Nuclear Receptors in Drug Metabolism, Drug Response and Drug Interactions

Chandra Prakash¹,²,¶, Baltazar Zuniga¹,³,¶, Chung Seog Song¹,¶, Shoulei Jiang¹, Jodie Cropper¹, Sulgi Park¹, and Bandana Chatterjee¹,4,*

¹Department of Molecular Medicine/Institute of Biotechnology, The University of Texas Health Science Center at San Antonio, Texas Research Park, 15355 Lambda Drive, San Antonio, Texas 78245
²William Carey University College of Osteopathic Medicine, 498 Tucsan Ave, Hattiesburg, Mississippi 39401
³University of Texas at Austin, 2100 Comal Street, Austin, Texas 78712
⁴South Texas Veterans Health Care System, Audie L Murphy VA Hospital, 7400 Merton Minter Boulevard, San Antonio, Texas 78229

Abstract

Orally delivered small-molecule therapeutics are metabolized in the liver and intestine by phase I and phase II drug-metabolizing enzymes (DMEs), and transport proteins coordinate drug influx (phase 0) and drug/drug-metabolite efflux (phase III). Genes involved in drug metabolism and disposition are induced by xenobiotic-activated nuclear receptors (NRs), i.e. PXR (pregnane X receptor) and CAR (constitutive androstane receptor), and by the 1α, 25-dihydroxy vitamin D₃-activated vitamin D receptor (VDR), due to transactivation of xenobiotic-response elements (XREs) present in phase 0-III genes. Additional NRs, like HNF4-α, FXR, LXR-α play important roles in drug metabolism in certain settings, such as in relation to cholesterol and bile acid metabolism. The phase I enzymes CYP3A4/A5, CYP2D6, CYP2B6, CYP2C9, CYP2C19, CYP1A2, CYP2C8, CYP2A6, CYP2J2, and CYP2E1 metabolize >90% of all prescription drugs, and phase II conjugation of hydrophilic functional groups (with/without phase I modification) facilitates drug clearance. The conjugation step is mediated by broad-specificity transferases like UGTs, SULTs, GSTs. This review delves into our current understanding of PXR/CAR/VDR-mediated regulation of DME and transporter expression, as well as effects of single nucleotide polymorphism (SNP) and epigenome (specified by promoter methylation, histone modification, microRNAs, long non coding RNAs) on the expression of PXR/CAR/VDR and phase 0-III mediators, and their impacts on variable drug response. Therapeutic agents that target epigenetic regulation and the molecular basis and consequences (overdosing, underdosing, or beneficial

*Corresponding Author : Bandana Chatterjee, Ph.D., Department of Molecular Medicine/Institute of Biotechnology, 15355 Lambda Drive, San Antonio, Texas 78245, chatterjee@uthscsa.edu; Tel: 210-567-7218, FAX: 210-567-7222.
¶Joint First Authors

Disclosure
Chandra Prakash, Baltazar Zuniga, and Chung Seog Song are joint first authors.

Conflict of Interest
No conflict of interest exists for any of the authors.
outcome) of drug-drug/drug-food/drug-herb interactions are also discussed. Precision medicine requires understanding of a drug’s impact on DME and transporter activity and their NR-regulated expression in order to achieve optimal drug efficacy without adverse drug reactions. In future drug screening, new tools such as humanized mouse models and microfluidic organs-on-chips, which mimic the physiology of a multicellular environment, will likely replace the current cell-based workflow.

Keywords
Nuclear receptors; PXR; CAR; Xenobiotic-response element; Gene induction; Phase 0-III mediators; Genetic polymorphism; Epigenetics; Drug interactions; Drug screening

1 Introduction

Drug metabolism, which occurs primarily in the liver and intestine, refers to the enzymatic modification and subsequent disposal of medicinally active compounds, originating either endogenously (as steroids, neurotransmitters, metabolic products like bile acids) or exogenously (as natural products or synthetic/semi-synthetic chemicals). Upon conversion to hydrophilic metabolites, drugs are eliminated from the body following biliary excretion and renal clearance by glomerular filtration and tubular secretion. Drug metabolism is also integral to the biotransformation of pro-drugs to pharmaco-active agents. Drug metabolism and disposition is coordinated by an array of liver- and intestine-expressed drug-metabolizing enzymes (DMEs) and drug-transporting proteins whose tissue abundance is transcriptionally regulated by specific nuclear receptors (NRs), which are ligand-activated transcription factors [1].

Of the 48 distinct receptors comprising the NR superfamily in humans, pregnane X receptor (PXR, NR1I2) and constitutive androstane receptor (CAR, NR1I3) are primary transcriptional regulators of the genes involved in the metabolism and elimination of drugs/drug metabolites [4, 2, 3]. PXR and CAR are generically referred as xenobiotic NRs, since structurally diverse drugs and environmental xenobiotics activate these two NRs. PXR and CAR are also activated by a number of endobiotics (steroids, sterols, retinoids, thyroid hormones, bile acids). In addition, PXR and CAR activation can result from receptor phosphorylation by various kinases that are activated in response to drug-mediated induction of specific intracellular signal cascades; in this case, drugs may not directly interact with the xenobiotic NRs [5]. Vitamin D receptor (VDR, NR1I1), beyond its classic role in calcium and phosphate homeostasis, has the ability to transcriptionally induce drug transporters and DMEs, especially in the enterocytes of intestinal tissue [6, 7]. In certain contexts, additional NRs, such as the bile acid-activated farnesoid X receptor (FXR, NR1H4); oxysterol-activated liver X receptor (LXR-α, NR1H3); fatty acid/eicosanoid-activated peroxisome proliferator activated receptor (PPAR-α, NR1C1), and retinoid-related orphan receptors (ROR-α, ROR-γ) regulate genes linked to drug absorption, distribution, metabolism and excretion (ADME) [8]. Hepatocyte nuclear factor (HNF4-α, NR2A1), a member of the NR superfamily, plays a synergizing role in the PXR- and CAR-mediated transactivation of DME- and transporter-encoding genes [11, 9, 10].
Altered activities of polymorphic variants of NRs and ADME-related gene products account for variable response to prescription medicine between individuals. Amino acid changes due to nucleotide polymorphisms in the coding region can influence protein stability or activity, while polymorphism at upstream, downstream or intragenic regulatory loci can alter NR-mediated ADME gene transactivation. Epigenetic, transcriptional and posttranslational regulation of xenobiotic NRs can further impact drug metabolism and clearance. Evaluation of drug-drug interactions (DDI), which result from changes in the level or activity of DMEs and/or transporters due to a second drug, is an essential component of drug development workflow.

In this review, we describe various classes of DMEs and transporters, present an overview of the molecular underpinnings for NR-mediated genetic and epigenetic regulation of ADME genes and consider roles for various NRs (especially PXR/CAR/VDR) and their target genes in differential drug response. Illustrative examples highlighting critical roles of xenosensing NRs, DMEs and transporters in DDI are also examined. Finally, we discuss current drug-screening platforms and their potential future improvements.

2 Drug Metabolizing Enzymes (DMEs) and Drug Transporters

The four phases of drug metabolism entail cellular uptake of therapeutic molecules (phase 0); their enzymatic oxidation (phase I) and conjugation (phase II), and efflux of drug metabolites for clearance (phase III). PXR and CAR activate genes involved in all four phases. General steps in drug metabolism and elimination are shown in Figure 1.2.

2.1 Phase 0 uptake proteins

Basolaterally located uptake proteins guide cellular entry of drugs from circulation; drug influx can be a rate-limiting step for drug metabolism and clearance [15, 12]. All uptake proteins are members of the solute carrier (SLC) protein family of which there are more than 300 members grouped under 52 subfamilies. Liver, intestine and kidneys are major sites of SLC expression. Most SLC proteins localize to cell membrane, although some may localize to mitochondria and other organelles. Proteins from nineteen SLC gene subfamilies have drug uptake activity. Most significant gene families of uptake transporters are SLC15 (oligopeptide transporter), SLC22 (organic anion/cation/zwitterion transporter), SLCO (organic anion transporting polypeptide) and SLC47 (organic cation transporter) [13, 14]. For example, OCT1 is a SLC22A1 encoded organic cation uniporter involved in the influx of the antiviral agent acyclovir, ganciclovir and the anti-diabetic drug metformin. Drug substrates for proteins encoded by SLC15, SLC22, SLCO, and SLC47 families have been described [13, 14, 15]. SLCs either serve as channels (uniporter) to guide drug diffusion down an electrochemical gradient, or drive drug transport against a diffusion gradient that is coupled to the sympport or antiport of small inorganic or organic ions.

2.2 Phase I DMEs

Heme-containing cytochrome P450s (CYPs), flavin-containing monooxygenases, monoamine oxidases and xanthine oxidase/aldehyde oxidases are examples of phase I DMEs, which generally localize to the endoplasmic reticulum of cells. CYP enzymes play
the most prominent role in phase I metabolism. Liver is the first pass and primary site of phase I metabolism, along with the gastrointestinal tract, kidneys, skin, and lung serving as additional sites; most tissues, however, express phase I DMEs. Addition or exposure of polar functional groups (e.g., −OH, hydroxyl; −COOH, carboxyl; −NH2, amine; −SH, sulfhydryl) to drug substrates enhances their bioavailability and solubility and promotes pro-drug biotransformation. Polar groups also arise by reduction of a ketone or aldehyde group to an alcohol; oxidation of an alcohol to an acidic group; hydrolysis of esters and amides; reduction of azo and nitro groups; oxidative dealkylation of N-alkyl, O-alkyl, and S-alkyl groups. When sufficiently polar, phase I metabolites can be pumped out of cells without additional modification.

CYPs are products of a multigene family, which for humans include 57 CYP genes [16]. Individual CYP is specified by the family (with an Arabic numeral), then the subfamily (with a letter) followed by the isozyme within the subfamily (with another Arabic numeral) and the allele number (with a preceding asterisk) of an individual gene within the subfamily. As an example, for CYP2D6*1, the *1 allele is wild type CYP2D6 with normal activity. Additional alleles of CYP2D6, marked with higher numbers preceded by *, exhibit aberrant functions [17]. CYPs are expressed in practically all tissues, with liver exhibiting the highest abundance and expressing largest number of individual CYPs. Enzymes of the CYP-1, -2, and -3 families metabolize majority of drugs and nondrug xenobiotics. The fraction of clinical drugs that are substrates for individual CYPs is schematically presented as Figure 2.2. CYP3A4, the most abundant CYP enzyme in human liver, acts on the greatest number of drugs and other xenobiotics. CYP2D6, although present at lower abundance, metabolizes numerous drugs. Substrate specificity is narrower for other members of the CYP family that are expressed in hepatic and extrahepatic tissues. They target endogenous substrates like sterols, fatty acids, eicosanoids and vitamins. A comprehensive list of drug substrates for CYPs has been reported ([18]; http://www.pharmacologyweekly.com/cytochrome-cyp-p450-enzyme-medication-herbs-substrates, updated May, 2015).

2.3 Phase II DMEs

Broad-specificity phase II transferases catalyze conjugative reactions. Common phase II modifications are glucuronidation by UDP-glucurononyltransferase (UGT), sulfonation by sulfotransferase (SULT), glutathionylation by glutathione S-transferase (GST), acetylation by N-acetyl transferase (NAT) and methylation by methyltransferase (MT). For any given transferase family, individual family members show predilection for a distinct set of substrates. Cofactors of phase II transferases react with functional groups that are either part of the parent drug or generated by phase I modification. In contrast to the enhanced potency of many phase I metabolites, phase II modified drug metabolites normally exhibit diminished function. PXR- and CAR-mediated gene regulation for a number of phase II transferases has been studied [9, 19].

2.4 Phase III efflux proteins

Members of the ATP binding cassette (ABC) superfamily, encoded by the ABCB, ABCC, and ABCG gene subfamilies, are broad-specificity exporters that pump drugs out of cells using energy from ATP hydrolysis. In hepatocytes, efflux proteins reside either in
canalicular/apical membranes or in blood-facing basolateral/sinusoidal membranes, guiding drugs, endobiotics and their metabolites for biliary excretion and efflux into systemic circulation. Multidrug-resistance associated proteins MRP2 (ABCC2), the bile salt export pump BSEP (ABCB11), the breast cancer resistance protein BCRP (ABCG2) are examples of ABC cassette family transporters which mediate apical efflux of drugs, steroids, bile acids and their conjugates. P-glycoprotein (MDR1, ABCB1) is an apical membrane transporter in hepatocytes [13]. Basolateral efflux of unconjugated and phase II-conjugated drugs, steroids, prostaglandin and bile acids from hepatocytes into sinusoidal blood is assisted by ABC transporters such as the multi-drug resistance associated proteins MRP3, MRP4, MRP5 and, also, by the ATP-independent OSTα/OSTβ complex that functions as an organic solute and steroid transporter. OST α contains seven transmembrane domains and OST β has a single transmembrane domain [20]; neither is part of the ABC transporter superfamily. MATE (multidrug and toxin extrusion) efflux transporters are H⁺-coupled antiporters, which transport structurally unrelated organic cations out of cells. They are members of the solute carrier subfamily SLC47, expressed primarily in the liver and kidney, and they localize at apical membranes of renal tubular epithelia and bile canaliculi. MATE1 (product of SLC47A1) mediates extrusion of organic cations into urine and bile. In the human kidney, the uptake transporter OCT2 (organic cation transporter, SLC22A2 encoded) promotes the import of cationic drugs (such as metformin, cisplatin, imatinib) from the blood at the basolateral membrane of the proximal tubule epithelial cells. MATE-1 and the isoform MATE-2K mediate secretion of cationic drugs across the brush-border membrane into the proximal tubule lumen [21].

3 Nuclear Receptors (NRs), Response Elements, Gene Regulation by PXR/CAR/VDR

NRs, upon association with DNA response elements, induce a cascade of protein-protein interactions that lead to the assembly of multiple classes of regulatory proteins (coactivators, corepressors, histone modifiers, chromatin remodeling complex) at the NR-bound chromatin region. Signal transmission from the coregulator assembly to the basal transcription machinery via a multi-protein mediator complex culminates in altered RNA polymerase II activity and transcriptional response of NR-regulated genes.

The NR superfamily of ligand-activated transcription factors in humans is defined by 48 receptors grouped into four classes (Type I–IV) based on the nature of activating ligands, preferred sequence organization of NR-binding DNA response elements in target genes and dimerization partner of the activated NR [22, 23]. Type I NRs reside in the cytoplasm in an inactive state in association with chaperone proteins. They are activated upon binding cognate steroid hormone ligands, translocated to the nuclear compartment and bind target gene response elements as homodimers to mediate gene regulation. Type II receptors, such as the vitamin D receptor (VDR), are activated by nonsteroid endocrine ligands (1α,25-dihydroxy vitamin D₃, retinoic acid-all trans, for RAR-α/β/γ; thyroid hormone for TR-α/β). Several Type II receptors are activated by intracrine ligands (e.g. bile acids activating FXR-α; oxysterols activating LXR-α/β; fatty acids/eicosanoids activating PPAR- α/γ/δ). Type II NRs in an inactive state remain tethered to corepressors.
as heterodimers with the obligate partner retinoid X receptor (RXR). Exchange of corepressors for coactivators initiates activation of ligand-bound Type II NRs. PXR and CAR, comprising the Type III subgroup, are transported from cytoplasm to the nucleus upon activation by chemical inducers. Nuclear PXR and CAR bind to DNA response elements as dimers with RXR to set the stage for subsequent regulation of target gene transcription.

Activation of CAR in most cases entails a ligand-binding independent mechanism, as reported for phenobarbital-like chemicals, which induce dephosphorylation of CAR at threonine-38, thereby activating CAR and promoting its nuclear translocation. Direct ligand binding and activation of CAR has also been reported for some xenobiotic compounds [24, 25]. Type IV NRs (e.g., LRH1, NGF1-B/NUR77, RORs) bind to DNA elements as a monomer, homodimer, or even as a heterodimer, partnering with RXR or another member of the same subfamily [26]. Although PXR, CAR and, to a significant extent VDR, are primary regulators of drug metabolism and disposition, NRs from all four classes are known to influence drug/xenobiotic response, as discussed under Section 3.3.

For all NRs, the primary structure specifies a common generalized organization based on functional domains [23, 2]. The highly variable amino-terminal A/B domain harbors constitutively active transactivation function (AF-1) and multiple autonomous transactivation domains. This is followed by a DNA-binding domain (DBD, C domain), through which an activated NR binds to a DNA response element. The ligand-binding domain (LBD, E domain) at the carboxyl end encompasses the activation function-2 region (AF-2). A less conserved flexible hinge domain (D) connects DBD and LBD. The hinge region contains a nuclear localization signal (NLS) sequence, which extends to the 3’ end of DBD. A variable F domain follows the LBD E domain in some but not all NRs.

X-ray crystallography, cryo electron microscopy and solution structure determination by various methods including small-angle X-ray scattering and hydrogen-deuterium exchange, revealed DBD and LBD structures of several NRs, such as the first and second zinc finger modules and DNA-binding specificity motif of DBD; receptor dimerization motif; twelve α-helices of LBD and ligand-induced helix-12 repositioning that creates an interaction surface for coactivator or corepressor recruitment [29, 28, 27].

DNA elements cognate to Type I-III NRs constitute repeats of the half-site consensus sequence RG(G/T)TCA (R: purine), configured as a direct repeat (DR), inverted repeat (IR), or everted repeat (ER) and separated by a varying number of nucleotides. Type I NRs recognize IR3-type palindromic elements; Type II and III NRs recognize specific repeat motifs of the consensus half site. Type IV NRs bind to a single hexamer consensus RG(G/T)TCA, which may contain a short preferred sequence 5’ to the hexameric site [26].

Preferred response elements for PXR, CAR and VDR are 3- or 4- nucleotide spaced direct repeats (DR3, DR4), as concluded from in vitro DNA-binding studies and response element-induced promoter activity in transfected cells. Numerous PXR/CAR/VDR target genes are also found to contain ER or IR motifs as response elements. Nevertheless, genome-wide chromatin immunoprecipitation (ChIP) and deep sequencing of immunoprecipitated DNAs (ChIP-Seq) identified DR4 as the most frequent PXR-associated recruitment sites in mouse liver [30]. DR4 in the human genome is a preferred DNA-binding site for the CAR/RXR heterodimer as well, as recently observed in a modified yeast one-hybrid assay [31]. DR3 is
the prevalent VDR-binding site at genomic regions that contain primary VDR target genes. These genomic regions are induced for chromatin opening in response to 1,25-D₃ signaling [32].

3.1 Regulation of PXR, CAR, VDR expression

PXR and CAR are the primary mediators of transcription regulation of ADME relevant genes. Pathological conditions negatively impact drug metabolism due to reduction of PXR and CAR activity. As an example, CYP3A4 expression is suppressed by inflammation in part due to interference of inflammation-activated NF-κB with PXR’s transactivation function, since the p65 subunit of NF-κB was found to disrupt DNA binding of the PXR/RXRα complex in the CYP3A4 gene [33]. Reduced PXR and CAR activity impairs drug metabolism under conditions of hepatic steatosis as well, since SREBP-1 (sterol regulatory element binding protein-1), activated in hepatocytes by lipogenesis- stimulated LXR-α, prevented p160 coactivator interaction with CAR or PXR, which curtailed phenobarbital-induced, PXR/CAR-mediated CYP3A4/CYP2B6 gene transactivation [34].

*PXR* and *CAR* gene expression is regulated by many transcription factors including various NRs [35, 25, 36]. Cholic acid-activated FXR robustly induced the mouse *Pxr* gene in the liver via four FXR-binding elements in the *Pxr* promoter [37]. HNF4α regulates xenobiotic response in mice during fetal liver development through *Pxr* gene activation [38]. GR regulated rat *Pxr* promoter in transfected primary hepatocytes and in hepatoma cells [39]. Human PXR expression in liver is transcriptionally regulated by PPARα [35] and HNF4-α [40]. Expression of CAR is induced by agonist ligands for GR, PPARα and functional binding sites for these NRs as well as a binding site for HNF4α were identified in the upstream sequence of the CAR promoter [25, 41]. Furthermore, in animal studies, CAR mRNA expression was induced by fasting and calorie restriction [41]. Additional mechanisms entailing genetic polymorphism, changes in the epigenetic landscape, post-transcriptional regulation by micro RNAs, and functional modulation through posttranslational modification (PTM) can have major impacts on the expression and activity of PXR and CAR. These examples are discussed under Sections 4.1 and 4.2.

VDR, upon activation by cognate ligands (i.e., 1,25-D₃ and lithocholic acid, LCA), can also induce ADME relevant genes, especially in the intestine. Examples for VDR-mediated induction of DMEs and drug transporters in 1,25-D₃ - or LCA-treated cells include *CYP3A4, CYP2B6, CYP2C9* [6, 42], *SULT2A1* [43], *OATP1A2* [44], *ABCA1* [45], and *MRP3, MRP2* [46]. Crystal structures of VDR bound to LCA- and 3-ketoLCA have been determined [47]. Seasonal differences in intestinal CYP3A4 levels are attributed to season-related fluctuations in sunlight exposure that lead to variations in serum levels of 25-hydroxy-D₃ and 1,25-D₃ [48].

Transcription of the *VDR* gene is under auto-regulation; 1,25-D₃ can increase *VDR* gene expression. Various endocrine factors including parathyroid hormone, retinoic acid, and glucocorticoids also regulate *VDR* expression [49]. Like PXR/CAR, VDR expression/activity is influenced by gene polymorphism, micro RNAs and by post-translational modification, as discussed in Section 4.
3.2 Xenobiotic response element (XRE)

At the chromatin level, XREs serve as sensors of xenobiotic (or endobiotic) signals by recruiting activated PXR/RXR and CAR/RXR to target genes. XRE activation is demonstrated by its activity in cis to induce promoter-directed reporter gene expression in transfected cells. Screening for XRE activation by synthetic or semi-synthetic chemicals is an integral part of the workflow for drug development. XREs also help identify other regulatory factors, which modulate PXR- and CAR-mediated expression of phase 0-III mediators. XREs in the phase II DME genes UGT1A1 (for the glucuronidating enzyme UDP glucuronosyltransferase, isoform A1, subfamily-1) and SULT2A1 (for the sulfotransferase enzyme, isoform A1, subfamily-2) are briefly described.

The phenobarbital responsive enhancer module (PBREM) in the human UGT1A1 includes three CAR-responsive XREs that are required for the optimal induction of UGT1A1 by phenobarbital (PB) [51, 50]. Protein-DNA interaction, analyzed by electrophoretic gel mobility shift assay (EMSA), revealed that CAR binds as a monomer to one of the functional XREs in the UGT1A1 PBREM, and similar to the CAR/RXR dimer, the DNA-bound CAR monomer can interact with coactivators and corepressors. Furthermore, binding of the monomeric CAR or CAR/RXR dimer to XRE is most favored when the hexamer repeat of the response element is preceded at the 5’ end by the dinucleotide AG. Arginine residues at positions 90 and 91, located within the carboxy-terminal extension of CAR’s DBD, mediate the dinucleotide-dependent binding preference [50].

XRE-dependent and PXR- and CAR-mediated induction of human SULT2A1 was investigated in our laboratory [9]. Preferred substrates for SULT2A1 are bile acids and dehydroepiandrosterone (DHEA) – the latter is the steroid precursor for testosterone and dihydrotestosterone. A major role for SULT2A1 in the enterohepatic tissue is to promote bile acid clearance as the sulfate conjugate. Notably, the prostate cancer drug Zytiga (abiraterone acetate) is hydrolyzed in vivo to the therapeutic metabolite abiraterone, which is cleared from the body after conversion by SULT2A1 to the inactive abiraterone sulfate, and by CYP3A4 to the inactive N-oxide abiraterone, which is then converted to a sulfated derivative (PubChem database, CID 132971). SULT2A1 expression is induced by VDR and the bile acid receptor FXR as well, which is in keeping with its role in bile acid homeostasis [52, 43]. A PXR/CAR- responsive composite XRE in the human SULT2A1 promoter and a synergizing role of HNF4-α in XRE- induced SULT2A1 expression is described below and is summarized schematically in Figure 3.2.

3.2.1 A composite XRE and HNF4-α-responsive DR1 element in the human SULT2A1 promoter—Induction of the SULT2A1 promoter by ligand-activated PXR and CAR in transfected liver and intestinal cells was shown to be mediated by an upstream xenobiotic-responsive composite element (XRE). Specific interaction of XRE with PXR/RXRα and CAR/RXRα was demonstrated by DNase1 footprinting and EMSA. The XRE from −190 to −131 positions, was defined by an inverted repeat and a direct repeat of the AG(G/T)TCA element, which are configured as IR2 (−190AACGCAAGCTCA-GATGACCCTAA−167) and DR4 (−55GATAAGTTCATGATTGCTCAACATC−131) [9]. XRE-mediated stimulation required both IR2 and DR4 elements; neither by itself was...
sufficient to cause robust SULT2A1 promoter induction. Thus XRE is a composite element. The composite XRE spanning −190 to −131 positions stimulated a heterologous promoter. Point mutations in the XRE prevented its interaction with PXR and CAR and abrogated induction of the SULT2A1 and the heterologous thymidine kinase promoter.

HNF4-\(\alpha\) plays a modifying role in the PXR- and CAR-mediated target gene transcription, since HNF4-\(\alpha\) potentiated PXR- and CAR-mediated transactivation of the SULT2A1 promoter. A DR1 element (\(\sim 63\) GTGACATGCTGGGACAGGTTAAAGATCG\(\sim 35\)) in the SULT2A1 gene promoter, located upstream of −30 nucleotide position, serves as an HNF4-\(\alpha\)-binding element. A schema on the regulation of SULT2A1 by PXR and CAR via the composite XRE, and the synergizing influence of DR1-bound HNF4-\(\alpha\) on xenobiotic-induced SULT2A1 expression is presented as schema in Figure 3.2.

**3.2.2 Sult2A1 induction by FXR via an IR0 element**—Apart from xenobiotic chemicals, bile acid overload induces SULT2A1 expression. For example, Sult2A1 mRNAs were induced in the mouse liver when animals were fed a cholic acid containing diet (Figure 4.3.2), and bile acid activated FXR robustly induced the Sult2A1 promoter through an FXR-bound IR0 element [52]. However, IR1 is the most abundantly encountered FXR-responsive element. An IR1 element drives FXR-mediated transactivation of ABCB11, the gene for the human bile salt export pump [53]. A number of other repeat motifs of the half site RG(G/T)TCA including DR1, ER6, ER8 are known to be FXR-responsive functional elements in FXR target genes. Assessment of genome-wide FXR binding in the mouse hepatic chromatin showed an IR1-type sequence as the preferred chromatin occupancy site for FXR in vivo [54]. FXR-occupied IR1 sites are frequently juxtaposed to a hexameric half-site consensus sequence, which binds a monomeric NR such as LRH-1 (liver receptor homolog-1). Positive interplay between FXR and LRH-1 for the gene encoding the small heterodimer partner (SHP), which is an atypical NR devoid of a DBD, as well as several other FXR target genes has been demonstrated [55]. The FXR/LRH-1/SHP axis plays a key role in bile acid homeostasis, as discussed in the next section.

In summary, above examples of XREs demonstrate that PXR, CAR, FXR bind a variety of repeat motifs of the consensus half site RG(G/T)TCA to induce genes involved in drug metabolism and disposition.

**3.3 NR, a drug target for diseases from disrupted bile acid/cholesterol homeostasis**

Bile acid synthesis is the primary pathway for cholesterol catabolism in liver, accounting for ~50% of daily cholesterol turnover. Cholesterol overload, the underlying cause for cholesterol stone, results from insufficient bile acid synthesis when bile acid saturation with cholesterol leads to the formation of cholesterol stone. On the other hand, bile acid accumulation leads to cholestasis due to reduction or stoppage of bile flow. Oral bile acid therapy is given to patients with cholesterol stones, and ursodeoxycholic acid is used to treat cholestasis of pregnancy and primary biliary cirrhosis (PBC), the autoimmune disease causing bile duct destruction. FXR and other NRs, such as LRH-1, HNF4-\(\alpha\), LXR-\(\alpha\), SHP, PXR, and VDR maintain bile acid/cholesterol homeostasis [56, 55].
CYP7A1 (cholesterol 7 α hydroxylase) is the rate-limiting enzyme for bile acid production from the catabolic breakdown of cholesterol. HNF4-α and LRH-1 are positive regulators of CYP7A1 expression. Some aspects of CYP7A1 regulation are, however, species-specific—a prominent example being the positive regulation of the basal expression of CYP7A1 by oxysterol-activated LXR-α in the rodent liver but not in human liver, since the LXR-binding site in the human promoter is mutated [57]. Toxic accumulation of bile acids, on the other hand, is prevented by FXR-imposed negative feedback regulation of CYP7A1. In this case, bile acid activated FXR induces SHP [58], and interference from SHP due to protein-protein interaction inhibits positive regulation of the CYP7A1 promoter by LRH-1 (an NR activated by phospholipids) [56]. SHP also interferes with the stimulatory interaction between HNF4-α and the coactivator PGC1-α (peroxisome proliferator activated receptor γ coactivator 1-α) on the CYP7A1 promoter [55]. In the ileum part of intestine, SHP plays a role in the CYP7A1 repression by the fibroblast growth factor-19 which, like SHP, is induced by FXR [56]. PXR blocks CYP7A1 expression by disrupting the PGC1α → HNF4 stimulatory axis [59]. Thus, like FXR, PXR also regulates bile acid homeostasis upon activation by drugs and certain bile acids. Drugs targeting FXR, SHP, LRH-1, PXR and HNF4-α have therapeutic potential against liver and biliary disorders. Small molecules that augment SHP activity may robustly reduce CYP7A1 expression to prevent bile acid overload. Small molecules, which elevate LRH-1 activity or increase PGC1-α ↔ HNF4-α interaction, would be useful in enhancing CYP7A1 expression, which then would promote cholesterol breakdown and reduce cholesterol build up.

Apart from FXR, TGR5, a transmembrane G protein coupled receptor, mediates bile acid signaling. TGR5 is located in intestinal epithelium, Kupffer cells, sinusoidal endothelium and bile duct cells. Both TGR5 and FXR are hotly pursued drug targets for diseases of errant bile acid and cholesterol metabolism [55]. The athero-protective effect of LXR-α arises in part from the LXR-α-mediated induction of efflux transporters in resident macrophages of the arterial wall, and this in turn promotes cholesterol efflux and reverse cholesterol transport to the liver and intestinal tissue and subsequent removal of cholesterol as part of excreta. Therefore, small molecule activators of LXR-α may normalize cholesterol homeostasis. Finally, VDR can regulate bile acid and cholesterol homeostasis, since agonist-activated VDR promotes cholesterol catabolism by repressing SHP and increasing CYP7A1 expression [60].

4 Genetics, Epigenetics and Interindividual Differences in Drug Response

4.1 Gene polymorphism and NR-regulated variable DME/drug transporter activity

Single nucleotide polymorphism (SNP) at regulatory loci of ADME related genes, or non synonymous SNPs in the coding region of NR itself, alter NR-mediated DME/transporter expression. A non-coding SNP at an HNF4-α binding site in the CYP2B6 promoter contributes to the interindividual variations in CYP2B6 expression [61], and a common African haplotype for an SNP at a PXR-binding enhancer in GSTA (encoding glutathione S-transferase A) causes hypersensitivity for GSTA induction by the human PXR ligand rifampin [62]. CYP2D6, which metabolizes a large number of drugs including antidepressants and β blockers, shows wide interindividual differences in expression and
activity. An HNF4-α variant having reduced binding to the CYP2D6 promoter and causing decreased CYP2D6 expression has been identified. The variant HNF4-α arises from a non synonymous SNP, which yields glycine → aspartic acid substitution at the position 60 (G60D). Compared to this variant, the wild-type HNF4α genotype is associated with higher CYP2D6 activity in the human liver [63, 10]. The G60D HNF4-α appears at low frequency in Asian populations; it has not been detected in Africans or Caucasians [63]. Variable CYP2D6 expression also results from gene amplification that ranges from 3 to 13 gene copies. CYP2D6 deficiency is an autosomal recessive trait in ~7% Caucasians and ~1% Orientals, making these individuals poor metabolizers of CYP2D6 drug substrates [64]. Pharmacogenomic tests for CYP2D6 variants are common practice for assessing the appropriateness and efficacy of a CYP2D6 drug substrate. Interindividual differences in drug response are managed by dosage adjustment based on the patient’s pharmacogenetic profile.

The basal level of CYP3A4 in the liver varies up to 60-fold between individuals, although SNPs in coding sequences and regulatory loci of CYP3A4 do not explain this variability [65]. Association analysis suggests that nonsynonymous SNPs of PXR and FOXA2 (aka HNF3-β, a liver-enriched transcription factor) contribute to CYP3A4 variation in the human liver, since the mRNA expression level for CYP3A4 in the human liver significantly relates to SNPs of PXR and FOXA2, and PXR expression itself is regulated by FOXA2. Binding sites for FOXA2 and PXR in the human CYP3A4 distal promoter were identified [66]. VDR polymorphism accounts for disparate intestinal CYP3A4 levels and variable first pass intestinal absorption and metabolism of CYP3A4-targeted drugs [48].

FXR, which regulates the expression of many uptake and efflux transporters, shows a common non-coding -1G>T polymorphism, where T replaces G at the -1 position of the translation start site causing reduced FXR expression. The FXR-1G>T SNP is associated with increased efficacy of the statin drug rosuvastatin in lowering hepatic cholesterol biosynthesis, thus affording greater LDL-cholesterol response [67]. Rosuvastain remains unmetabolized in hepatocytes and ABCG2 (BCRP), an apical ABC cassette efflux transporter, plays a major role in the biliary clearance of rosuvastatin. ABCC2 (MRP2) and possibly ABCC11 (BSEP) also contribute to rosuvastatin disposition from human liver. Mechanistically, low expression of the variant FXR accounts for reduced expression of the transporters ABCG2, ABCC2, ABCC11, which leads to a blockade in the biliary clearance of rosuvastatin and longer residency of the drug in hepatocytes – hence a more potent effect of this statin on hypercholesterolemic patients who carry the FXR-1G>T SNP [67].

A large number of SNPs for PXR (NR1I2)- and CAR (NR1I3)- encoding genes are known, several of which are associated with altered expression and/or function of these receptors [69, 68]. For the NR1I2 SNP 63396C>T, located in a putative transcription factor binding site, the 63396T variant associates with elevated PXR expression, increased CYP3A4 expression and decreased plasma levels of the CYP3A4 substrate atazanavir (an anti-retroviral drug). Natural PXR variants, which harbor single amino acid changes, confer altered transactivation response of the CYP3A4 promoter [65]. Among the 22 naturally occurring splice variants of CAR, some are nonfunctional due to nonsense mutations. For the CAR (NR1I3) SNP rs2307424C>T, the T allele is associated with a low plasma level of the anti-retroviral drug efavirenz, which is a CYP2B6 and CYP3A4 substrate [70]. Extensive
VDR gene polymorphism has been reported [71], and it has been reported that intestinal CYP3A4 expression levels are functions of VDR polymorphisms [48].

4.2 Epigenetic machinery and drug response

Roles for DNA methylation, histone modification and microRNAs in the regulation of a large number of mediators of phase 0-III processes and their NR regulators (PXR, VDR, HNF4-α) have been reported [73, 72]. Epigenetic factors confer heritable changes in chromatin structure and function, caused by mechanisms other than DNA sequence alteration at the coding or non-coding region of a gene. An integral role of epigenetics in health and disease is revealed by the tragic history of the synthetic estrogen diethyl stilbesterol (DES) as a birth control pill. In utero DES exposure caused vaginal tumors and breast cancer in adult females. In mice, DES altered gene-specific DNA methylation, expression of epigenetic enzymes (DNMT3A, MBD2, HDAC2, EZH2), and the abundance of HOTAIR, a lncRNA [74]. Epigenetic systems are briefly discussed and current information on their roles in drug metabolism and drug response is presented.

4.2.1 DNA methylation, ADME gene activity, interindividual differences—DNA methylation at the 5’ cytosine of the CpG sequence (5mC) is an epigenetic mark for gene activity [75]. Gene repression is linked to hypermethylated promoters when 5mC methylation occurs within long stretches of CpG repeats (CpG island) at proximal promoters, although 5C-methylation at low CpG density (CpG shores) or even at single CpG sites can mark reduced gene expression. Of 3 major DNA methyltransferases (DNMTs) in mammals, DNMT1 is the maintenance methyltransferase; DNMT-3a and -3b are de novo enzymes, essential for the genome-wide methylation of DNA following embryo implantation. Gene repression by DNA hypermethylation is aided by the interaction of DNMTs with the polycomb repressor complex (PRC2), especially with EZH2 (Enhancer of Zeste homolog 2), the histone methyltransferase component of PRC2 [75]. Cancer development is associated with genome-wide DNA hypomethylation, which activates proto-oncogenes. For many tumor suppressors, site-specific hypermethylation contributes to gene silencing [75]. 5-hydroxymethylcytosine (5hmc) modification of DNA, on the other hand, is an activation mark, linked to active gene transcription [76]. Notably, the paternal sperm DNA methylation pattern has been linked to autism risks in an autism-dense cohort [77]. Extensive interindividual differences in the genome-wide DNA methylation pattern have been reported [78].

Acquired drug resistance has been linked to altered DNA methylation of NRs and NR-regulated ADME genes, as observed in i) drug-induced demethylation of MDR1 and BCRP, which leads to their overexpression causing multidrug resistance (MDR) of cancer cells [79, 80]; ii) drug-induced methylation of the estrogen receptor (ER-α) encoding ESR1 gene promoter, causing reduced ER-α expression and tamoxifen resistance in breast cancer [81]; and iii) resistance to progesterone therapy in endometrial cancer due to reduced expression caused by enhanced methylation of the gene encoding progesterone receptor isoform A (PR-A) [82]. Methylation of the PXR gene promoter attenuated PXR expression and reduced CYP3A4 expression in colon cancer cells [83]. In colon and endometrial cancers, the VDR gene is aberrantly methylated [83]; differential methylation of PXR and FXR at CpG
promoter sites has been reported in cholestatic pregnancy versus normal healthy pregnancy [84].

A role for DNA methylation in the expression of a number of DMEs and drug transporters has been reviewed [73, 85]. A few representative examples are discussed here. 1) CYP3A4/5/7 expression is dependent upon the methylation status of these genes, since their expression was altered when human hepatoma cells were treated with 5-aza-2′-deoxycytidine (a DNA demethylating agent). CYP3A4 induction is associated with reduced 5mC at CpG-rich regions located at or near the binding sites for PXR, CAR, and VDR, which are well-known regulators of CYP3A4 [73]. 2) Altered CYP1A1 expression in response to cigarette smoking is associated with changes in the methylation status of CYP1A1. 3) Development stage-dependent CYP2E1 expression is influenced by the methylation status of this gene. 4) Phase II genes including UGT1A1, GSTP1, SULT1A1 and genes for efflux transporters like MDR1, BCRP and members of the OATP family of uptake transporters are epigenetically regulated due to DNA methylation [86, 87, 89, 88].

4.2.2 Histone marks; impact on NR-regulated ADME genes—Post-translational modification (PTM) of histones (methylation, acetylation, phosphorylation, ubiquitinylation, sumoylation, ADP-ribosylation and several other modifications), especially acetylation and methylation at the amino-terminal histone tails for histone H3 and H4, are well-characterized epigenetic signatures that influence gene activity. PTMs are also known for histone H2A and H2B and the linker histone H1. More than 10 different PTMs at ~80 sites on histone tails, histone core domains and on the H1 linker histone have been identified [90]. Gene-activating histone marks include H4 lysine-16 acetylation (H4K16ac); H3 trimethylation at lysine-4 (H3K4me3) and lysine 36 (H3K36me3), and H3 phosphorylation at serine-10. Among repression marks, trimethylated histone H3 at lysine-9, lysine-27 and lysine-20 are most well characterized [91]. Histone deacetylases (HDACs, subgrouped as class I to IV), histone acetyltransferases (HATs), histone methyltransferases (HMTs) and histone demethylases (HDMs) are important drug targets. Numerous lysine-specific HMTs (SET7/9, MLL, EZ2H2) and lysine-specific HDMs (LSD1/KDM-1, JMJD2A/KDM4A, JARID1A/KDM5A) have been characterized. Arginine methylation of histones mediated by protein arginine methyltransferases (PRMTs) also regulates gene activity, as seen for histone H3 Arg-17, H4 Arg-3. Histone marks are the “codes” that are “read” by chromatin remodelers (such as SWI/SNF containing complexes) and histone-modifying enzyme complexes (such as PRC2) in order to prepare chromatin for positive or negative transcriptional response. Enzymes that mark histones through PTM are “writers”; those involved in removing histone marks are “erasers”; and protein/ enzyme complexes, which recognize histone codes, are “readers”. Crosstalk of HMTs with DNMT1 influences epigenetic regulation [75].

Among the ADMEs whose genes are known to be regulated by histone modification include the phase I DMEs CYP3A4, CYP2E1, phase II DMEs SULT2B1, UGT1A1, the efflux transporters MDR1, BCRP, the OATP family of uptake transporters and the SLC5A5 encoded iodine uptake transporter sodium/iodide symporter [86, 73]. Histone modification can have long-lasting effects on ADME genes. For example, the CAR target genes Cyp2b10 and Cyp2c37, when neonatally exposed to the CAR ligand TCPOBOP, remained induced in
adult mouse livers; as a result, adult mice were much less sensitive to the Cyp2b10 substrate zoxazolamine [92]. H3K4 methylation and H3K9 demethylation at CAR-responsive elements in the Cyp genes, detected in the neonatal liver, persisted in the adult mouse liver. It was concluded that the early-life methylation status of histone H3 played a role in the Cyp gene induction in the adult liver, since hepatocytes isolated from the livers of mice receiving neonatal CAR activation were significantly more sensitive to low TCPOBOP concentrations for Cyp gene induction than hepatocytes from control mice lacking neonatal exposure to this inducer [92]. Also, the possibility remains that the long-lasting stability and biological potency of TCPOBOP in vivo, with activity persisting in mouse liver for 6 months or more [93], contributed to the hepatic Cyp induction in adult mice when receiving a single dose of TCPOBOP as neonates. PXR-mediated CYP3A4 induction was regulated by PRMT1, which methylated histone H4 at arginine-3 that is located within a PXR-responsive chromatin region in the CYP3A4 gene [94]. In a rat model of chronic kidney disease, reduced Cyp2C and Cyp3A expression, and associated reduction in PXR and HNF4-α binding to cognate sites in the Cyp2C11 and Cyp3A2 promoters, was accompanied by reduced histone H4 acetylation at the Cyp3A2 promoter regulatory region and reduced histone H3 acetylation at the PXR- and HNF4-α-bound regulatory loci of Cyp2C11 and Cyp3A2 promoters [95].

Given the reversible nature of chromatin modifications by DNA methylation and histone PTM, drugs targeting epigenetic enzymes (“epi drugs”), especially DNMT, HAT, HDAC, HMT and HDM, are being developed. 5-azacytidine (Azacitidine) and 5-aza-2′-deoxycytidine (Decitabine) are nucleoside analogs and DNA demethylating agents that are clinically used against myelodysplastic syndromes, chronic myelomonocytic leukaemia and acute myeloid leukaemia. Second-generation DNA de-methylating agents (SGI-110, CP-4200) are under development [96].

Valproic acid, a class I HDAC inhibitor and an anticonvulsant, activates CAR and PXR to induce CYP3A4, CYP2B6 and MDR1 expression [97, 99, 98]. Valproic acid also enhances tissue sensitivity to estrogen and progesterone by potentiating estrogen receptor (ER) and progesterone receptor (PR) activity due to HDAC1 inhibition [100]. Vorinostat (a hydroxamate) and romidepsine (a depsipeptide) are orally administered pan-HDAC inhibitors, which are used in combination therapy with chemotherapeutics like paclitaxel, doxorubicin. Vorinostat resistance is thought to develop from increased expression of efflux transporters, since MDR1, BCRP, and MRPs were detected at elevated levels in vorinostat-treated cells [73]. Inhibition of ABC transporters in this case may improve vorinostat’s therapeutic efficacy. Epi drugs, which target histone methylation, are at various stages of development [101].

4.2.3 Non-coding RNA-mediated regulation of PXR, CAR, VDR and ADME gene expression—Non-coding RNAs (ncRNAs), best characterized for micro RNAs, are integrally linked to epigenetic machinery. Transcripts of more than 90% of the human genome represent ncRNAs, many of which regulate gene expression at transcriptional and posttranscriptional levels. Short (<30 nucleotides) ncRNAs, best characterized for micro RNAs (miRNAs), and long ncRNAs (lnc RNAs) with >200-nucleotide lengths are two major categories of ncRNAs. The list for micro RNAs, which influence ADME gene expression, is steadily growing [83, 103, 102].
The miRbase lists as many as 2555 unique mature human miRNAs (database version 20; June 2013 release). Base pairing of the miRNA nucleotide sequence with a cognate sequence in the 3' untranslated region (3'-UTR) of a target messenger RNA (mRNA) within the RNA-induced silencing complex leads to either mRNA degradation (in the case of a perfectly complemented base pairing), or translational suppression of the target mRNA when base pairing is not 100% complementary. A single miRNA can target 3'UTRs of multiple messenger RNAs.

Studies in cell culture show that the messenger RNAs for ADME related genes are targeted directly or, via upstream regulatory NR and other transcription factors, by one or more miRNAs. Regulation of CYP1B1 and CYP3A4 mRNAs by miR-27b; CYP2E1 mRNA by miR-378; the MDR1 transporter by miR-451; and the BCRP transporter by miR-328, miR-519C, miR-520h underscores the impact that miRNAs may have on drug metabolism and disposition, provided these miRNA-dependent regulations are upheld in vivo. While miR-27b directly regulates CYP3A4 expression, the VDR level is regulated by this micro RNA as well, so that miR-27b can both directly and indirectly influence CYP3A4 expression. PXR expression is regulated also by miR-148a. The miRNA-dependent regulation of several epigenetic enzymes including DNMTs, HDACs, EZH2, and epigenetic enzymes that regulate miRNA-specifying genes (which produce miRNA precursor transcripts) have been reported [104, 105]. Such cross talks provide a miRNA-dependent additional regulatory cascades that may alter DME/transporter expression. The abundance of specific miRNAs may predict drug response, since miR-21 levels in pancreatic cancer biopsies correlated with gemcitabine responsiveness, and ectopic miR-21 expression caused gemcitabine resistance in pancreatic cancer cells [106].

A lncRNA known as AIR is indirectly involved in the inactivation of the mouse organic cation transporter (OCT) genes Slc22a2 and Slc22a3, since AIR plays a role in silencing the Igf2R gene cluster and Slc22a2 and Slc22a3 are located within this cluster [107]. LncLSTR, a recently reported liver-enriched lncRNA, is a regulator of Cyp8b1, which is involved in bile acid biosynthesis [108]. The lncRNAs PCA3 and PCGEM1 are elevated in human prostate cancer. PCGEM1 coactivates activities of the androgen receptor and cMYC oncoprotein. [109]. Whether lncRNAs directly regulate ADME-relevant genes remains to be determined.

5 Drug Interactions: A Role for Xenosensing NRs

5.1 Drug-drug, drug-food, drug-herb interaction

Drug-drug interaction (DDI) reflects changes in target drug pharmacokinetics or bioavailability in the presence of a co-administered drug. By activating PXR and CAR, the interfering drug renders changes in one or more components of the drug metabolizing and disposition machinery. DDI is assessed quantitatively by the pharmacokinetic parameters C_{max}, which refers to the peak plasma drug concentration at post-dosing; and AUC (area under the time-plasma drug concentration curve), which defines total serum drug levels over time. DDI has three possible outcomes: i) overdosing and potential toxicity due to increased half-life of a target drug caused by one or more of the following – excessive pro-drug bioactivation; attenuated DME activity; increased uptake activity and reduced efflux activity.
of transporters; ii) underdosing resulting in low drug efficacy, which is due to reduced drug uptake and/or reduced bioactivation; enhanced metabolism and/or accelerated drug efflux; iii) a boost in medicinal potency. CYP-mediated DDI led to the withdrawal of numerous drugs from clinical use, such as terfenadine (the antihistamine Seldane) and cerivastatin (a cholesterol-lowering statin). Dietary ingredients (e.g., furanocoumarins in grapefruit juice) or phytochemicals in medicinal herbs (e.g., hyperforin in St John’s Wort) can modulate a drug’s efficacy and engender potentially fatal drug-food and drug-herb interactions. CYP3A4/3A5 and CYP2D6 are most frequent participants in DDI [18, 110].

Desirable outcomes may also result from drug interactions, as seen in the hepatoprotective effect of ginger extracts against diverse drugs including high-dose acetaminophen [111]. DDI is not a concern for peptide or antibody based therapeutics, since they do not activate PXR and CAR. Recently approved PCSK9 inhibitors are antibody-based drugs, which aid in LDL-cholesterol clearance from circulation by preventing PCSK9-mediated degradation of the LDL receptor [112].

5.2 Linking xenobiotic NRs to drug interactions

Association of the xenobiotic NR activity with clinical DDI has been reported [1, 113, 114]. Orally delivered drugs, which are CYP3A4 and/or MDR1 transporter substrates, can exhibit markedly altered pharmacokinetics in response to rifampicin co-administration. For example, increased enterohepatic expression of CYP3A4, triggered by the long-term treatment with the human PXR agonist rifampicin caused a 96% decrease in the oral bioavailability of the CYP3A4 substrate (S)-verapamil and loss of the anti-hypertensive effect of this drug in patients [115]. Cyp2C9 induction by rifampicin-activated PXR reduced plasma concentrations of CYP2C9 substrates such as warfarin (anticoagulant) and sulfonylurea (antidiabetic) [116]. Binding and activation of PXR by hyperforin, a bioactive component of St. John’s Wort, leads to the transcriptional induction of CYP3A4 and widely prevalent clinical DDI due to increased metabolism and hence decreased efficacy of numerous drugs including oral contraceptives, the immune suppressant drug cyclosporine and the anti-HIV protease inhibitor indinavir [117].

Apart from acting as direct ligands, certain drugs induce phosphorylation of PXR and CAR by activating signal pathways that lead to activation of kinases such as PKA, PKC, CDK2, CDK5, and p70S6K [5]. One such PXR activator is forskolin, a diterpene constituent of the Indian plant C. forskohlii, which is used for the treatment of glaucoma, asthma and various other diseases. Forskolin induces PXR phosphorylation through PKA activation, and enhances PXR-coactivator interaction upon its direct binding to the PXR LBD [118]. Additionally, forskolin is a constituent of an herbal mixture marketed over-the-counter for weight loss. DDI/drug-herb interaction may interfere with forskolin’s therapeutic value.

In the case of CAR, metformin induces phosphorylation of this xenosensing NR at threonine-38, mediated by activated AMPK and the MAP kinase ERK1/2. Thr-38 phosphorylation restricts nuclear translocation of CAR and disruption of coactivator-CAR interaction, thereby preventing CAR-mediated induction of target genes such as CYP2B6 [25]. As a result, co-administration of metformin is known to cause altered pharmacokinetics for CYP2B6 drug substrates [119]. Reciprocally, reduced CYP2B6 and CAR activity may...
render a negative impact on the renal clearance of metformin as a result of reduced expression of the renal OCT2/MATE transporter system. Pronounced DDI may be expected as a result of such negative interplays.

Additional examples below underscore how PXR and CAR may play roles in clinical DDI events. Several reviews on NR-regulated drug interactions provide further elaborations on this subject [120, 121, 15, 114].

5.3. Drug-drug interaction

5.3.1 Statin-induced myopathy, DDI: a likely role for NRs—Uptake transporters of the OATP family are predominantly involved in the hepatic import of statins [15], and common variants in SLCO1B1, which encodes OATP1B1, are strongly linked to an increased risk for statin-induced myopathy [122]. As an example of adverse DDI, cyclosporine A, which competitively inhibits OATP-mediated hepatic statin uptake, caused skeletal muscle statin overload and muscle damage upon co-administration with pitavastatin or rosuvastatin. Extreme statin overload is linked to the fatal condition of rhabdomyolysis [123].

Various statins, however, differ significantly in pharmacokinetic characteristics due to differences in ADME. It is, therefore, conceivable that additional to altered uptake activity, the PXR-/CAR-regulated expression of uptake transporters may influence the hepatic uptake of some form of statins. Indeed, long-term treatment of rifampin reduced atorvastatin bioavailability due to induced expression of CYP3A4 and efflux transporters by rifampin-activated PXR [124], whereas short-term rifampicin administration caused inhibition of OATP-mediated hepatic uptake of atorvastatin and caused elevated AUC for this statin [125]. PXR- and CAR-responsive functional XREs are present in genes for many drug transporters including OATPs and various efflux transporters [126].

5.3.2 Prostate cancer, ZYTIGA®, DDI with rifampicin—Zytiga (abiraterone acetate), the anti-androgen drug against recurrent metastatic prostate cancer, is a CYP3A4 and SULT2A1 substrate. In a DDI trial, serum Zytiga exposure decreased by 55% in the presence of rifampin, indicating a need for higher dosage of this drug when a PXR activator is co-administered [127]. DDI is likely caused by increased CYP3A4 and SULT2A1 expression by rifampicin-activated PXR, since inhibition of CYP3A4 activity by co-administered ketoconazole (a strong CYP3A4 inhibitor) did not significantly alter Zytiga pharmacokinetics (Clinical Pharmacol 12.3; FDA Drug Safety Reporting, 2015).

5.4. Drug-food, drug-herb interactions

5.4.1 Grapefruit juice, CYP3A4, drug transporters, PXR/CAR—Grapefruit and several other citrus fruits contain furanocoumarins in addition to other phytochemicals. Furanocoumarins, which inhibit OATPBs and CYP3A4, elevate the bioavailability of CYP3A4/OATPB substrates including cyclosporine, midazolam, calcium channel blockers and certain statins [128]. Although in humans furanocoumarins predominantly inhibit CYP3A4 activity, Cyp 1, 2, 3 expression and activity in mice was induced by isopimpinellin (a furanocoumarin) in a Pxr- and Car-dependent manner [129]. To settle whether species
specificity explains such differences, effects of furanocoumarins on PXR and CAR activity should be re-assessed in humanized PXR-CAR-CYP3A4/5 mice where human counterparts replace rodent Pxr, Car and Cyp genes [131, 130].

5.4.2 Pomegranate juice, SULT2A1, Zytiga® activity—Punicalagin, a polyphenol constituent of pomegranate, impairs sulfoconjugation of drugs in the intestine [132], which leads to reduced clearance and thus overdosing of orally delivered Zytiga (abiraterone acetate) which, as a CYP3A4 and SULT2A1 substrate, is normally metabolized to abiraterone sulfate and N-oxide abiraterone sulfate. Inhibition of CYP2C9 by punicalagin has also been reported. It is not known whether this polyphenol influences PXR or CAR activity.

5.4.3 St. John’s Wort, PXR, CAR, CYP3A4—Hyperforin, which confers the antidepressant activity of St. John’s wort, is a ligand for human PXR and CAR [117, 133]. Hyperforin-activated PXR/CAR induces CYP3A4, other CYP genes (CYP2B6, CYP2C9, CYP2C19), as well as MDR1. Acute rejection of transplanted hearts in patients due to self-medication with St. John’s Wort is an example of serious drug-herb interactions. Rejection was caused by a drop in plasma levels of cyclosporine, which is a CYP3A4 and MDR1 substrate [134].

5.4.4 Garlic, CYP2C9, Warfarin—Garlic extracts suppressed CYP2C9 mRNA expression and activity in the human hepatocyte-derived Fa2N-4 cell line; furthermore, garlic extract can competitively inhibit CYP2C9 activity [135]. Increased systemic exposure of CYP2C9 drug substrates such as warfarin in the presence of garlic extract has been reported. Reduced warfarin metabolism may enhance the possibility for uncontrolled bleeding. Since the diallyl sulfide in garlic extracts can activate CAR [136], CYP2C9 gene suppression may be driven by a CAR-dependent mechanism.

5.4.5 Protection from acetaminophen toxicity by garlic extracts: a role for CAR-induced SULT—The hepato-protective effect of organo-sulfurs in garlic extracts against acetaminophen-induced liver injury is due to two mechanisms: 1) reduction of hepatic CYP2E1 expression and inhibition of CYP2E1-mediated acetaminophen biotransformation to a toxic metabolite [137]; 2) increased acetaminophen clearance as a sulfate metabolite by SULT activity. It has been reported that CAR, activated by diallyl sulfide (a garlic constituent), promotes acetaminophen conversion to a sulfated metabolite by inducing SULT2A1 and other SULTs (SULT1A1, SULT1A3/4, SULT1E1) [139, 138]. Reduced build up of acetaminophen prevents GSTpi induction by acetaminophen-activated CAR. The net result is diminished oxidative stress from glutathione depletion and consequent reduction of oxidant-induced liver injury [140].

Additional NRs can potentially generate drug interactions. VDR-mediated regulation of DMEs and transporters and a modifier role of HNF4 in the expression of ADME-relevant genes have been reported [43, 9, 126, 6, 141]. Whether long-term use of vitamin D supplements would cause adverse drug interactions should be evaluated. Drug interaction from activated glucocorticoid receptor (GR) is a distinct possibility, since ligand-activated GR induces CAR and PXR expression; a GR-responsive element has been identified in the
CAR gene promoter [142]. Dexamethasone, a synthetic glucocorticoid, promotes nuclear translocation of CAR and PXR and induces PXR/CAR target genes [142, 143]. Ketoconazole, an anti-fungal agent and GR antagonist, prevented rifampin- and phenobarbital-mediated PXR/CAR activation and induction of their target genes [144]. Thus, under ketoconazole co-medication, a primary drug may respond with altered pharmacokinetics.

5.5 Platforms for screening drug candidates

Early assessment of drug candidates can avoid late-stage failure of clinical trials due to DDI and help minimize costs for developing and marketing a new drug. Candidate drugs are routinely screened in a cell based workflow for their impact on DME activity and PXR/CAR-mediated transactivation of XREs. Humanized mouse models, where Pxr, Car and Cyp rodent genes are replaced by corresponding human genes, are better suited for drug testing since these models provide in vivo relevance and they approximate as human surrogates. The humanized PXR-CAR-CYP3A4/3A7 mouse strain is commercially available. A new hPXR-hCAR-hCYP3A4/3A7-hCYP2C9-hCYP2D6 mouse strain, with human PXR and CAR genes substituted for the rodent Pxr and Car genes and the gene clusters Cyp3a, Cyp2c and Cyp2d replaced by counterpart human genes, has been reported [145].

In the not-to-distant future, microfluidic organs-on-chips may be adopted as a preferred platform for drug testing, replacing animal models. In a microfluidic device, live cells on chips, organized in continuously perfused chambers, mimic the complex multicellular environment so that bioavailability, efficacy and toxicity of test molecules could be assessed in a context which, in part, recapitulates human tissue and organ physiology [146, 147]. The future drug discovery pipeline may also include a workflow that assesses drug-induced PTM profiles of PXR and CAR determined through liquid chromatography-coupled-tandem mass spectrometry, and examines how PTM alters PXR/CAR activity using an approach similar to that reported recently for PXR [148].

6 Summary and Perspectives

PXR and CAR, the two nuclear receptors that are activated by drugs and other xenobiotics, coordinate both metabolism of orally administered drugs in the liver and intestine and excretion of drug metabolites by mediating transcriptional induction of genes encoding phase I/phase II drug-metabolizing enzymes (DMEs) and transporters which regulate drug influx (phase 0) and efflux (phase III) of drug metabolites. Phase 0-III mediators are also induced by ligand-activated VDR, especially in the enterocytes of intestine. Additional nuclear receptors, especially FXR, HNF4-α, LRH-1 and SHP regulate expression of the enzymes and transporters involved in cholesterol and bile acid homeostasis. More than 90% of all known drugs are metabolized by a subset of cytochrome P450s (CYPs) – CYP3A4/3A5, CYP2D6, CYP2B6, CYP2C9, CYP2C19, CYP1A2, CYP2C8, CYP2A6, CYP2J2, and CYP2E1. In the human liver and intestinal epithelium, CYP3A4 and its functionally indistinguishable isoform CYP3A5 are the most abundant CYP enzymes and together, they metabolize more than half of all prescription medicines. Overdosing or underdosing leading to drug toxicity or reduced drug efficacy, respectively, is the
consequence of interference from a co-administered second drug (DDI, i.e. drug-drug interaction) or from a dietary or herbal agent (drug-food/drug-herb interaction). Adverse (or beneficial) drug interaction results from i) enhanced gene transactivation for DMEs or transporters due to PXR/CAR activation by the interfering drug or other agent; and/or ii) altered DME or transporter activity. In order to minimize late-stage failure of clinical trials, an essential routine at early stages of drug development is to evaluate candidate molecules for effects on the activities and expression of a select set of CYP isozymes; for PXR and CAR activation and for DDI. Humanized mouse strains, as in hPXR-hCAR-hCYP3A4-hCYP3A7 mice (available commercially), or recently reported hPXR-hCAR-hCYP3A4/3A7-hCYP2C9-hCYP2D6 mice, may replace a cell-based workflow for screening candidate drugs. A humanized mouse model provides human-like drug metabolism machinery and in vivo relevance. A microfluidic organ-on-a chip platform, which mimics human physiology at tissue and organ levels, may be used in the near future as a preferred alternative to animal models for screening drug candidates (Figure 5.6).

Disparate drug response among individuals results from altered activity or expression of DMEs/transporters due to single nucleotide polymorphisms (SNPs) in coding regions or in PXR-/CAR-/VDR/HNF4-α-regulated genomic loci; it can also be due to SNPs of PXR/CAR/VDR/HNF4-α that lead to variable expression or activity of these nuclear receptors. An epigenome signature is specified by DNA methylation, chromatin histone marks for transcription activation/repression (largely defined by lysine acetylation and lysine/arginine methylation of the amino-terminal tails of H3 and H4 histones), and by non-coding regulatory RNAs (microRNAs, long non-coding RNAs). The signature can have a profound impact on drug metabolism and disposition due to changes in PXR/CAR/VDR mediated transactivation of phase 0-III genes. The epigenome landscape also contributes to interindividual variations in drug response, since such a landscape is shaped by endogenous regulatory molecules and exogenous factors that are as varied as lifestyle, food habits, pollution and psychological disposition.

An integrated scheme linking genetic and epigenetic factors to drug metabolism/disposition, and interindividual variations in drug response is presented in Figure 6.6. In the era of personalized medicine, all of these regulatory factors must be taken into consideration before deciding on a medicinal regimen that provides optimal therapeutic efficacy and minimal toxicity, while preventing adverse drug reactions.

Acknowledgments

This work was supported by a VA Research Career Scientist (RCS) Award to BC; a DOD Grant (W81XWH-14-1-0606); a VA Merit-Review grant (201BX000280-05A1); and Morrison Trust Foundation, San Antonio. B Zuniga was supported by a summer undergraduate research fellowship from Cancer Prevention Research Institute in Texas (CPRIT). We thank past members of our laboratory for their contributions and Ms. Deborah Siller for assistance in manuscript preparation.

Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| NR           | nuclear receptor |
| DBD          | DNA-binding domain |
LBD  ligand-binding domain
XRE  xenobiotic response element
PXR  pregnane X receptor
CAR  constitutive androstanee receptor
VDR  vitamin D receptor
FXR  farnesoid X receptor
LXR  liver X receptor
CYP  cytochrome P450
DME  drug-metabolizing enzyme
ADME  absorption, distribution, metabolism, excretion
DDI  drug-drug interaction
PTM  post-translational modification
MDR  multi-drug resistance
ABC  ATP-binding cassette
HDAC  histone deacetylase
HAT  histone acetyltransferase
HMT  histone methyltransferase
HMD  histone demethylase
DNMT  DNA methyltransferase
SNP  single nucleotide polymorphism

References

1. Willson TM, Kliwer SA. PXR, CAR and drug metabolism. Nat Rev Drug Discov. 2002; 1:259–266. [PubMed: 12120277]
2. Evans RM, Mangelsdorf DJ. Nuclear receptors, RXR and the big bang. Cell. 2014; 157:255–266. [PubMed: 24679540]
3. Tzameli I, Moore DD. Role reversal: new insights from new ligands for the xenobiotic receptor CAR. Trends Endocrinol Metab. 2001; 12:7–10. [PubMed: 11137034]
4. Xie W, Evans RM. Orphan nuclear receptors: the exotics of xenobiots. J Biol Chem. 2001; 276:37739–37742. [PubMed: 11459851]
5. Smutny T, Mani S, Pavek P. Post-translational and post-transcriptional modifications of pregnane X receptor (PXR) in regulation of the cytochrome P450 superfamily. Curr Drug Metab. 2013; 14(10):1059–1069. [PubMed: 24329114]
6. Makishima M, Lu TT, Xie W, Whitfield GK, Domoto H, Evans RM, Haussler MR, Mangelsdorf DJ. Vitamin D receptor as an intestinal bile acid sensor. Science. 2002; 296:1313–1316. [PubMed: 12016314]
7. Chatterjee B, Echchgadda I, Song CS. Vitamin D receptor regulation of the steroid/bile acid sulfoconjugase SULT2A1. Methods Enzymol. 2005; 400:165–191. [PubMed: 16399349]
8. Xie W, Chiang JY. Nuclear receptors in drug metabolism and beyond. Drug Metab Rev. 2013; 45:1–2. [PubMed: 23330537]
9. Echchgadda I, Song CS, Oh T, Ahmed M, De La Cruz II, Chatterjee B. The xenobiotic-sensing nuclear receptors pregnane X receptor, constitutive androstane receptor, and orphan nuclear receptor hepatocyte nuclear factor 4alpha in the regulation of human steroid-/bile acid-sulfotransferase. Mol Endocrinol. 2007; 21:2099–2111. [PubMed: 17595319]
10. Hwang-Verslues WW, Sladek FM. HNF4α–role in drug metabolism and potential drug target? Curr Opin Pharmacol. 2010; 10:698–705. [PubMed: 20833107]
11. Tirona RG, Lee W, Leake BF, Lan LB, Cline CB, Lamba V, Parviz F, Duncan SA, Inoue Y, Gonzalez FJ, Schuetz EG, Kim RB. The orphan nuclear receptor HNF4alpha determines PXR- and CAR-mediated xenobiotic induction of CYP3A4. Nat Med. 2003; 9:220–224. [PubMed: 12514743]
12. Döring B, Petzinger E. Phase 0 and phase III transport in various organs: combined concept of phases in xenobiotic transport and metabolism. Drug Metab Rev. 2014; 46:261–282. [PubMed: 24483608]
13. Nigam SK. What do drug transporters really do? Nat Rev Drug Discov. 2015; 14:29–44. [PubMed: 25475361]
14. Russel, FGM. Enzyme- and Transporter-Based Drug–Drug Interactions. Rodrigues, AD.; Peter, RM., editors. Springer; Pang, KS: 2010. p. 27-49.
15. König J, Müller F, Fromm MF. Transporters and drug-drug interactions: important determinants of drug disposition and effects. Pharmaco 2013; 65:944–966. [PubMed: 23686349]
16. Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacol Ther. 2013; 138:103–141. [PubMed: 23333322]
17. Wijnen PAH, Op den Buijsch RAM, Drent M, Kuijpers PM, Neef C, Bast A, Bekers O, Koek GH. Review article: the prevalence and clinical relevance of cytochrome P450 polymorphisms. Aliment Pharmacol Ther. 2007; 26(Suppl 2):211–219. [PubMed: 18081664]
18. Lynch T, Price A. The effect of cytochrome P450 metabolism on drug response, interactions, and adverse effects. Am Fam Physician. 2007; 76:391–396. [PubMed: 17708140]
19. Tolson AH, Wang H. Regulation of drug-metabolizing enzymes by xenobiotic receptors: PXR and CAR. Adv Drug Deliv Rev. 2010; 62(13):1238–1249. [PubMed: 20723777]
20. Ballatori N. Biology of a novel organic solute and steroid transporter, OSTalpha-OSTbeta. Exp Biol Med (Maywood). 2005; 230:689–698. [PubMed: 16246895]
21. Motohashi, H.; Inui, K. AAPS J. Vol. 15. AAPS; 2013. Organic cation transporter OCTs (SLC22) and MATEs (SLC47) in the human kidney; p. 581-588.
22. Mangelsdorf DJ, Thummler C, Beato M, Herrlich P, Schütz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P, Evans RM. The nuclear receptor superfamily: the second decade. Cell. 1995; 83:835–839. [PubMed: 8521507]
23. Sever R, Glass CK. Signaling by nuclear receptors. Cold Spring Harb Perspect Biol. 2013; 5 Article ID a016709.
24. Mutoh S, Sobhany M, Moore R, Perera L, Pedersen L, Sueyoshi T, Negishi M. Phenobarbital indirectly activates the constitutive active androstane receptor (CAR) by inhibition of epidermal growth factor receptor signaling. Sci Signal. 2013; 6:ra31. [PubMed: 2362203]
25. Yang H, Garzel B, Heyward S, Moeller T, Shapiro P, Wang H. Metformin represses drug-induced expression of CYP2B6 by modulating the constitutive androstane receptor signaling. Mol Pharmacol. 2014; 85:249–260. [PubMed: 24252946]
26. Mullican SE, Dispirito JR, Lazar MA. The orphan nuclear receptors at their 25-year reunion. J Mol Endocrinol. 2013; 51:T115–T140. [PubMed: 24096517]
27. Carlberg C, Campbell MJ. Vitamin D receptor signaling mechanisms: integrated actions of a well-defined transcription factor. Steroids. 2013; 78:127–136. [PubMed: 23178257]
28. Helsen C, Claessens F. Looking at nuclear receptors from a new angle. Mol Cell Endocrinol. 2014; 382:97–106. [PubMed: 24055275]

Nuc Receptor Res. Author manuscript; available in PMC 2016 July 27.
29. Pawlak M, Lefebvre P, Staels B. General molecular biology and architecture of nuclear receptors. 
Curr Top Med Chem. 2012; 12(6):486–504. [PubMed: 22242852]

30. Cui JY, Gunewardena SS, Rockwell CE, Klaassen CD. ChIPing the cistrome of PXR in mouse 
liver. Nucleic Acids Res. 2010; 38:7943–7963. [PubMed: 20693526]

31. Hosoda K, Kanno Y, Sato M, Inajima J, Inouye Y, Yanai K. Identification of CAR/RXRa 
heterodimer binding sites in the human genome by a modified yeast one-hybrid Assay. Adv Biol 
Chem. 2015; 5:83–97.

32. Seuter S, Neme A, Carlberg C. Characterization of genomic vitamin D receptor binding sites 
through chromatin looping and opening. PLoS One. 2014; 9 Article ID e96184.

33. Gu X, Ke S, Liu D, Sheng T, Thomas PE, Rabson AB, Gallo MA, Xie W, Tian Y. Role of Nf-
kappaB in regulation of PXR-mediated gene expression: a mechanism for the suppression of 
cytochrome P-450 3A4 by proinflammatory agents. J Biol Chem. 2006; 281:17882–17889. 
[PubMed: 16608838]

34. Roth A, Looser R, Kaufmann M, Meyer UA. Sterol regulatory element binding protein 1 interacts 
with pregnane X receptor and constitutive androstane receptor and represses their target genes. 
Pharmacogenet Genomics. 2008; 18:325–337. [PubMed: 18334917]

35. Aouabdi S, Gibson G, Plant N. Transcriptional regulation of the PXR gene: identification and 
characterization of a functional peroxisome proliferator-activated receptor alpha binding site 
within the proximal promoter of PXR. Drug Metab Dispos. 2006; 34:138–144. [PubMed: 
16243957]

36. Kumari S, Mukhopadhyay G, Tyagi RK. Transcriptional regulation of mouse PXR gene: an 
interplay of transregulatory factors. PLoS One. 2012; 7:e44126. [PubMed: 22952895]

37. Jung D, Mangelsdorf DJ, Meyer UA. Pregnane X receptor is a target of farnesoid X receptor. J Biol 
Chem. 2006; 281:19081–19091. [PubMed: 16682417]

38. Kamiya A, Inoue Y, Gonzalez FJ. Role of the hepatocyte nuclear factor 4alpha in control of the 
pregnane X receptor during fetal liver development. Hepatology. 2003; 37:1375–1384. [PubMed: 
12774017]

39. Shi D, Yang D, Yan B. Dexamethasone transcriptionally increases the expression of the pregnane X 
receptor and synergistically enhances pyrethroid esfenvalerate in the induction of cytochrome 
P450 3A23. Biochem Pharmacol. 2010; 80:1274–1283. [PubMed: 20599767]

40. Iwazaki N, Kobayashi K, Morimoto K, Hirano M, Kawashima S, Furuihata T, Chiba K. Involvement 
of hepatocyte nuclear factor 4 alpha in transcriptional regulation of the human pregnane X receptor 
gene in the human liver. Drug Metab Pharmacokinet. 2008; 23:59–66. [PubMed: 18305375]

41. Ding X, Staudingler JL. 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. 2005; 
312:849–856. [PubMed: 15459237]

42. Drocourt L, Ourlin J-C, Pascussi J-M, Maurel P, Vilarem M-J. Expression of CYP3A4, CYP2B6, 
and CYP2C9 is regulated by the vitamin D receptor pathway in primary human hepatocytes. J Biol 
Chem. 2002; 277:25125–25132. [PubMed: 11991950]

43. Echchgadda I, Song CS, Roy AK, Chatterjee B. DHEA-sulfotransferase is a target for 
transcriptional induction by the vitamin D receptor. Mol Pharmacol. 2004; 65:720–729. [PubMed: 
14978251]

44. Eloranta JJ, Hiller C, Jüttner M, Kullak-Ublick GA. The SLCO1A2 gene, encoding human organic 
anion-transporting polypeptide 1A2, is transactivated by the vitamin D receptor. Mol Pharmacol. 
2012; 82:37–46. [PubMed: 22474172]

45. Tachibana S, Yoshinari K, Chikada T, Toriyabe T, Nagata K, Yamazoe Y. Involvement of Vitamin 
D receptor in the intestinal induction of human ABCB1. Drug Metab Dispos. 2009; 37:1604–1610. 
[PubMed: 19460946]

46. Fan J, Liu S, Du Y, Morrison J, Shipman R, Pang KS. Up-regulation of transporters and enzymes 
by the vitamin D receptor ligands, 1alpha,25-dihydroxyvitamin D3 and vitamin D analogs, in the 
Caco-2 cell monolayer. J Pharmacol Exp Ther. 2009; 330:389–402. [PubMed: 19416242]

47. Masuno H, Ikura T, Morizono D, Orita I, Yamada S, Shimizu M, Ito N. Crystal structures of 
complexes of vitamin D receptor ligand-binding domain with lithocholic acid derivatives. J Lipid 
Res. 2013; 54:2206–2213. [PubMed: 23723390]
48. Thirumaran RK, Lamba JK, Kim RB, Urquhart BL, Gregor JC, Chande N, Fan Y, Qi A, Cheng C, Thummel KE, Hall SD, Schuetz EG. Intestinal CYP3A4 and midazolam disposition in vivo associate with VDR polymorphisms and show seasonal variation. Biochem Pharmacol. 2012; 84:104–112. [PubMed: 22484315]

49. Pike JW, Meyer MB. The vitamin D receptor: new paradigms for the regulation of gene expression by 1,25-dihydroxyvitamin D3. Endocrinol Metab Clin North Am. 2010; 39:255–269. [PubMed: 20511050]

50. Frank C, Gonzalez MM, Oinonen C, Dunlop TW, Carlberg C. Characterization of DNA complexes formed by the nuclear receptor constitutive androstane receptor. J Biol Chem. 2003; 278:43299–43310. [PubMed: 12896978]

51. Sugatani J, Kojima H, Ueda A, Kakizaki S, Yoshinari K, Gong Q-H, Owens IS, Negishi M, Sueyoshi T. The phenobarbital response enhancer module in the human bilirubin UDP-glucuronosyltransferase UGT1A1 gene and regulation by the nuclear receptor CAR. Hepatology. 2001; 33:1232–1238. [PubMed: 11343253]

52. Song CS, Echchgadda I, Baek BS, Ahn SC, Oh T, Roy AK, Chatterjee B. Dehydroepiandrosterone sulfotransferase gene induction by bile acid activated farnesoid X receptor. J Biol Chem. 2001; 276:42549–42556. [PubMed: 11533040]

53. Plass JR, Mol O, Heegsma J, Geuken M, Faber KN, Jansen PL, Müller M. Farnesoid X receptor and bile salts are involved in transcriptional regulation of the gene encoding the human bile salt export pump. Hepatology. 2002; 35:589–596. [PubMed: 11870371]

54. Chong HK, Infante AM, Geuken M, Faber KN, Jansen PL, Müller M. Farnesoid X receptor and bile salts are involved in transcriptional regulation of the gene encoding the human bile salt export pump. Hepatology. 2002; 33:1232–1238. [PubMed: 11870371]

55. Schaap FG, Trauner M, Jansen PL. Bile acid receptors as targets for drug development. Nat Rev Gastroenterol Hepatol. 2014; 11(1):55–67. [PubMed: 23982684]

56. Kir S, Zhang Y, Gerard RD, Kliever SA, Mangelsdorf DJ. Nuclear receptors HNF4a and LRH-1 cooperate in regulating Cyp7a1 in vivo. J Biol Chem. 2012; 287:41334–41341. [PubMed: 21303826]

57. Handschin C, Meyer UA. Regulatory network of lipid-sensing nuclear receptors: roles for CAR, PXR, LXR, and FXR. Arch Biochem Biophys. 2005; 433:387–396. [PubMed: 15581595]

58. Goodwin B, Jones SA, Price RR, Watson MA, Mckee DD, Moore LG, Galardi C, Wilson JG, Lewis MC, Roth ME, Willson TM, Kliever SA. A regulatory cascade of the nuclear receptors FXR, HNF4a, and LRH-1 increases bile acid biosynthesis. Mol Cell. 2000; 6:517–526. [PubMed: 11030332]

59. Li T, Chiang JY. Mechanism of rifampicin and pregnane X receptor inhibition of human cholesterol 7 alpha-hydroxylase gene transcription. Am J Physiol Gastrointest Liver Physiol. 2005; 288:G74–G84. [PubMed: 15331348]

60. Chow EC, Magomedova L, Quach HP, Patel R, Durk MR, Fan J, Maeng HJ, Iroadi K, Anakk S, Moore DD, Cummins CL, Pang KS. Vitamin D receptor activation down-regulates the small heterodimer partner and increases CYP7A1 to lower cholesterol. Gastroenterology. 2014; 146(4): 1048–1059. [PubMed: 24365583]

61. Lamba V, Lamba J, Sasuda K, Strom S, Davila J, Hancock ML, Fackenthal JD, Rogan PK, Ring B, Wrighton SA, Schuetz EG. Hepatic CYP2B6 expression: gender and ethnic differences and relationship to CYP2B6 genotype and CAR (constitutive androstane receptor) expression. J Pharmacol Exp Ther. 2003; 307:906–922. [PubMed: 14551287]

62. 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, Ahituv N. Genome-wide discovery of drug-dependent human liver regulatory elements. PLoS Genet. 2014; 10 Article ID 1004648.

63. Lee SS, Cha E-Y, Jung H-J, Shon J-H, Kim E-Y, Yeo CW, Shin J-G. Genetic polymorphism of hepatocyte nuclear factor-4alpha influences human cytochrome P450 2D6 activity. Hepatology. 2008; 48:635–645. [PubMed: 18666237]

64. Bertilsson L, Dahl ML, Dalén P, Al-Shurbaji A. Molecular genetics of CYP2D6: clinical relevance with focus on psychotropic drugs. Br J Clin Pharmacol. 2002; 53:111–122. [PubMed: 11851634]
65. Hustert E, Zibat A, Presecan-Siedel E, Eiselt R, Mueller R, Fuss C, Brehm I, Brinkmann U, Eichelbaum M, Wojnowski L, Burk O. Natural protein variants of pregnane X receptor with altered transactivation activity toward CYP3A4. Drug Metab Dispos. 2001; 29:1454–1459. [PubMed: 11602521]

66. Lamba V, Panetta JC, Strom S, Schuetz EG. Genetic predictors of interindividual variability in hepatic CYP3A4 expression. J Pharmacol Exp Ther. 2010; 332:1088–1099. [PubMed: 19934400]

67. Hu M, Lui SS, Tam L-S, Li EK, Tomlinson B. The farnesoid X receptor -1G>T polymorphism influences the lipid response to rosuvastatin. J Lipid Res. 2012; 53:1384–1389. [PubMed: 22534644]

68. Meyer zu Schwabedissen HE, Kim RB. Hepatic OATP1B transporters and nuclear receptors PXR and CAR: interplay, regulation of drug disposition genes, and single nucleotide polymorphisms. Mol Pharm. 2009; 6:1644–1661. [PubMed: 19558188]

69. Swart M, Whitehorn H, Ren Y, Smith P, Ramesar RS, Dandara C. PXR and CAR single nucleotide polymorphisms influence plasma efavirenz levels in South African HIV/AIDS patients. BMC Med Genet. 2012; 13:112. [PubMed: 23173844]

70. Wyen C, Hendra H, Siccardi M, Platten M, Jaeger H, Harrer T, Esser S, Bogner JR, Brockmeyer NH, Bieniek B, et al. Cytochrome P450 2B6 (CYP2B6) and constitutive androstane receptor (CAR) polymorphisms are associated with early discontinuation of efavirenz-containing regimens. J Antimicrob Chemother. 2011; 66:2092–2098. [PubMed: 21715435]

71. Uitterlinden AG, Fang Y, Van Meurs JB, Pols HA, Van Leeuwen JP. Genetics and biology of vitamin D receptor polymorphisms. Gene. 2004; 338:143–156. [PubMed: 15315181]

72. Ingelman-Sundberg M, Zhong X-B, Hankinson O, Beedanagari S, Yu A-M, Peng L, Osawa Y. Potential role of epigenetic mechanisms in the regulation of drug metabolism and transport. Drug Metab Dispos. 2013; 41:1725–1731. [PubMed: 23918665]

73. Ivanov M, Barragan I, Ingelman-Sundberg M. Epigenetic mechanisms of importance for drug treatment. Trends Pharmacol Sci. 2014; 35:384–396. [PubMed: 24993164]

74. Nilsson EE, Skinner MK. Environmentally induced epigenetic transgenerational inheritance of disease susceptibility. Transl Res. 2014; 165(1):12–17. [PubMed: 24657180]

75. Sharma S, Kelly TK, Jones PA. Epigenetics in cancer. Carcinogenesis. 2010; 31:27–36. [PubMed: 19752007]

76. Ivanov M, Kals M, Kacevska M, Barragan I, Kasuga K, Rane A, Metspalu A, Milani L, Ingelman-Sundberg M. Ontogeny, distribution and potential roles of 5-hydroxymethylcytosine in human liver function. Genome Biol. 2013; 14:R83. [PubMed: 23958281]

77. Feinberg JI, Bakulski KM, Jaffe AE, Tryggvadottir R, Brown SC, Goldman LR, Croen LA, Hertz-Picciotto I, Newshaffer CJ, Fallin MD, Feinberg AP. Paternal sperm DNA methylation associated with early signs of autism risk in an autism-enriched cohort. Int J Epidemiol. 2015; 44(4):1199–1210. [PubMed: 25878217]

78. Zhang D, Cheng L, Badner JA, Chen C, Chen Q, Luo W, Craig DW, Redman M, Gershon ES, Liu C. Genetic control of individual differences in gene-specific methylation in human brain. Am J Hum Genet. 2010; 86:411–419. [PubMed: 20215007]

79. Bram EE, Stark M, Raz S, Assaraf YG. Chemotherapeutic drug-induced ABCG2 promoter demethylation as a novel mechanism of acquired multidrug resistance. Neoplasia. 2009; 11:1359–1370. [PubMed: 20019844]

80. Ivanov M, Kacevska M, Ingelman-Sundberg M. Epigenomics and interindividual differences in drug response. Clin Pharmacol Ther. 2012; 92:727–736. [PubMed: 23093317]

81. Pathiraja TN, Stearns V, Oesterreich S. Epigenetic regulation in estrogen receptor positive breast cancer–role in treatment response. J Mammary Gland Biol Neoplasia. 2010; 15(1):35–47. [PubMed: 20101445]

82. Shao R. Progesterone receptor isoforms A and B: new insights into the mechanism of progesterone resistance for the treatment of endometrial carcinoma. ecaner. 2013; 7 Article ID 2013381.

83. Kacevska M, Ivanov M, Ingelman-Sundberg M. Epigenetic-dependent regulation of drug transport and metabolism: an update. Pharmacogenomics. 2012; 13:1373–1385. [PubMed: 22966887]

84. Cabreri R, Castaño GO, Burgueño AL, Fernández Gianotti T, Gonzalez Lopez Ledesma MM, Flichman D, Pirola CJ, Sookoian S. Promoter DNA methylation of farnesoid X receptor and
pregnane X receptor modulates the intrahepatic cholestasis of pregnancy phenotype. PLoS One. 2014; 9 Article ID e87697.

85. Gomez A, Ingelman-Sundberg M. Pharmacoepigenticits: its role in interindividual differences in drug response. Clin Pharmacol Ther. 2009; 85:426–430. [PubMed: 19242404]

86. Imai S, Kikuchi R, Kusuhara H, Sugiyama Y. DNA methylation and histone modification profiles of mouse organic anion transporting polypeptides. Drug Metab Dispos. 2013; 41:72–78. [PubMed: 23033256]

87. Imai S, Kikuchi R, Tsuruya Y, Naoi S, Nishida S, Kusuhara H, Sugiyama Y. Epigenetic regulation of organic anion transporting polypeptide 1B3 in cancer cell lines. Pharm Res. 2013; 30:2880–2890. [PubMed: 23812637]

88. Reed K, Hembrow SL, Sproll JA, Parisent AM. The temporal relationship between ABCB1 promoter hypomethylation, ABCB1 expression and acquisition of drug resistance. Pharmacogenomics. 2010; 10:499–504. [PubMed: 20125118]

89. Xie FY, Peng Y-H, Chen X, Chen X, Li J, Yu Z-Y, Wang W-W, Ouyang X-N. Regulation and expression of aberrant methylation on irinotecan metabolic genes CES2, UGT1A1 and GUSB in the in-vitro cultured colorectal cancer cells. Biomed Pharmacother. 2014; 68:31–37. [PubMed: 2449671]

90. Cohen I, Poręba E, Kamieniarz K, Schneider R. Histone modifiers in cancer: friends or foes? Genes Cancer. 2011; 2:631–647. [PubMed: 21941619]

91. Kouzarides T. Chromatin modifications and their function. Cell. 2007; 128:693–705. [PubMed: 17320507]

92. Chen WD, Fu X, Dong B, Wang YD, Shah S, Moore DD, Huang W. Neonatal activation of the nuclear receptor CAR results in epigenetic memory and permanent change of drug metabolism in mouse liver. Hepatology. 2012; 563:1499–1509. [PubMed: 22488010]

93. Smith G, Henderson CJ, Parker MG, White R, Bars RG, Wolf CR. 1,4-Bis[2-(3,5-dichloropyridyloxy)]benzene, an extremely potent modulator of mouse hepatic cytochrome P-450 gene expression. Biochem J. 1993; 289:807–813. [PubMed: 8435079]

94. Xie Y, Ke S, Ouyang N, He J, Xie W, Bedford MT, Tian Y. Epigenetic regulation of transcriptional activity of pregnane X receptor by protein arginine methyltransferase 1. J Biol Chem. 2009; 284:9199–9205. [PubMed: 19144646]

95. Velan J, Feere DA, Sohi G, Hardy DB, Urquhart BL. Decreased nuclear receptor activity and epigenetic modulation associates with down-regulation of hepatic drug-metabolizing enzymes in chronic kidney disease. FASEB J. 2014; 28:5388–5397. [PubMed: 25208844]

96. Jordheim LP, Duranet D, Zoulim F, Dumontet C. Advances in the development of nucleoside and nucleotide analogues for cancer and viral diseases. Nat Rev Drug Discov. 2013; 12:447–464. [PubMed: 23722347]

97. Cerveny L, Svecova L, Anzenbacherova E, Vrazil R, Staud F, Dvorak Z, Ulrichova J, Anzenbacher P, Pavek P. Valproic acid induces CYP3A4 and MDR1 gene expression by activation of constitutive androstane receptor and pregnane X receptor pathways. Drug Metab Dispos. 2010; 38:1032–1041. [PubMed: 17392393]

98. Xi N, Li L, Pan G. HDAC inhibitor-induced drug resistance involving ATP-binding cassette transporters (Review). Oncol Lett. 2015; 9:515–521. [PubMed: 25624882]

99. Takizawa D, Kakizaki S, Horiguchi N, Tojima H, Yamazaki Y, Ichikawa T, Sato K, Mori M. Histone deacetylase inhibitors induce cytochrome P450 2B by activating nuclear receptor CAR. Drug Metab Dispos. 2010; 38:1493–1498. [PubMed: 20516253]

100. Rieger JK, Klein K, Winter S, Zanger UM. Expression variability of absorption, distribution, metabolism, excretion-related microRNAs in human liver: influence of nongenetic factors and

Nucl Receptor Res. Author manuscript; available in PMC 2016 July 27.
association with gene expression. Drug Metab Dispos. 2013; 41:1752–1762. [PubMed: 23733276]

103. Yu A-M, Pan Y-Z. Noncoding microRNAs: small RNAs play a big role in regulation of ADME? Acta Pharm Sin B. 2012; 2:93–101.

104. Wang J, Bian Y, Wang Z, Li D, Wang C, Li Q, Gao X. MicroRNA-152 regulates DNA methyltransferase1 and is involved in the development and lactation of mammary glands in dairy cows. PLOS One. 2014; 9 Article ID e101358.

105. Chuang JC, Jones PA. Epigenetics and microRNAs. Pediatr Res. 2007; 61:24R–29R.

106. Giovannetti E, Funel N, Peters GJ, Del Chiario M, Erozencli IA, Vasile E, Leon LG, Pollina LE, Groen A, Falcone A, Danesi R, Campani D, Verheul HM, Boggi U. MicroRNA-21 in pancreatic cancer: correlation with clinical outcome and pharmacologic aspects underlying its role in the modulation of gemcitabine activity. Cancer Res. 2010; 70:4528–4538. [PubMed: 20460539]

107. Sleutels F, Zwart R, Barlow DP. The non-coding Air RNA is required for silencing autosomal imprinted genes. Nature. 2002; 415:810–813. [PubMed: 11845212]

108. Li P, Ruan X, Yang L, Kiesewetter K, Zhao Y, Luo H, Chen Y, Gucek M, Zhu J, Cao H. A liver-enriched long non-coding RNA, IncLSTR, regulates systemic lipid metabolism in mice. Cell Metab. 2015; 21:455–467. [PubMed: 25738460]

109. Hung C-L, Wang L-Y, Yu Y-L, Chen H-W, Srivastava S, Petrovics G, Kung H-J. A long noncoding RNA connects c-Myc to tumor metabolism. Proc Natl Acad Sci USA. 2014; 111:18697–18702. [PubMed: 25512540]

110. Shirasaka Y, Chang S-Y, Grubb MF, Peng C-C, Thummel KE, Isoherranen N, Rodrigues AD. Effect of CYP3A5 expression on the inhibition of CYP3A-catalyzed drug metabolism: impact on modeling CYP3A-mediated drug-drug interactions. Drug Metab Dispos. 2013; 41:1566–1574. [PubMed: 23723360]

111. Haniadka R, Saxena A, Shivashankara AR, Fayad R, Palatty PL, Nazreth N, Francis A, Arora R, Baliga MS. Ginger Protects the Liver against the Toxic Effects of Xenobiotic Compounds: Preclinical Observations. J Nutr Food Sci. 2013; 3(5) Article ID 1000226.

112. Roth EM, McKenney JM, Hanotin C, Asset G, Stein EA. Atorvastatin with or without an antibody to PCSK9 in primary hypercholesterolemia. N Engl J Med. 2012; 367:1891–1900. [PubMed: 23113833]

113. Ihunnah CA, Jiang M, Xie W. Nuclear receptor PXR, transcriptional circuits and metabolic relevance. Biochim Biophys Acta. 2011; 1812:956–963. [PubMed: 21295138]

114. Yu J, Ritchie TK, Zhou Z, Raguenneau-Majlessi I. Key findings from preclinical and clinical drug interaction studies presented in new drug and biological license applications approved by the FDA in 2014. Drug Metab Dispos. 2015; 44(1):83–101. [PubMed: 26424199]

115. Fuhr U. Induction of drug metabolising enzymes: pharmacokinetic and toxicological consequences in humans. Clin Pharmacokinet. 2000; 38:493–504. [PubMed: 10885586]

116. Niemi M, Backman JT, Fromm MF, Neuvonen PJ, Kivistö KT. Pharmacokinetic interactions with rifampicin: clinical relevance. Clin Pharmacokinet. 2003; 42:819–850. [PubMed: 12882588]

117. Moore LB, Goodwin B, Jones SA, Wisely GB, Serahjit-Singh CJ, Willson TM, Collins JL, Kliewer SA. St. John’s wort induces hepatic drug metabolism through activation of the pregnane X receptor. Proc Natl Acad Sci USA. 2000; 97:7500–7502. [PubMed: 10852961]

118. Staedinger JL, Ding X, Lichit K. Pregnane X receptor and natural products: beyond drug-drug interactions. Expert Opin Drug Metab Toxicol. 2006; 2:847–857. [PubMed: 17125405]

119. Zamek-Gliszczynski MJ, Mohutsky MA, Rehmel JLF, Ke AB. Investigational small-molecule drug selectively suppresses constitutive CYP2B6 activity at the gene transcription level: physiologically based pharmacokinetic model assessment of clinical drug interaction risk. Drug Metab Dispos. 2014; 42:1008–1015. [PubMed: 24658455]

120. Harmsen S, Meijerman I, Beijnen JH, Schellens JHM. The role of nuclear receptors in pharmacokinetic drug-drug interactions in oncology. Cancer Treat Rev. 2007; 33:369–380. [PubMed: 17451886]

121. Neuvonen PJ. Drug interactions with HMG-CoA reductase inhibitors (statins): the importance of CYP enzymes, transporters and pharmacogenetics. Curr Opin Investig Drugs. 2010; 11:323–332.
122. Link E, Parish S, Armitage J, Bowman L, Heath S, Matsuda F, Gut I, Lathrop M, Collins R. SLCO1B1 variants and statin-induced myopathy—a genomewide study. N Engl J Med. 2008; 359:789–799. [PubMed: 18650507]

123. Tomaszewski M, Stepiń KM, Tomaszewska J, Czuczwar SJ. Statin-induced myopathies. Pharmacol Rep. 2011; 63:859–866. [PubMed: 22001973]

124. Backman JT, Luurila H, Neuvonen M, Neuvonen PJ. Rifampin markedly decreases and gemfibrozil increases the plasma concentrations of atorvastatin and its metabolites. Clin Pharmacol Ther. 2005; 78:154–167. [PubMed: 16084850]

125. Lau YY, Huang Y, Frassetto L, Benet LZ. Effect of OATP1B transporter inhibition on the pharmacokinetics of atorvastatin in healthy volunteers. Clin Pharmacol Ther. 2007; 81:194–204. [PubMed: 17192770]

126. Tirona, RG. Drug Transporters: Handbook of Experimental Pharmacology, 201 of Handbook of Experimental Pharmacology. Springer-Verlag; Berlin: 2010. p. 373-402.

127. Beckett RD, Rodeffer KM, Snodgrass R. Abiraterone for the treatment of metastatic castrate-resistant prostate cancer. The Annals of Pharmacotherapy. 2012; 46:1016–1024. [PubMed: 22714819]

128. Hanley MJ, Cancalon P, Widmer WW. Greenblatt DJ. The effect of grapefruit juice on drug disposition. Expert Opin Drug Metab Toxicol. 2011; 7(3):267–286. [PubMed: 21254874]

129. Kleiner HE, Xia X, Sonoda J, Zhang J, Pontius E, Abey J, Evans RM, Moore DD, DiGiovanni J. Effects of naturally occurring coumarins on hepatic drug-metabolizing enzymes in mice. Toxicol Appl Pharmacol. 2008; 232:337–350. [PubMed: 18692084]

130. Cheng J, Ma X, Gonzalez FJ. Pregnan e X receptor- and CYP3A4-humanized mouse models and their applications. Br J Pharmacol. 2011; 163:461–468. [PubMed: 21091656]

131. Xie W, Barwick JL, Downes M, Blumberg B, Simon CM, Nelson MC, Neuschwander-Tetri BA, Brunt EM, Guzelian PS, Evans RM. Humanized xenobiotic response in mice expressing nuclear receptor SXR. Nature. 2000; 406:435–439. [PubMed: 10935643]

132. Saruwatari A, Okamura S, Nakajima Y, Narukawa Y, Takeda T, Tamura H. Pomegranate juice inhibits sulfoconjugation in Caco-2 human colon carcinoma cells. J Med Food. 2008; 11(4):623–628. [PubMed: 19053852]

133. Chang TKH. Activation of pregnane X receptor (PXR) and constitutive androstane receptor (CAR) by herbal medicines. The AAPS Journal. 2009; 11(3):590–601. [PubMed: 19688601]

134. Ruschitzka F, Meier PJ, Turina M, Lüscher TF, Noll G. Acute heart transplant rejection due to Saint John’s wort. Lancet. 2000; 355:548–549. [PubMed: 10683008]

135. Ho BE, Shen DD, McCune JS, Bui T, Risler L, Yang Z, Ho RJY. Effects of garlic on cytochromes P450 2C9- and 3A4-mediated drug metabolism in human hepatocytes. Sci Pharm. 2010; 78:473–481. [PubMed: 20936048]

136. Sueyoshi T, Green WD, Vinal K, Woodrum TS, Moore R, Negishi M. Garlic extract diallyl sulfide (DAS) activates nuclear receptor CAR to induce the Sult1e1 gene in mouse liver. PLoS One. 2011; 6 Article ID e21229.

137. Park KA, Kweon S, Choi H. Anticarcinogenic effect and modification of cytochrome P450 2E1 by dietary garlic powder in diethylnitrosamine-initiated rat hepatocarcinogenesis. J Biochem Mol Biol. 2002; 35(6):615–622. [PubMed: 12470597]

138. Adjei AA, Gaedigk A, Simon SD, Weinshilboum RM, Leeder JS. Interindividual variability in acetaminophen sulfation by human fetal liver: implications for pharmacogenetic investigations of drug-induced birth defects. Birth Defects Res A Clin Mol Teratol. 2008; 82(3):155–165. [PubMed: 18232020]

139. McGill MR, Jaeschke H. Metabolism and disposition of acetaminophen: recent advances in relation to hepatotoxicity and diagnosis. Pharm Res. 2013; 30(9):2174–2187. [PubMed: 23462933]

140. Zhang J, Huang W, Chua SS, Wei P, Moore DD. Modulation of acetaminophen-induced hepatotoxicity by the xenobiotic receptor CAR. Science. 2002; 298:422–424. [PubMed: 12376703]
141. Knauer MJ, Girdwood AJ, Kim RB, Tirona RG. Transport function and transcriptional regulation of a liver-enriched human organic anion transporting polypeptide 2B1 transcriptional start site variant. Mol Pharmacol. 2013; 83:1218–1228. [PubMed: 23531488]

142. Pascussi JM, Busson-Le Coniat M, Maurel P, Vilarem MJ. Transcriptional analysis of the orphan nuclear receptor constitutive androstane receptor (NR1I3) gene promoter: identification of a distal glucocorticoid response element. Mol Endocrinol. 2003; 17:42–55. [PubMed: 12511605]

143. Sugatani J, Nishitani S, Yamakawa K, Yoshinari K, Sueyoshi T, Negishi M, Miwa M. Transcriptional regulation of human UGT1A1 gene expression: activated glucocorticoid receptor enhances constitutive androstane receptor/pregnane X receptor-mediated UDP-glucuronosyltransferase 1A1 regulation with glucocorticoid receptor-interacting protein 1. Mol Pharmacol. 2005; 67:845–855. [PubMed: 15557560]

144. Duret C, Daujat-Chavanieu M, Pascussi JM, Pichard-Garcia L, Balaguer P, Fabre JM, Vilarem MJ, Maurel P, Gerbal-Chaloin S. Ketoconazole and miconazole are antagonists of the human glucocorticoid receptor: consequences on the expression and function of the constitutive androstane receptor and the pregnane X receptor. Mol Pharmacol. 2006; 70:329–339. [PubMed: 16608920]

145. Kapelyukh, Y.; Scheer, N.; McLaughlin, L.; McMahon, M.; Rode, A.; Henderson, C.; Wolf, R. Characterization of hPXR-hCAR-hCYP3A4/A7-hCYP2C9-hCYP2D6 mouse model. NCRI Cancer Conference; 2–5 November; Liverpool UK: The BT Convention Center; 2014.

146. Bhatia SN, Ingber DE. Microfluidic organs-on-chips. Nat Biotechnol. 2014; 32:760–772. [PubMed: 25093883]

147. Reardon S. ‘Organs-on-chips’ go mainstream. Nature. 2015; 523:266. [PubMed: 26178942]

148. Elias A, High AA, Mishra A, Ong SS, Wu J, Peng J, Chen T. Identification and characterization of phosphorylation sites within the pregnane X receptor protein. Biochem Pharmacol. 2014; 87:360–370. [PubMed: 24184507]
Figure 1. Steps involved in drug metabolism and disposition
(1) Uptake transporter mediates drug entry into the cell. (2) Drug activates xeno-sensing NR in the cytoplasm or nucleus. (3) NR binds to XREs in target genes that are involved in drug metabolism and clearance. (4) Coactivator association with the DNA-bound NR and a cascade of activating steps, which culminate in gene transcription for DMEs, transporters. (5) Expression of phase 0-III mediators. (6) Phase I enzyme adds water-soluble functional groups to the drug structure. (7) A phase II conjugative transferase adds hydrophilic groups to drug/drug metabolite. (8) Phase III efflux transporter moves to plasma membrane. (9) Transporter-assisted drug efflux. (10) Drug clearance through biliary and urinary excretion.
Figure 2. Percentage of all prescription drugs metabolized in human liver by a particular CYP enzyme
(adapted from [16]).
Figure 3. Induction of the human SULT2A1 promoter by PXR, CAR and a synergizing effect of HNF4-α

Schema showing a PXR- and CAR-binding composite XRE comprised of IR2 and DR4 elements, and an HNF4-α-binding DR1 element located downstream of XRE. Dotted, upward arrows signify promoter induction. Interaction between DR1-bound HNF4-α and XRE-bound PXR/RXR, CAR/RXR has a synergistic effect (triple upward arrows) on SULT2A1 induction. (based on results described in [9]).
Figure 4. SULT2A1 mRNA induction by cholic acid in mouse liver
Sult2A1 mRNAs in mouse livers were assayed by semi-quantitative RT-PCR. Cholic acid, a primary bile acid, was added to diet at 1% w/w. Data are for 3 individual mice (6-month-old, male) from the control and experimental group. Levels of β-actin mRNAs served as the normalization control (B. Chatterjee & CS Song, unpublished).
Candidate drugs are screened for effects on the activity and expression of a select set of CYPs (e.g., CYP3A4, and several other CYPs). Workflow for traditional screening (shown at left) relies on cell-based high throughput assay to identify and narrow down candidates with potential for optimal drug activity. Microfluidic organ-on-a chip constitutes an emerging technology that may replace cell-based screening as the primary assay platform. In cross screening, cells are co-administered with a test drug and a second drug or a non-drug xenobiotic agent (such as a medicinal herb or a foodstuff) in order to reveal drug-drug or drug-herb or drug-food interactions. Subsequently, drugs are tested in mice. A humanized mouse model (transgenic mice with human PXR, CAR and CYP genes replacing the counterpart rodent genes) can serve as a human surrogate for the examination of drug interactions in the preclinical stage of drug screening.
Figure 6. NR-mediated regulation of drug metabolism, drug disposition: control at multiple steps
Transcriptional regulation primarily dictates NR expression and its cellular abundance (box at the upper right corner). Post-translational modification modulates NR stability and NR activity (box at upper left corner). (A) Drugs activate xenobiotic NRs (PXR, CAR), which in turn modulate the expression of phase 0-III mediators via induction of XREs. Ligand-activated VDR also induces DME and transporter expression. (B) Drug-drug, drug-herb, drug-food interactions cause altered NR expression/activity leading to altered expression of DME/transporter. An interfering agent (such as a second drug or a dietary constituent) may also modulate DME/transporter activity via competitive or allosteric regulation. (C) Histone modification and DNA methylation modulate NR expression; they also modulate NR-regulated DME/transporter expression due to epigenetic changes at or near XREs. (D) SNP at an XRE or at an alternate regulatory locus of phase 0-III genes leads to a change in the NR interaction with the response element, which alters DME and transporter expression. SNP in coding regions of PXR/CAR/VDR/HNF4-α, DMEs or transporters can alter the activity or cellular abundance of these proteins/enzymes. (E) Micro RNAs and long non-coding RNAs (lncRNAs) regulate the cellular abundance of NRs and mediators of phase 0-III processes. (F) Interindividual differences in drug response stem from SNP at an XRE, at another NR-interacting regulatory locus of the target gene, or at the coding region of NRs (PXR/CAR/VDR/HNF4-α) or phase 0-III mediators.