of pharmaceutical substrates, a CYP gene-inducing drug is potentially capable of affecting the metabolism and clearance of co-consumed drugs. As mentioned earlier, the identification of RIF as a potent hPXR agonist has provided an explanation as to why this antibiotic drug is prone to drug–drug interactions. In another example, SJW, a popular herbal remedy for depression, has been reported to trigger severe adverse interactions with several clinical drugs, such as oral contraceptives, the HIV-protease inhibitor indinavir and the immunosuppressant ciclosporin. Such drug–drug interactions are likely to be the result of activation of PXR and consequent induction of CYP3A by SJW and the subsequent increased metabolism and/or decreased bioavailability of co-metabolized drugs. In the case of birth control pills, the use of SJW enhances drug clearance, increasing contraceptive failure and thus the birth of ‘miracle

babies'. The identification of SJW as a PXR agonist offered a plausible explanation for the propensity of SJW to cause drug–drug interactions [12].

Several traditional Chinese medicines (TCMs) have also been implicated in drug–drug interactions. TCMs are essential components of alternative medicines. One clinical concern of herbal product use is the effect of herbal products on the metabolism of co-administered drugs. We showed that two TCM herbs, Wu Wei Zi (*Schisandra chinensis* Baill) and Gan Cao (*Glycyrrhiza uralensis* Fisch), can activate PXR and induce the expression of several drug-metabolizing enzymes and transporters, including CYP3A and CYP2C9 and the MRP2 in reporter gene assay and in primary hepatocyte cultures [36]. The anticoagulant warfarin is known to be metabolized by CYP2C9 in humans [37]. As expected, administration of Wu Wei Zi and Gan Cao extracts in rats resulted in an increased metabolism of co-administered warfarin, reinforcing concerns involving the safe use of herbal medicines and other nutraceuticals to avoid PXR-mediated drug–drug interactions [36].

The recent development in the identification and development of PXR antagonists also has its potential implications in drug metabolism. It is conceivable that PXR antagonists may be useful to prevent drug–drug interactions and fine-tune the efficacy of therapeutics. There is a growing list of large- and small-molecule PXR antagonists that includes ET-743 [31], some polychlorinated biphenyls [38], ketoconazole [39], fluconazole and enilconazole [40], sulforaphane [41], coumestrol [42] and the HIV-protease inhibitor A-792611 [43]. Recent studies suggest that most of the known PXR antagonists interact on the outer surface of PXR at the AF-2 domain and disrupt the recruitment of co-activators [44]. PXR antagonist pharmacophore models were developed using computational methods based on the three azole antagonists and biphenyls [45]. The properties of the pharmacophore for PXR antagonists binding site showed an equal balance between hydrogen-bond acceptor and hydrophobic features, differing from the predominantly hydrophobic pharmacophores for agonists. This pharmacophore also suggested an overall small binding site that was identified on the outer surface of the receptor at the AF-2 site and validated by docking studies. Using these computational approaches, several new smaller antagonists of PXR were discovered with the antagonist pharmacophore [44]. Further studies are necessary to examine the clinical relevance of the PXR antagonists. An outstanding challenge is to determine whether these antagonists have applications in modulating PXR activity and downstream gene expression *in vivo* for preventing harmful drug–drug interactions and improving therapeutic efficacy.

Endobiotic function of PXR

Even though PXR was initially identified as a 'xenobiotic receptor', emerging evidence has pointed to an equally important role of PXR as an 'endobiotic receptor' that responds to a wide array of endogenous chemicals (endobiotics). Moreover, the activation of PXR by endogenous or xenobiotic ligands has implications in several important physiological and pathological conditions. For this reason, there have been discussions as to whether or not PXR can be explored as a therapeutic target [27,46].

PXR in bile acid detoxification & cholestasis—One family of endogenous PXR ligands identified shortly after the cloning of PXR are bile acids. Bile acids are catabolic end products of cholesterol metabolism. They are physiologically important in the formation of bile and solubilizing biliary lipids and promoting their absorption. However, excessive bile acids are potentially toxic. For example, the secondary bile acid lithocholic acid (LCA) has been shown to cause cholestasis in experimental animals, and has long been suspected of doing the same in humans. As an average human releases 600 ml of bile a day, the potential for disrupting bile flow (cholestasis) and the resultant accumulation of toxic byproducts is significant. Therefore, excess bile acid should be efficiently eliminated to avoid the toxic effect.

The PXR has been demonstrated to act as a LCA sensor and play an essential role in detoxification of cholestatic bile acids [20,47]. Studies in different animal models showed that activation of PXR protected against severe liver damage induced by LCA. Pretreatment of wild-type mice, but not the PXR null mice, with PCN reduced the toxic effects of LCA. Moreover, genetic activation of PXR by expressing the activated PXR in the liver of transgenic mice was sufficient to confer resistance to the hepatotoxicity of LCA. The cholestatic preventive effect of PXR was initially reasoned to be due to the activation of CYP3A, an important CYP enzyme responsible for bile acid hydroxylation [20,47]. Subsequent identification of SULT2A, a bile acid detoxifying hydroxysteroid sulfotransferase, as a PXR target gene suggested that additional PXR target genes may have also contributed to the phenotype [48]. Several follow-up studies, including those using mice with individual or combined loss of PXR and constitutive androstane receptor (CAR; NR1I2), have suggested that PXR-responsive bile acid transporter regulation may also play a role in preventing cholestasis [47,49–51]. Since activation of PXR was sufficient to prevent cholestasis, it has been suggested that PXR agonists may prove useful in the treatment of human cholestatic liver disease, a notion that has been supported by several clinical observations. Both RIF and SJW have been empirically used to treat cholestatic liver diseases [6]. The relief from cholestasis-associated pruritis and amelioration of cholestasis by RIF was associated with increased 6 α -hydroxylation of bile acids, which in turn facilitates glucuronidation by the UGTs at the 6 α -hydroxy position. Both RIF and SJW are potent agonists of hPXR and both *CYP3A* and *UGT* are PXR target genes, suggesting the anticholestatic effects of RIF and SJW are mediated by PXR.

PXR in bilirubin detoxification & clearance—Bilirubin is the catabolic byproduct of heme proteins, such as β -globin and CYP enzymes. Accumulation of bilirubin in the blood is potentially neurotoxic. For example, an insufficiency in expression of UGT1A1, a key enzyme for the conjugation of bilirubin in the Crigler–Najjar syndrome and Gilbert's diseases, results in severe hyperbilirubinemia. Deficiency of MRP2, a drug transporter responsible for the hepatic excretion of conjugated bilirubin, leads to Dubin–Johnson syndrome, characterized by the accumulation of glucuronidated bilirubin. PXR has been shown to induce the expression of multiple key components in the clearance pathway, including UGT1A1, OATP2, GSTA1 and 2 and MRP2. OATP2 facilitates bilirubin uptake from blood into hepatocytes [52]. GSTA1 and 2 reduce bilirubin back efflux from hepatocytes into blood. MRP2 promotes the canalicular efflux of conjugated bilirubin. Consistent with the pattern of gene regulation, activation of PXR in transgenic mice has been shown to prevent experimental hyperbilirubinemia [53]. It should be noted that activation of the xenobiotic receptor CAR has also been shown to be protective from hyperbilirubinemia [54].

PXR in adrenal steroid homeostasis & drug–hormone interactions—The PXR plays an important endobiotic role in adrenal steroid homeostasis. Our recent study showed that genetic (VP-hPXR transgene) and pharmacological (ligand, RIF) activation of hPXR in mice markedly increased plasma concentrations of corticosterone and aldosterone, the respective primary glucocorticoid and mineralocorticoid in rodents. The increased levels of corticosterone and aldosterone were associated with activation of adrenal steroidogenic enzymes, including *Cyp11a1*, *Cyp11b1*, *Cyp11b2* and 3β -*Hsd*. The PXR-activating transgenic mice also exhibited hypertrophy of the adrenal cortex, loss of glucocorticoid circadian rhythm, and lack of glucocorticoid responses to psychogenic stress [55].

Interestingly, the VP-hPXR transgenic mice had normal pituitary secretion of adrenocorticotropic hormone (ACTH), and the corticosterone-suppressing effect of dexamethasone was intact, suggesting a functional hypothalamus–pituitary–adrenal (HPA) axis despite a severe disruption of adrenal steroid homeostasis. The ACTH-independent hypercortisolism in the PXR-activating transgenic mice is reminiscent of the pseudo-Cushing's

syndrome in patients, the clinical hallmark of which is the normal dexamethasone suppression despite a high circulating level of glucocorticoid. Pseudo-Cushing's syndrome is most seen in alcoholic, depressed or obese subjects. It is of interest to know whether or not these susceptible patients are associated with increased expression and/or activity of PXR. The glucocorticoid effect appeared to be PXR-specific, as the activation of CAR in transgenic mice had little effect on the homeostasis of the glucocorticoids. We propose that PXR is a potential endocrine-disrupting factor that may have broad implications in steroid homeostasis and drug–hormone interactions [55].

PXR in lipid metabolism—The PXR has also recently been shown to play an endobiotic role by impacting lipid homeostasis [56,57]. Expression of an activated PXR in the livers of transgenic mice resulted in an increased hepatic deposit of triglycerides. This PXR-mediated lipid accumulation was independent of the activation of the lipogenic transcriptional factor sterol regulatory element-binding protein 1c (SREBP-1c) and its primary lipogenic target enzymes, including fatty acid synthase (FAS) and acetyl CoA carboxylase 1 (ACC-1). Instead, the lipid accumulation in transgenic mice was associated with an increased expression of the free fatty acid transporter CD36 and several accessory lipogenic enzymes, such as stearoyl CoA desaturase-1 (SCD-1) and long-chain free fatty acid elongase (FAE). Studies using transgenic and knockout mice showed that PXR is both necessary and sufficient for *Cd36* activation. Promoter analyses revealed a DR-3 type of PXR response element in the mouse *Cd36* gene promoter, establishing *Cd36* as a direct transcriptional target of PXR. The hepatic lipid accumulation and *Cd36* induction was also seen in the hPXR ‘humanized’ mice treated with the hPXR agonist RIF. The activation of PXR was also associated with an inhibition of pro- β -oxidative genes, such as peroxisome proliferator-activated receptor α (PPAR α) and thiolase, and an upregulation of PPAR γ , a positive regulator of CD36. The crossregulation of CD36 by PXR and PPAR γ suggests that this fatty acid transporter may function as a common target of orphan nuclear receptors in their regulation of lipid homeostasis [57]. Dai and colleagues recently showed that the PXR-mediated lipid accumulation is required for the hepatic regenerative response to liver resection [58], suggesting that PXR is essential for normal progression of liver regeneration by modulating lipid homeostasis. The role of PXR in lipid metabolism has also been supported by another recent study by Nakamura [59], in which the authors showed activated PXR represses the FoxA2 activity to downregulate gene expressions of carnitine palmitoyltransferase 1A (*Cpt1a*) and mitochondrial 3-hydroxy-3-methylgluturate-CoA synthase 2 (*Hmgcs2*), while activating that of *Scd1*. Thus, activated PXR represses hepatic energy metabolism by increasing triglyceride synthesis and decreasing β -oxidation and ketogenesis in the fasting mice.

PXR in inflammation & inflammatory bowel disease—RIF, an hPXR agonist, has been known as an immunosuppressant to suppress humoral and cellular immunological responses in liver cells, and its immunosuppressive role has also been well-documented in humans [60]. Recent studies show that activation of PXR by RIF in humans inhibits the activity of NF- κ B, a key regulator of inflammation and the immune response. In PXR knockout mice, the expression of NF- κ B target genes is substantially upregulated in multiple tissues, and small bowel inflammation is significantly increased, thereby demonstrating a direct link between PXR and drug-mediated antagonism of NF- κ B [61,62].

Inflammatory bowel disease (IBD) refers to a chronic inflammatory condition of the digestive tract occurring as one of two major types, ulcerative colitis and Crohn's disease. Ulcerative colitis is limited to the colon, while Crohn's disease most commonly affects the small intestine and/or the colon, but can involve any part of the gastrointestinal tract from the mouth to the anus. However, the etiology of IBD is unknown. Recently, PXR was identified as a gene strongly associated with the susceptibility to IBD in humans [63]. In patients with IBD, decreased expression of PXR and PXR target genes was also noted [64,65]. Thus, identification

of the role of PXR in IBD might provide new stratagem for IBD therapeutics. In the dextran sulfate sodium (DSS)-induced IBD mouse acute colitis model, treatment with the PXR ligand PCN protected against DSS-induced colitis compared with vehicle-treated mice, as defined by body weight loss, diarrhea, rectal bleeding, colon length and histology. However, this treatment did not decrease the severity of DSS-induced colitis in *Pxr*-null mice, indicating a role for PXR in protection against IBD [66]. It has recently been reported that hepatic SCD1 is down regulated in mice with DSS-induced colitis and that this leads to elevated levels of proinflammatory saturated fatty acids and reduced levels of anti-inflammatory unsaturated fatty acids [67]. It should be noted that *SCD1* was upregulated in mice by PXR activation [56], and thus PXR activation should be expected to ameliorate the symptoms of DSS-induced colitis in mice having low levels of expression of SCD1 through increased production of unsaturated fatty acids.

PXR in bone homeostasis—Vitamin D is essential for the maintenance of calcium homeostasis and for the development and maintenance of bones. 1,25-dihydroxyvitamin D₃ (1,25[OH]₂D₃), the physiologically active form of vitamin D in humans, elicits most of its biological effects by binding to a high-affinity receptor, the vitamin D receptor (VDR; NR1I1). CYP24 is a multifunctional 24-hydroxylase and the major vitamin D catabolic enzyme that directs the side-chain oxidation and cleavage of 1,25(OH)₂D₃ and 25(OH)D₃ to catabolic carboxylic acid end products. Recently, *CYP24* was identified as a PXR target gene by both *in vivo* and *in vitro* studies, suggesting that drugs such as PXR ligands can modulate *CYP24* gene expression and alter the homeostasis of 1,25(OH)₂D₃ [68]. The PXR target *CYP3A4* catalyzes 23- and 24-hydroxylation of 1,25(OH)₂D₃, resulting in production of the biologically inactive metabolites 1,23R,25-trihydroxyvitamin D₃ (1,23R,25[OH]₃D₃), 1,23S,25-trihydroxyvitamin D₃ (1,23S,25[OH]₃D₃), and 1,24S,25-trihydroxyvitamin D₃ (1,24S,25[OH]₃D₃). Compared with CYP24, CYP3A4 expression in humans is very abundant, especially in liver. However, the affinity and efficiency of CYP3A4 in 1,25(OH)₂D₃ metabolism are approximately tenfold lower than that of CYP24, as revealed by enzyme kinetics studies [69]. These studies suggest that activation of PXR by some drugs may be responsible for the acceleration of vitamin D catabolism through the upregulation of CYP3A4 and CYP24, leading to vitamin D deficiency and, eventually, to osteopenia or osteomalacia.

PXR in retinoid acid metabolism—The vitamin A metabolite retinoic acid (RA) modulates the transcription of a set of genes associated with cellular apoptosis, growth and differentiation by binding to retinoic acid receptors (RARs) and retinoid X receptors (RXRs). RA displays distinct anticarcinogenic activities and is currently used in or is being explored as a therapeutic agent in several human cancers, while a major drawback to its clinical application is the prompt development of resistance. The acquired resistance to RA might be explained in part by the concomitant administration of PXR ligands through activation of the PXR–CYP3A pathway, accelerating the catabolism of RA by induced CYP3A. Moreover, other induced PXR target transporter genes such as *MDR1A*, *MRP3* and *OATP2* might also be involved [70].

Implications of genetic variation of human PXR

Studies over the last two decades have shown significant interindividual variation in the hepatic and intestinal expression of CYP3A4 and the MDR1, two of the main transcriptional targets of PXR [71–74]. CYP3A4 has been estimated to play a role in the metabolism of over 50% of prescription medications on the market in the USA [75]. Due to the clinical importance of CYP3A4 and P-glycoprotein in drug pharmacokinetics, a number of research groups have investigated the possible impact of genetic variation of PXR on drug metabolism and clearance. Genetic variants of PXR could effect protein expression, ability to bind target DNA, or activation of PXR by ligands. A more recent study has also shown that PXR is a main regulator of the basal expression of multiple CYPs in primary cultured human hepatocytes [76],

suggesting that PXR expression *in vivo* may significantly influence both baseline and inducible metabolism of drugs and endogenous compounds. There is also increasing data on PXR crosstalk with the vitamin D receptor [77,78], and of the role of PXR in the homeostasis of steroid hormones [55]. Lastly, PXR interacts with the FOXA2 and FOXO1 pathways important in glucose homeostasis [79,80]. Consequently, genetic variation of PXR could potentially influence a wide range of physiologic pathways.

Variation of PXR sequences across vertebrate species

PXR genes have been cloned and functionally characterized from a variety of vertebrate species, including mammals, chickens, frogs and teleost fish [7]. A striking feature is high cross-species sequence divergence in the LBD. The LBD of PXR shares amino acid identities of only 75% between human and rodent sequences and only 50% between human and chicken or fish sequences. These sequence identities are unusually low in the nuclear hormone receptor superfamily, for which the corresponding sequence identities tend to be 10–20% higher [7, 81,82]. There are substantial differences in the ligand selectivity of PXR across animal species [7,83–86]. The high sequence divergence of the PXR LBD has been speculated to be an adaptation to cross-species differences in toxic exogenous (e.g., dietary) or endogenous PXR ligands (e.g., bile salts) [7,83,87,88].

PXR genes also demonstrate evidence of positive selection across vertebrate species. This has been shown by maximum likelihood analyses that estimate the rate of nonsynonymous (changes amino sequence) and synonymous (does not change amino acid sequence) nucleotide variation in the *PXR* coding region [82,83,89]. The high degree of sequence variation in the PXR LBD across species suggests that variation could also occur between human populations as an adaptation to different environmental and dietary conditions. An elegant example of inter-population genetic variation in humans has been proposed for CYP3A5 as an adaptation allowing for salt retention for human populations living in hot, arid climates [90].

Human *PXR* gene structure

The *hPXR* gene is located on chromosome 3q12q13.3 and is 38 kb [91]. The gene consists of nine exons, with exons 2 through 9 containing the coding regions. From analysis of expressed sequence tags (ESTs), the most abundant *hPXR* mRNA transcript (designated *hPXR.1*) contains exon 1a and uses a CTG (as compared with the more common ATG) initiator in exon 2. Bertilsson *et al.* described a variant they termed *hPAR2* that has an additional 177 nucleotides and 39 amino acid residues in the amino terminal end [3]. *hPAR2* results from the use of an ATG initiator in exon 1b. Using a CYP3A4-based luciferase reporter plasmid, no differences in ligand activation were found between *hPXR.1* and *hPAR2* [3].

Splice variants of human *PXR*

In addition to *hPAR2*, two additional splice variants of *hPXR* involve the 5' end of exon 5 [92,93]. *hPXR.2* was originally detected in breast tissue and results from a cryptic splice acceptor within exon 5, creating an in-frame deletion of 37 amino acid residues (174–210) relative to *hPXR.1* [93]. *hPXR.3* results from deletion of 123 nucleotides from the 5' end of exon 5, leading to an in-frame loss of 41 amino acids in the LBD. A splice variant similar to *hPXR.3* was found for mouse PXR [1]. One study found that *hPXR.2* and *hPXR.3* represented 6.7 and 0.3%, respectively, of total PXR transcripts in human liver. Transcripts of *hPXR.2* and *hPXR.3* were also detected in the adrenal gland, bone marrow, adult brain (medulla, pons, thalamus and spinal cord), fetal brain, colon, heart and stomach. In every tissue examined, *hPXR.1* was the most abundant transcript [92]. Interestingly, *hPXR.2* and *hPXR.3* lack portions of the LBD that are also not present in CAR, liver X receptor- α (LXR α ; NR1H3), LXR β (NR1H2), farnesoid X receptor (FXR; NR1H4), retinoid X receptor- α (RXR α ; NR2B1) and estrogen receptor- α (ER α , NR3A1) [92]. Five of the missing amino acid residues are known

to be part of the hPXR ligand-binding pocket from x-ray crystallographic structures of the hPXR LBD [5,94].

Some of the PXR slicing variants have been shown to possess differential functions. For example, in cell-based assays using LS174 cells, hPXR.2 showed marked reduction of basal and ligand-induced (corticosterone and RIF) transactivation of a CYP3A4-based reporter [95]. Similar findings were observed with the mouse ortholog of hPXR.2 [1]. An additional study found that hPXR.2 was unable to induce UGT1As in Caco-2 cells [96].

In addition, seven splice variants were identified by Fukuen *et al.* [97]. A total of five of the seven splice variants had an alteration in exon 5. All seven splice variants were expressed at significantly lower levels than PXR.1, PXR.2 and PXR.3. None have been functionally characterized.

Genetic variants in coding regions of human PXR

Several groups have performed resequencing studies of the exons of hPXR. To date, 227 SNPs of hPXR have been deposited in dbSNP [201]. Table 1 lists some of the PXR polymorphisms that have been characterized (see also Figure 2). Some of the PXR nonsynonymous SNPs described to date (E18K, P27S, D163G and A370T) have only been detected in African–American populations and have not yet been found in European or Asian populations. To prioritize SNPs for further characterization or association studies, some research groups have used *in silico* approaches, such as Sorting Intolerant From Tolerant (SIFT) [202] and Polyphen [203] to predict the probability that a SNP affects PXR function. SIFT and Polyphen use amino acid biochemical and structural properties along with sequence conservation data to estimate the likelihood that a given polymorphism affects function. In addition, evolutionary tests for investigating positive (Darwinian) selection have also been used on human resequencing data.

P27S (PXR*2) and G36R (PXR*3) SNPs in exon 2 were originally observed by Zhang *et al.* and Hustert *et al.* [91,95]. P27S was predicted to affect protein function by two possible mechanisms: change in hydrophobicity and creation of a serine phosphorylation site. Interestingly, P27S has not yet been observed in Caucasian populations, but was observed fairly frequently (15–20%) in the African–American populations analyzed [91,95]. P27S was not associated with any differences in liver CYP3A4 content [91]. The G36R polymorphism occurs in a region of lower evolutionary conservation than P27S. Transient transfection assays did not detect any differences between PXR*1, *2 and *3 in transactivation of a CYP3A4-based reporter [91,95].

To date, the greatest number of SNPs in the hPXR coding region have been detected in exon 4, which encodes the C-terminal portion of the DBD and part of the N-terminal portion of the LBD. A total of five SNPs in exon 4 are nonsynonymous: R122Q (PXR*4) [91], V140M (PXR*10) [95], R148Q (PXR*6) [98,99], Q158K [100] and D163G (PXR*11) [95]. R122Q was only detected in one individual homozygous for this variant. PXR*10 was identified in Caucasian individuals, while PXR*6 and *11 were identified in African–American individuals. Expression of the PXR*10 variant in LS174T cells revealed significantly higher basal but lower corticosterone- and RIF-induced transactivation in a reporter assay compared with hPXR.1. By contrast, the PXR*11 allele had complete loss of basal transactivation for CYP3A4/DR3 response elements but higher RIF-inducible transactivation relative to hPXR.1 [95]. Q158K has so far only been detected in Chinese populations and is associated with lower induction by paclitaxel and RIF in luciferase-based reporter assays using LS174T and HepG2 cells [100]. The functional analyses of the PXR*10 and *11 variants emphasize some of the challenges in assessing PXR polymorphisms, in that some of the effects were promoter-specific. The PXR*9 (E18K) variant, when transfected into LS174 cells, resulted in lower protein expression, but similar response to hPXR.1 in a transactivation assay [95].

Several nonsynonymous polymorphisms were identified by Koyano *et al.* in a Japanese population [99]. *PXR*5* (R98S) occurred at very low frequency, but was associated with a dramatic phenotype in functional assays. When transfected transiently in Cos-7 and HepG2 cells, *PXR*5* showed no basal or inducible transactivation. Furthermore, *PXR*5* has markedly reduced binding to an ER6 DNA response element in a gel shift assay [95,98]. *PXR*4* is predicted to reside within the C-terminal extension to the DBD, adjacent to a highly conserved Cys residue of the second zinc finger [5]. Disruption of this portion of the DBD would explain the lack of DNA binding observed in cellular studies of *PXR*5*. Other nonsynonymous polymorphisms of *hPXR* are summarized in Table 1.

The analysis of nonsynonymous polymorphisms in the *hPXR* LBD have the caveat that the functional effects have mainly been assessed using prototypical ligands (e.g., corticosterone, RIF) and CYP3A4-based reporters. These polymorphisms may have effects on ligands not yet studied or have promoter- or tissue-specific interactions that have yet to be appreciated.

Genetic variants within the promoter and other noncoding regions of human *PXR*

The limited number of nonsynonymous variants within *hPXR* has prompted research groups to resequence noncoding regions of the gene. In the first detailed characterization of the *hPXR* gene structure, Zhang *et al.* detected 14 SNPs in the introns [91]. Only C8357G in intron 7 was predicted to have a high probability of alternative splicing using a computational algorithm [204] for assessing the likelihood of creating cryptic splice sites or disrupting existing splice sites. However, analysis of liver cDNAs harboring the intron 7 SNP did not detect any aberrant splicing [91]. Several intronic SNPs were associated with differences in CYP3A4 expression or metabolic capacity (Table 1). A7635G in intron 5 was associated with higher intestinal expression of CYP3A, as well as increased induction of intestinal CYP3A4 by RIF. Similarly, the C8055T variant was also associated with increased induction of intestinal CYP3A4 by RIF. The mechanism by which these SNPs could affect intestinal expression of CYP3A4, or whether these SNPs are in linkage disequilibrium with other genetic variants, remains to be determined. More recent studies have found an association of G252A (intron 2) with increased midazolam clearance (a measure of CYP3A metabolizing activity) [101] and a weak association of A7635G with the onset of primary sclerosing cholangitis [102].

A total of nine SNPs have been reported in the 3'-UTR of *hPXR*. The 10620T and 10799A variants were associated with twofold lower testosterone 6 β -hydroxylase activity (a measure of CYP3A metabolism) in primary human hepatocytes [91]. Along with A7635G, the polymorphism in intron 5 described above, two other polymorphisms in the 3'-UTR (10620, rs1054190; 11193, rs3814058) were also associated with onset of primary sclerosing cholangitis [102].

Zhang *et al.* was the first to report sequence variation within the *hPXR* promoter [91]. This study noted an association between -831T and a higher RIF-induced increase in the erythromycin breath test, a marker of hepatic CYP3A4 activity [73]. Individuals who were -831TT had twofold higher RIF-induction than individuals with the CC genotype. The -831 position falls within a predicted NF- κ B binding element.

More recent studies have shown associations between *hPXR* promoter polymorphisms and expression of CYP3A4 in the liver. Basal activity of liver CYP3A4 was related to several promoter SNPs that are known to be in linkage disequilibrium with one another, including -1359T>C [103]. A second study demonstrated that females with the -1359TT genotype have threefold higher testosterone 6 β -hydroxylase activity (a measure of CYP3A activity) than those with the -1359CC genotype.

A SNP at -24381 has been associated with higher P-glycoprotein and CYP3A4 expression in colon tumors [104]. This SNP was originally observed by Zhang *et al.* and found not to be associated with differences in expression of PXR transcriptional targets in pathologically normal intestines or livers [91]. This variant warrants further investigation. A SNP at -566 (A>C) was found to be significantly associated with both MDR1 and CYP3A4 expression [104]. A 6-bp deletion in intron 1a (-24020) has been reported that is found with a frequency of approximately 28% in the Japanese population [103,105]. This variant is associated with total loss of PXR promoter activity when expressed recombinantly in the HepG2 human liver cell line [105]. An intron 1 SNP (-63396C>T) that is in linkage disequilibrium with multiple other SNPs is associated with threefold higher CYP3A4 activity in the liver.

Several studies have shown that HNF3 β (also called fork head transcription factor FoxA2), HNF1 and HNF4 influence transcription activity of PXR. Moreover, HNF4 has been shown to regulate expression of PXR in the liver [106–108]. Interestingly, several SNPs associated with interindividual differences in CYP3A4 reside within sites predicted by *in silico* analysis to be HNF binding sites. For example, 69789A>G polymorphism disrupts a predicted HNF4 binding site and has been associated with decreased transcription of CYP3A4, MDR1 and PXR in the liver [103].

Association of PXR genetic variation with inflammatory bowel disease & primary sclerosing cholangitis

Characterization of PXR knockout mice showed significant intestine inflammation [61]. In addition, PXR ligand activation has been shown to decrease hepatic fibrosis [109], cause hepatic steatosis [56], and reduce the inflammatory response [61]. Consistent with the animal models, PXR expression is lower in the pathologically-involved tissue of patients with inflammatory bowel disease (Crohn's disease or ulcerative colitis) [64,65]. Further investigation found an association between the hPXR 45005C genotype and inflammatory bowel disease [63]. Curiously, the promoter SNP at 45005, which is within predicted NF- κ B and ISFG-2 sites, was originally found to be associated with PXR mRNA in the liver, but with the 45005C genotype associated with higher mRNA levels [91]. The contrasting results suggest the possibility that the 45005 SNP may have different consequences in liver versus colon, or in the inflammatory versus noninflammatory state (perhaps by affecting NF- κ B pathways).

Another study found an association with a SNP in the 3'-UTR of hPXR (rs1054190) and disease course in patients with primary sclerosing cholangitis, an inflammatory disease of the hepatic bile ducts that leads to fibrosis and eventually liver cirrhosis [102].

Conclusion & future perspective

Genetic variation of PXR was originally studied for possible effects on enzymes and transporters important in xenobiotic and endogenous compound metabolism and clearance. To date, a number of polymorphisms in both coding and noncoding regions have been discovered that are associated with differences in expression of PXR itself or of the major transcriptional targets of PXR such as CYP3A4 and P-glycoprotein. More recent investigations have linked PXR with pathways involved in inflammation as well as homeostasis of glucose, lipids, steroid hormones and fat-soluble vitamins. PXR knockout mice show a higher incidence of intestinal inflammation. Preliminary studies have shown associations between PXR polymorphisms and inflammatory bowel disease and primary biliary sclerosing sclerosis. Identification of the role of PXR in IBD might provide new stratagem for IBD therapeutics. However, the mechanism by which the PXR polymorphisms modulate these diseases is currently unclear. Future studies should focus on more extensive genetic studies and on the mechanism by which PXR genetic variants influence physiology.

Executive summary

Implications of pregnane X receptor (PXR)-mediated gene regulation in drug metabolism, physiology & pathophysiology

- The nuclear receptor PXR functions as a species-specific xenobiotic-sensing receptor mediating the induction of phase I and phase II drug-metabolizing enzymes and transporters. This regulation has a broad implication in drug metabolism, drug–drug interactions, and drug toxicity.
- PXR also functions as an endobiotic receptor and is implicated in normal physiology and diseases, such as hepatic steatosis, vitamin D homeostasis, bile acids homeostasis, steroid hormones homeostasis and inflammatory bowel diseases.
- Cross-species comparisons of PXR show surprising differences in the amino acid sequence of their ligand-binding domains (LBDs), which explains the striking differences in the activation profiles of PXR across species. The species specificity of PXR ligand also underscores the significance of human PXR humanized mice, in which the mouse PXR is genetically replaced by its human counterpart.

Implications of genetic variation of human PXR

- PXR is subject to alternative splicing, and the differences in the expression of splice variants could play a role in interindividual variability in PXR responsiveness.
- Genetic variants in coding regions, the promoter and other noncoding regions of human PXR have been identified and these variants could effect protein expression, the ability to bind target DNA or activation of PXR by ligands.
- PXR genetic variation is associated with inflammatory bowel disease and primary sclerosing cholangitis.

Conclusion & future perspective

- PXR has been established a ‘master’ xenobiotic receptor regulating the expression of phase I and phase II enzymes, as well as drug transporters.
- Subsequent studies have also revealed important endobiotic functions of PXR, ranging from liver diseases to inflammation and the homeostasis of lipids, steroid hormones and the bone.
- A number of polymorphisms have been discovered that are associated with differences in expression of PXR itself or of the major transcriptional targets of PXR.
- Future studies should focus on more extensive genetic studies and on the mechanism by which PXR genetic variants influence physiology.

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Figure 1. Summary of the biological functions of PXR

PXR is activated by many xeno- and endo-biotics, regulating the expression of drug-metabolizing enzymes and transporters. Upon ligand binding, PXR forms a heterodimer with the RXR and the regulation is achieved by binding of the PXR–RXR heterodimer to the PXRE in the target gene promoters. The regulatory function of PXR is implicated in many physiological pathways and diseases.

PXRE: PXR-responsive element; RXR: Retinoid X receptor.

Figure 2. Location of *PXR* polymorphisms in the coding region and 3'-UTR

See Table 1 for more detailed information on the biological effects of these polymorphisms. The two 3'-UTR polymorphisms have been associated with onset of primary sclerosing cholangitis. DBD: DNA-binding domain; LBD: Ligand binding-domain; UTR: Untranslated region.

Table 1

Selected list of *PXR* polymorphisms

Location in Genbank accession number AF364606	Name (nonsynonymous polymorphisms only)	Functional consequences or putative disease correlations	Population allele frequency	Ref.
C79T (exon 2)	<i>PXR</i> *2 (P27S, DBD)	None observed in transactivation assays	AA = 0.20 Cauc = 0.0	[91]
G106A (exon 2)	<i>PXR</i> *3 (G36R, DBD)	None observed in transactivation assays	AA = 0.03 Cauc = 0.01	[91]
C2904T (exon 3)	<i>PXR</i> *5 (R98S)	Loss of DNA binding and transactivation in HepG2 cells	J = 0.0024	[98]
G4321A (exon 4)	<i>PXR</i> *4 (R122Q)	Reduced affinity for <i>PXR</i> binding sequences (EMSA)	AA = 0.0 Cauc = 0.01	[91]
G4374A (exon 4)	<i>PXR</i> *10 (V140M, LBD)	Reduced ligand activation (transactivation assays) 50% decrease in <i>PXR</i> protein expression in LS174T cells Increased basal transcription activity but reduced induction by RIF and corticosterone	AA = 0.0, Cauc = 0.002	[95]
A4444G (exon 4)	<i>PXR</i> *11 (D163G, LBD)	No basal transactivation activity in LS174T cells	AA = 0.014 Cauc = 0.0	[95]
A7635G (intron 5)		Higher intestinal expression of CYP3A and increased induction of intestinal CYP3A by RIF. Associated with onset of PSC	AA = 0.77 Cauc = 0.35	[91,102]
C8055T (intron 6)		Increased induction of intestinal CYP3A by RIF	AA = 0.18, Cauc = 0.15	[91,102]
C10620T (3'-UTR)		Increased 6 β -hydroxylation activity in primary human hepatocytes.	AA = 0.14 Cauc = 0.11	[91,102]
C10799A (3'-UTR)		Increased 6 β -hydroxylation activity in primary human hepatocytes	AA = 0.14 Cauc = 0.13	[91]
-25385C>T		Affects NF- κ B and ISGF-3 binding sites. Associated with IBD	Cauc = 0.69 T = 0.31	[63,91,102]

AA: African-American; Cauc: Caucasian; IBD: Inflammatory bowel disease; J: Japanese; PSC: Primary sclerosing cholangitis; *PXR*: Pregnane X receptor; RIF: Rifampicin; T: Total; UTR: Untranslated region.