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Drug-Metabolizing Enzyme, Transporter and Nuclear Receptor Genetically Modified Mouse Models

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Abstract

Determining the in vivo significance of a specific enzyme, transporter or xenobiotic receptor in drug metabolism and pharmacokinetics may be hampered by gene multiplicity and complexity, levels of expression and interaction between various components involved. The development of knockout (loss-of-function) and transgenic (gain-of-function) mouse models opens the door to the improved understanding of gene function in a whole body system. There is also growing interest in the development of humanized mice to overcome species difference in drug metabolism and disposition. This review, therefore, aims to summarize and discuss some successful examples of drug-metabolizing enzyme, transporter, and nuclear receptor genetically modified mouse models. These genetically modified mouse models have proven as invaluable models for understanding in vivo function of drug-metabolizing enzymes, transporters and xenobiotic receptors in drug metabolism and transport, as well as predicting potential drug-drug interaction and toxicity in humans. Nevertheless, concerns remain about interpretation of data obtained from such genetically modified mouse models in which the expression of related genes is altered significantly.

Keywords

Transgenic; knockout; humanized; drug metabolism; pharmacokinetics; drug interaction; toxicity; cancer

Introduction

Understanding the metabolism and disposition of drugs is critical for efficient drug discovery and development. Drugs may be structurally altered by Phase 1 oxidative enzymes [e.g., cytochrome P450 (P450 or CYP) and flavin-containing monooxygenase (FMO)] and Phase 2 conjugative enzymes [e.g., uridine 5'-diphospho-glucuronosyltransferase (UGT), sulfotransferase (SULT) and glutathione S-transferase (GST)] in liver, small intestine or other tissues. Drug accessibility, through intestinal absorption, tissue distribution, cell uptake and efflux, and renal and biliary excretion could be ultimately limited or facilitated by efflux [e.g., adenosine triphosphate (ATP)-binding cassette (ABC) family] or influx [e.g., solute carrier (SLC) family] drug transporters. Furthermore, levels of expression of enzyme and transporter proteins are subject to complex regulation by xenobiotic receptors [e.g., aryl

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hydrocarbon receptor (AhR), pregnane X receptor (PXR), and constitutive androstane receptors (CAR)], as well as epigenetic factors recognized recently (Gonzalez and Yu, 2006; Ingelman-Sundberg et al., 2007; Yu, 2007; Gomez and Ingelman-Sundberg, 2009; Yu, 2009). Differences in the functions or the degree of regulation of enzymes and transporters inevitably affect pharmacokinetic profiles, which may result in considerable inter-individual variability in drug response or toxicity.

Animals are common models used to predict drug metabolism and disposition in humans because they share many similarities in anatomy, physiology and genetics. Mouse models are of particular interest. The small size features relative low husbandry and maintenance costs. The fairly short life span, large litter size and fast breeding rate allow for growth of a large number of animals in a relatively short time, and therefore many studies can be carried out in a desired period. Mice are also of great value for genetic modification through transgenesis and targeted gene disruption; a target gene may be disrupted and a new gene inserted into the mouse genome (Manis, 2007). The resultant gene knockout and transgenic mouse models have been widely used for improved understanding of the role of specific enzyme, transporter or xenobiotic receptor in drug metabolism and disposition at the systemic level (Gonzalez, 2003; Gonzalez and Kimura, 2003; Henderson et al., 2003b; Henderson and Wolf, 2003; Dai and Wan, 2005; Xia et al., 2007; Muruganandan and Sinal, 2008; Lagas et al., 2009; Ou et al., 2010). In addition, species difference is a major confounding factor in metabolism and disposition of many xenobiotics such as chemical carcinogens and medications. The overall metabolic stability of the same drug could be very different across species. There are also human-specific metabolites that cannot be produced or produced at similar levels in wild-type mouse models, where pharmacological and toxicological properties of a particular metabolite need to be understood. Since species differences in drug metabolism and disposition are generally caused by the difference in composition, expression and/or function of enzymes, transporters or xenobiotic receptors, one approach to overcome this issue is to use humanized transgenic mouse models that can be developed by breeding a knockout mouse with a transgenic mouse created separately, or directly replacing a target mouse gene with the corresponding human gene or cDNA ("knock-in"). Studies with these humanized mouse models may not only provide mechanistic understanding of species difference but also improve prediction of risks of xenobiotics in humans (Henderson and Wolf, 2003; Liggett, 2004; Gonzalez and Yu, 2006). The following provides a brief review on enzyme, transporter and xenobiotic receptor gene knockout, transgenic and humanized mouse models that have been developed and used for studies on drug metabolism and disposition of xenobiotics or endobiotics.

Drug-Metabolizing Enzyme Genetically Modified Mouse Models

CYP1A1/CYP1A2

A number of *Cyp1a1*($-/-$) and *Cyp1a2*($-/-$) mouse lines have been generated and used to study the function of human CYP1A orthologues in xenobiotic metabolism (Pineau et al., 1995; Liang et al., 1996; Dalton et al., 2000), given a high degree of interspecies conservation in CYP1A1/1A2 expression, function and gene regulation. The *Cyp1a2*($-/-$) mouse (Pineau et al., 1995) was among the first reported P450-knockout mouse models (Table 1), which was created by disruption of the exon 2 of *Cyp1a2*, and exhibited neonatal lethality associated with severe respiratory distress symptom, that ultimately was bred out yielding a mouse that was phenotypically indistinguishable from a wild-type mouse. Another *Cyp1a2*($-/-$) mouse model was created by targeted disruption of the exons 2–5 of *Cyp1a2* (Liang et al., 1996) (Table 1). The human and rodent CYP1A enzymes do exhibit some difference in metabolic capacity and substrate selectivity (Bogaards et al., 2000), and thus extrapolation of rodent data to humans is prone to error. Therefore, *CYP1A1_1A2* humanized mice were generated by crossing the *Cyp1a1*($-/-$), *Cyp1a2*($-/-$) or

Cyp1a1/1a2 (−/−) mice with the *CYP1A1_1A2* (+/+) mice, which the latter were developed by incorporating a bacterial artificial chromosomes (BAC) containing human *CYP1A1* and *CYP1A2* into mouse genome (Jiang et al., 2005; Dragin et al., 2007) (Table 1). These knockout and humanized mouse models have been successfully used for studying the role of CYP1A1/1A2 in the toxicology of a number of environmental carcinogens including polycyclic aromatic hydrocarbons and heterocyclic aromatic amines/amides (Uno et al., 2004; Cheung et al., 2005a; Uno et al., 2006), and in the pharmacokinetics of many drugs including caffeine and theophylline (Buters et al., 1996; Derkenne et al., 2005).

CYP2A6/Cyp2a5

Human CYP2A6 and the mouse orthologue Cyp2a5 share many common substrates (e.g., coumarin, nicotine and cotinine). To assess the role of CYP2A6 in drug metabolism in vivo, a *CYP2A6* (+/+) mouse model with liver-specific expression of human *CYP2A6* was generated using the *CYP2A6* cDNA, driven by a mouse liver-specific transthyretin promoter/enhancer (Zhang et al., 2005) (Table 1). More recently, a *Cyp2a5* (−/−) mouse model, in which the exon 9 of *Cyp2a5* was disrupted with a *tk-neo*-bpA cassette, was developed on the C57BL/6J background (Zhou et al., 2009) (Table 1). *CYP2A6* transgenic and *Cyp2a5* knockout mice, showing no apparent developmental defects with normal viability and fertility, were used to establish that CYP2A6/Cyp2a5 is involved in the clearance of various drugs including coumarin, nicotine and cotinine (Zhang et al., 2005; Zhou et al., 2009).

CYP2C19

CYP2C19 metabolizes many drugs including antidepressants and proton pump inhibitors and has many genetic variations in different populations. A *CYP2C18/2C19* transgenic mouse model was created using a *CYP2C18/2C19*-containing BAC clone (Lofgren et al., 2008; Lofgren et al., 2009) (Table 1). Gender difference in CYP2C18/C19 expression was observed at the mRNA level in transgenic mice, while only CYP2C19 protein was detectable in heterozygous *CYP2C18/19* (+/−) mice. In addition, *CYP2C18/19* (+/−) mouse liver microsomes showed higher catalytic capacity in metabolism of *R*-omeprazole and *S*-mephentoin. These findings suggest this mouse line as an appropriate model for assessing hormonal regulation of *CYP2C* in vivo, as well as CYP2C19-mediated drug metabolism (Lofgren et al., 2008; Lofgren et al., 2009).

CYP2D6

There is remarkable species difference in xenobiotic metabolism between mice and humans mediated by the CYP2D family of P450s. Thus a humanized *CYP2D6* (+/+) (Tg-*CYP2D6*) mouse model was generated with a λ phage genomic clone carrying wild-type *CYP2D6* gene (Corchero et al., 2001) (Table 1). Functional CYP2D6 protein was expressed in *CYP2D6* (+/+) mouse liver, intestine and kidney, resulting in an enhanced debrisoquine metabolism and clearance. Furthermore, urinary debrisoquine metabolic ratio (UMR) profiles (debrisoquine/4-hydroxydebrisoquine) in *CYP2D6* (+/+) versus wild-type mice closely resembled those in human CYP2D6 extensive metabolizers versus poor metabolizers, suggesting that *CYP2D6* (+/+) mice could be good animal models for studying CYP2D6 pharmacogenetics (Yu et al., 2004). Indeed, the *CYP2D6* (+/+) mice have been used to successfully define the role of CYP2D6 in pinoline metabolism (Jiang et al., 2009), as well as the effects of CYP2D6 status on harmaline pharmacokinetics and pharmacodynamics (Figure 1) (Wu et al., 2009). In contrast, when desipramine (DMI), another well-known CYP2D6 substrate, was administered to Tg-*CYP2D6* and wild-type control mice, the UMRs of DMI were similar in the two genotyped mice (Shen and Yu, 2009). The results indicate that mouse enzymes have notable contribution to DMI 2-hydroxylation, which is mainly catalyzed by CYP2D6 in humans. In addition, this mouse model was used to identify potential

endogenous substrates for CYP2D6 (Yu et al., 2003a; Yu et al., 2003b; Yu et al., 2003c; Yu et al., 2004), indicating its application to the assessment of CYP2D6-mediated reactions in vivo.

CYP2E1

To evaluate CYP2E1-mediated biotransformation in vivo, a *Cyp2e1* (−/−) mouse model was generated (Lee et al., 1996) (Table 1). The *Cyp2e1* (−/−) mice were less sensitive to acetaminophen (APAP) than wild-type mice, thus establishing an important role for Cyp2e1 in the activation of APAP to a hepatotoxic metabolite. Since species differences in CYP2E1 function and regulation were documented between mice and humans (Bogaards et al., 2000), a *CYP2E1*-humanized mouse model was developed by introducing a *CYP2E1*-containing BAC clone into the *Cyp2e1* (−/−) mice (Cheung et al., 2005b) (Table 1). Comparative studies revealed that *CYP2E1*-humanized and wild-type mice did differ in APAP toxicity. The *CYP2E1*-humanized mice should be useful in assessing the pharmacological and toxicological effects of CYP2E1 substrates.

CYP3A4

CYP3A4 is the most abundant P450 enzyme in human liver and gut, and contributes to the metabolism of many different drugs including benzodiazepines, HIV antivirals, immunomodulators and steroids. To develop a *CYP3A4* transgenic mouse model, a BAC clone containing complete *CYP3A4* gene was used (Granvil et al., 2003) (Table 1). In contrast to constitutive CYP3A4 expression in small intestines, CYP3A4 expression in transgenic mouse livers was subject to developmental and hormonal regulation (Figure 2) (Yu et al., 2005; Cheung et al., 2006). Pharmacokinetic studies in adult male mice with CYP3A4 expression only in the gut supported a role for CYP3A4 in first-pass metabolism of midazolam (Granvil et al., 2003). Furthermore, the impact of ketoconazole on midazolam pharmacokinetics in transgenic mice seems to be closer to that in humans (Table 2). This *CYP3A4*-transgenic mouse model may offer better assessment of intestinal CYP3A4-mediated drug metabolism and drug-drug interaction. Nevertheless, the expression of some mouse P450 enzymes (e.g., Cyp3a and Cyp2c) was altered in *CYP3A4*-transgenic mouse livers (Figure 2), which may be due to the change of epigenetic regulation (Li et al., 2009; Pan et al., 2009). The change in murine P450s might complicate interpretation of data obtained from these transgenic mouse models (Yu et al., 2005; Felmlee et al., 2008).

Another *CYP3A4*-transgenic mouse model was created with an ApoE promoter-HCR1 driven expression cassette containing *CYP3A4* cDNA (van Herwaarden et al., 2005) (Table 1). CYP3A4 was selectively expressed in transgenic mouse livers, leading to a reduced systemic exposure to intravenously-administered midazolam and cyclosporin A. A villin-*CYP3A4*-transgenic mouse model was also developed with selective CYP3A4 expression in small intestines (van Herwaarden et al., 2007). Furthermore, *Cyp3a* (−/−) mice lacking all functional murine *Cyp3a* genes were generated and bred with the ApoE- and villin-*CYP3A4*-transgenic mice to produce liver (*Cyp3a*−/−A) and intestine (*Cyp3a*−/−V) *CYP3A4*-humanized mice, respectively (van Herwaarden et al., 2007) (Table 1). Studies in the *CYP3A4*-genetically modified mice showed that docetaxel pharmacokinetics and toxicity were largely affected by Cyp3a. In addition, intestinal CYP3A4 was shown to determine the systemic exposure to orally administered triazolam, as well as the drug-drug interaction between ketoconazole and triazolam (van Waterschoot et al., 2009). These genetically modified mouse lines are useful models for understanding Cyp3a-mediated drug metabolism and drug-drug interaction. Nevertheless, change in mouse Cyp2c expression was observed in *Cyp3a* (−/−) mice (van Waterschoot et al., 2008). Since Cyp2c enzymes contribute to the metabolism of many Cyp3a substrates, it is necessary to be cautious when extrapolating data obtained from the related mouse models.

Cytochrome P450 reductase (CPR)

Disruption of CPR is anticipated to cause a global inactivation of metabolism catalyzed by all microsomal P450s because CPR is the sole electron donor for microsomal P450s. Indeed, ubiquitous disruption of *Cpr* led to embryonic lethality (Shen et al., 2002; Otto et al., 2003). The *Cre-loxP* approach was thus utilized to generate conditional knockout mouse lines lacking *Cpr* expression in livers (Gu et al., 2003; Henderson et al., 2003a; Wu et al., 2003) (Table 1). Other tissue-specific *Cpr* ($-/-$) mouse models were also created for intestine (Finn et al., 2007; Zhang et al., 2009), lung (Weng et al., 2007) and cardiomyocytes (Fang et al., 2008b), besides the global *Cpr*-low expression mice (Wu et al., 2005; Gu et al., 2007). These *Cpr* genetically modified mouse models were used to investigate the physiological role of *Cpr* (Gu et al., 2003; Wu et al., 2005; Mutch et al., 2006), and the importance of *Cpr* in the metabolic clearance or detoxification of many xenobiotic drugs (Pass et al., 2005; Henderson et al., 2006; Weng et al., 2007; Fang et al., 2008a; Fang et al., 2008b; Zhang et al., 2009).

Glutathione S-transferase pi (GSTP)

Human GSTP is one of the most important GST enzymes catalyzing the conjugation of glutathione (GSH) with electrophilic compounds and being responsible for cellular defense against endobiotic and xenobiotic toxins. A *GstP1/P2* ($-/-$) mouse model was created to study the function of GSTP in vivo (Henderson et al., 1998) (Table 1). Mice lacking *GstP* did not show any obvious phenotype, although some *GstP* null mice had higher body weights than controls. Studies using the *GstP* knockout mouse model have demonstrated that *GstP* could be an important metabolic determinant of 7,12-dimethylbenz anthracene (DMBA)-induced carcinogenesis (Henderson et al., 1998), APAP-induced hepatotoxicity (Henderson et al., 2000), and tobacco-induced endothelial dysfunction (Conklin et al., 2009).

Sulfotransferase 1E1 (SULT1E1)

Estrogen sulfotransferase (SULT1E1) catalyzes the sulfation of estrogens at the 3-hydroxyl position and contributes to estrogen homeostasis. A *Sult1e1* ($-/-$) mouse model was developed to examine the physiological role of *Sult1e1* (Qian et al., 2001) (Table 1). Male *Sult1e1* ($-/-$) mice showed age-dependent Leydig cell hypertrophy/hyperplasia and seminiferous tubule damage, which could be recapitulated by estradiol supplementation. Further studies in *Sult1e1* ($-/-$) female mice demonstrate that *Sult1e1* is a critical estrogen modulator in placenta and affects estrogen levels and placental thrombosis (Tong et al., 2005). This unique mouse model has not yet been used to study xenobiotic metabolism.

Uridine 5'-diphospho-glucuronosyltransferase 1 (UGT1)

A *UGT1*-transgenic mouse model was created using a BAC clone containing the entire human UGT1 locus (Chen et al., 2005) (Table 1). UGT1A proteins were differentially expressed in transgenic mouse liver and gastrointestinal tract. *UGT1* transgene was inducible by AhR, PXR and PPAR α ligands (Chen et al., 2005; Bonzo et al., 2007; Senekeo-Effenberger et al., 2007). The disruption of murine *Ugt1* gene cluster resulted in severe hyperbilirubinemia in newborn *Ugt1* ($-/-$) mice, which resembles Crigler-Najjar Type I disease in humans (Nguyen et al., 2008) (Table 1). Furthermore, humanized mice expressing the Gilbert's UGT1A1*28 allele or the wild-type UGT1A1*1 allele were developed on the *Ugt1*-null background to evaluate UGT1A-dependent drug clearance (Cai et al., 2010), as well as the role of UGT1 in developmental hyperbilirubinemia and CNS toxicity (Fujiwara et al., 2010) (Table 1). These UGT1 genetically modified mice would be useful models to investigate regulation and function of UGT1 enzymes.

Drug Transporter Genetically Modified Mouse Models

P-glycoprotein (P-gp/MDR1/ABCB1)

Human P-gp, expressed at high levels on the apical membrane of enterocytes, biliary surface of hepatocytes, and the luminal (apical) side of kidney proximal tubule cells, largely affects drug absorption, distribution and excretion. To investigate the role of P-gp in vivo, mice deficient in *Mdr1a* [*Mdr1a*(-/-)] (Schinkel et al., 1994) or both *Mdr1a* and *Mdr1b* [*Mdr1a/b*(-/-)] (Schinkel et al., 1997) were generated (Table 3). Comparative studies in wild-type versus knockout mice demonstrated the importance of *Mdr1a* or *Mdr1a/1b* in brain uptake, pharmacokinetics and toxicity of many drugs including ivermectin, vinblastine, dexamethasone, digoxin, cyclosporine A, ondansetron, loperamide and rhodamine (Schinkel et al., 1994; Schinkel et al., 1995; Schinkel et al., 1996; Schinkel et al., 1997). For instance, *Mdr1a*-knockout mice showed 100-fold higher sensitivity to the neurotoxic pesticide ivermectin (Figure 3) and 3-fold higher sensitivity to the carcinostatic drug vinblastine (Schinkel et al., 1994). The *Mdr1* knockout mice are useful animal models to evaluate the pharmacological and toxicological role of P-gp. Nevertheless, the obvious increase in *Mdr1b* expression in *Mdr1a*(-/-) mice (Schinkel et al., 1994) reminds one to be cautious when translating data from transgenic mice into humans.

Bile salt export pump (BSEP/ABCB11)

A *Bsep* knockout mouse model (Table 3) was developed after disruption of the coding region of Walker A of the N-terminal ATP-binding domain of *Bsep* (Wang et al., 2001). The *Bsep*(-/-) mice were viable and fertile, but showed growth retardation. In addition, the *Bsep*(-/-) mice exhibited various phenotypic characteristics including intrahepatic cholestasis, reduced secretion of cholic acid and output of bile salt, liver necrosis, high mortality, and change of the expression of many liver genes, supporting the critical role of *Bsep* in the transport of bile acid and lipid homeostasis (Wang et al., 2001; Wang et al., 2003). However, no study on drug disposition in this *Bsep*(-/-) mouse model has been reported.

Multidrug resistance-associated proteins (MRP/ABCC)

MRP1/ABCC1 is ubiquitously expressed in humans and has an important role in disposition of endobiotic and xenobiotic agents. Two different *Mrp1*(-/-) mouse lines (Table 3) were generated by targeted disruption of either the exons encoding the first ATP-binding domain of mouse *Mrp1* gene (Wijnholds et al., 1997) or part of the second putative ATP-binding domain (Lorico et al., 1997). Both mouse lines exhibited increased sensitivity to anticancer drugs such as etoposide and vincristine (Lorico et al., 1997; Wijnholds et al., 1997), indicating the critical role of *Mrp1* in drug disposition. Two *Mrp2*(-/-) mouse lines were also developed (Chu et al., 2006; Vlaming et al., 2006) (Table 3). Absence of *Mrp2* resulted in a decreased biliary excretion of *Mrp2* substrates and an increased systemic exposure to several drugs and carcinogens after oral administration, illustrating the critical role of *Mrp2* in disposition of these substances.

To examine the function of MRP3 in vivo, two *Mrp3* knockout mouse models (Table 3) were developed after targeted disruption of exons 6–8 (Belinsky et al., 2005) or exons 2–8 (Zelcer et al., 2006). The resulting *Mrp3*(-/-) mice have been employed to assess the function of *Mrp3* in disposition of endogenous bile acids and bilirubin glucuronide (Belinsky et al., 2005; Zelcer et al., 2006), as well as morphine, morphine-6-glucuronide and APAP (Manautou et al., 2005; Zelcer et al., 2006). A *Mrp4* knockout mouse model (Table 3) was also generated by disruption of exon 27, and utilized to evaluate the role of *Mrp4* in renal elimination and brain penetration of topotecan (Leggas et al., 2004). The *Mrp4*(-/-) mouse model was further employed to demonstrate the contribution of *Mrp4* to renal

elimination of antiviral drugs (e.g., adefovir and tenofovir) (Imaoka et al., 2007), and cytotoxicity and tissue distribution of 9-(2-(phosphonomethoxy)ethyl)-adenine (Takenaka et al., 2007).

Breast cancer resistance protein (BCRP/ABCG2)

BCRP/ABCG2 plays an important role in disposition of many xenobiotic drugs (e.g., mitoxantrone, doxorubicin and topotecan) and metabolites (e.g., estrone-3-sulfate and estradiol-17- β -D-glucuronide). Two *Bcrp1* ($-/-$) mouse models (Table 3) were created to investigate the physiological and pharmacological function of BCRP/ABCG2 (Jonker et al., 2002; Zhou et al., 2002). Studies using *Bcrp1* ($-/-$) mouse models have revealed a critical role for Bcrp1 in protection of hematopoietic stem cells from toxins (Zhou et al., 2002), and in prevention of diet-dependent phototoxicity (Jonker et al., 2002). In addition, the *Bcrp1* ($-/-$) mice were used to demonstrate the effects of Bcrp1 expression on absorption, distribution, and elimination of dietary carcinogens (e.g., PhIP) and medications (e.g., nitrofurantoin) (van Herwaarden et al., 2003; Breedveld et al., 2005; Jonker et al., 2005; Merino et al., 2005; Merino et al., 2006). The *Bcrp1* ($-/-$) mice may serve as unique animal models for understanding the disposition of drugs transported by BCRP.

Organic solute transporter α (OST α)

OST α and OST β are coexpressed on the intestinal basolateral membrane, forming a functional heteromer to transport many organic compounds such as taurocholate, digoxin and prostaglandin E1. An *Osta* knockout mouse model (Table 3) was developed to evaluate the function of the Osta-Ost β heteromeric transporter in intestinal bile acid reabsorption after deletion of the proximal promoter and exons 1 and 2 of murine *Osta* gene (Rao et al., 2008). The transileal transport study using the everted gut sac prepared from *Osta* ($-/-$) mice demonstrated that Osta-Ost β heteromer is critical for intestinal transport of bile acids.

Peptide transporter 2 (PEPT2/SLC15A2)

A constitutive *Pept2*-deficient mouse model and a conditional *Pept2* knockout mouse model (Table 3) were developed to examine the physiological and pharmacological role of PEPT2 (Rubio-Aliaga et al., 2003; Shen et al., 2003). Comparative studies in knockout versus wild-type mice demonstrated a critical role for PEPT2 in uptake of dipeptides (Rubio-Aliaga et al., 2003; Shen et al., 2003). Further studies revealed that renal PEPT2 was fully responsible for reabsorption of cefadroxil in kidney, and choroid plexus PEPT2 limited brain penetration of cefadroxil (Shen et al., 2007), indicating that the *Pept2* knockout mouse model could be useful in the development of peptidomimetic drugs.

Organic cation transporters (OCT/SLC22A)

A number of *Oct*-knockout mouse models (Table 3) were developed to examine the physiological and pharmacological functions of OCT transporters (Jonker et al., 2001; Zwart et al., 2001; Jonker et al., 2003). Studies in the *Oct1* ($-/-$) mice demonstrated an important role for Oct1/Slc22a1 in the distribution and excretion of tetraethylammonium, as well as hepatic accumulation of neurotoxin 1-methyl-4-phenylpyridium and anticancer drug metaiodobenzylguanidine (Jonker et al., 2001). Studies in *Oct2* single knockout and *Oct1/2* double knockout mice indicated that Oct1 and Oct2/Slc22a2 together were critical for renal secretion of organic cations (Jonker et al., 2003). The mouse homolog of *OCT3* was also disrupted to generate an *Orct3* ($-/-$) mouse line, which was used to reveal a critical role for Oct3/Slc22a3 in the accumulation of 1-methyl-4-phenylpyridium in the heart (Zwart et al., 2001).

Organic anion transporters (OAT/SLC22A)

OAT/SLC22 transporters are located on the barrier epithelia of diverse tissues such as the proximal tubule of kidney and choroid plexus of brain, where they mediate the absorption and excretion of many organic anions (e.g., β -lactam antibiotics). Disruption of murine *Oat1/Slc22a6* or *Oat3/Slc22a8* did not alter the fertility of the resulting gene knockout mice (Sweet et al., 2002; Eraly et al., 2006) (Table 3). Studies with *Oat1*($-/-$) mice demonstrated a critical role for Oat1 in the renal excretion of organic anions and drugs (Eraly et al., 2006). The *Oat3*($-/-$) mice were utilized to investigate the contribution of Oat3 to renal and brain uptake of several drugs including *para*-aminohippurate and estrone sulfate taurocholate (Sweet et al., 2002; Sykes et al., 2004).

Organic anion transporting polypeptides (OATP/SLCO)

To investigate the role of Oatp1b2 in hepatic uptake of xenobiotic drugs, *Oatp1b2*($-/-$) mouse models (Table 3) were developed by the targeted disruption of exons 10–12 (Zaher et al., 2008) or exon 3 of *Oatp1b2/Slco1b2* (Lu et al., 2008). The *Oatp1b2*($-/-$) mice were used to demonstrate an important role for Oatp1b2 in the hepatic uptake, systemic disposition and toxicity of many xenobiotic drugs including phalloidin, microcystin-LR, pravastatin, rifampicin and lovastatin (Chen et al., 2008; Lu et al., 2008; Zaher et al., 2008). Recently, a human *OATP1B1*-transgenic mouse model with liver-specific OATP1B1 expression (Table 3) was generated and found to have significantly higher hepatic accumulation of methotrexate than wild-type control mouse (van de Steeg et al., 2009). These genetically modified mouse models should be of great value for defining the role of OATP in drug disposition.

Multidrug and toxin extrusion 1 (MATE1/SLC47A1)

MATE1/SLC47A1 is a proton-dependent transporter mainly expressed on the luminal side of renal proximal tubules and bile canaliculi, and mediates the excretion of many exogenous or endogenous organic cations. Targeted disruption of murine *Mate1/Slc47a1* did not alter fertility of the resultant knockout mice (Tsuda et al., 2009) (Table 3). However, lack of Mate1 expression caused a sharp decrease in renal clearance of metformin and a considerable increase in systemic exposure to metformin, indicating an important role for Mate1 in metformin disposition.

Xenobiotic Receptor Genetically Modified Mouse Models

Aryl hydrocarbon receptor (AHR)

A number of *Ahr* knockout mouse models (Table 4) were developed independently through disruption of exon 1 or exon 2 (Fernandez-Salguero et al., 1995; Schmidt et al., 1996; Mimura et al., 1997). These *Ahr*-null mouse strains showed some physiological changes including lower growth rate, decreased fertility, and/or liver deficiency. Studies in *Ahr*($-/-$) versus wild-type mice demonstrated a crucial role for Ahr in induction of Cyp1a enzymes, dioxin-induced toxicity and carcinogenesis. In addition, an *AHR*-humanized mouse model (Table 4), in which murine *Ahr* was replaced by human *AHR* after homologous recombination, was generated to investigate species difference in dioxin toxicity (Moriguchi et al., 2003). Compared with wild-type *Ahr*($+/+$) mice, the *AHR*-humanized mice indeed exhibited a different induction profile following the treatment of AHR ligands. These genetically modified mice are useful models for predicting the risk of xenobiotic drugs in humans.

Pregnane X receptor (PXR/NR1I2)

PXR regulates the expression of many enzymes (e.g., CYP3A4 and UGT1A) and transporters (e.g., ABCB1) important in drug metabolism and disposition. Some *Pxr* (−/−) mouse models (Table 4) were developed to study its physiological and pharmacological role after targeted disruption of exons 2–3 or exon 1 (Xie et al., 2000; Staudinger et al., 2001). Induction of various drug-metabolizing enzymes and transporters by PXR ligands was abolished in mice lacking *Pxr* expression (Xie et al., 2000; Staudinger et al., 2001). In addition, human PXR-transgenic mice were generated using *PXR* cDNA or a BAC clone, and then crossbred with *Pxr*-null mice to produce *PXR*-humanized mouse models (Xie et al., 2000; Ma et al., 2007) (Table 4). Differential regulation of drug metabolism by human PXR and mouse *Pxr* was demonstrated by studies in wild-type versus *PXR*-humanized mice where they exhibited differences in ligand specificity (Figure 4). These findings indicate that *PXR*-humanized and *Pxr*-knockout mice are unique animal models to study PXR ligands and PXR-mediated drug-drug interaction.

Constitutive androstane receptor (CAR/NR1I3)

CAR is an important xenobiotic receptor regulating the expression of various enzymes (e.g., CYP2B6, UGT1A1 and GSTA1) and transporters (e.g., ABCC2). A *Car* (−/−) mouse model was developed to study the function of CAR in vivo (Wei et al., 2000) (Table 4). The absence of Cyp2b10 induction by *Car* activators (e.g., phenobarbital) in *Car* (−/−) mice supports the essential role for CAR in the regulation of Cyp2b10 expression (Wei et al., 2000). The *Car* (−/−) mice were resistant to APAP hepatotoxicity, indicating CAR as a modulator in APAP-induced toxicity (Zhang et al., 2002). A transgenic mouse model [VP-CAR(+/+)] with human CAR expression was also developed (Saini et al., 2004) (Table 4), which showed higher hepatic sulfation activity toward lithocholic acid (LCA) and increased resistance to LCA-induced hepatotoxicity, compared to wild-type mice (Saini et al., 2004). Another *CAR*-transgenic mouse model was created using human *CAR* cDNA, and crossbred with *Car* (−/−) mice to produce a *CAR*-humanized mouse model (Huang et al., 2004) (Table 4). Interestingly, meclizine was revealed as an agonist for mouse *Car* and an inverse agonist for human CAR by studies in wild-type, *Car* (−/−) and *CAR*-humanized mice, illustrating the potential application of these mouse models to preclinical assessment of CAR-mediated regulation of drug metabolism.

Peroxisome proliferator-activated receptor α (PPAR α /NR1C1)

PPAR α regulates CYP4A expression and provokes peroxisome proliferation and hepatocarcinogenesis in rodents. A *Ppara* (−/−) mouse model was developed after targeted disruption of exon 8 of murine *Ppara* (Lee et al., 1995) (Table 4). The absence of peroxisome proliferation and Cyp4a induction in *Ppara* (−/−) mice treated with *Ppara* ligands (e.g., clofibrate and Wy-14,643) demonstrated a critical role for PPAR α in peroxisome proliferation and regulation of target genes (Lee et al., 1995; Gonzalez et al., 1998). In addition, a *PPAR α* -humanized mouse model (Table 4) was generated to investigate the species difference in PPAR α functionality (Cheung et al., 2004). Despite peroxisome proliferator induced hypolipidemia and many enzymes in fatty acid oxidation, synthesis, transport in both wild-type and *PPAR α* -humanized mice, the increase in peroxisome and induction of cell cycle genes, hepatocellular proliferation and hepatomegaly were not observed in *PPAR α* -humanized mice but only in wild-type mice (Cheung et al., 2004; Morimura et al., 2006; Foreman et al., 2009) (Table 5). Thus the *PPAR α* -humanized mice may be a better animal model to predict risk of peroxisome proliferators (e.g., lipid lowering fibrate drugs) in humans.

Retinoid X receptor α (RXR α /NR2B1)

The RXR α is a heterodimer partner for PXR, CAR and PPAR α regulating liver functions. The conventional *Rxra*($-/-$) mice (Table 4) developed by targeted disruption of exon 3 or exon 4 of *RXRA/NR2B1* showed embryonic lethality, which was probably due to cardiac or placental developmental defects (Kastner et al., 1994; Sucov et al., 1994). Therefore, a *Rxra*($-/-$)_{hep} mouse model (Table 4), in which RXR α is conditionally and selectively disrupted in hepatocytes, was developed for investigation of the physiological functions of RXR α (Wan et al., 2000). While no gross liver abnormalities were found in the *Rxra*($-/-$)_{hep} mice, many hepatic metabolic pathways mediated by heteromerization between RXR α and xenobiotic receptors (e.g., PPAR α , CAR and PXR) were changed (Wan et al., 2000; Wu et al., 2004; Dai et al., 2005). The *Rxra*($-/-$)_{hep} mouse line is an unparalleled animal model for studying the function of RXR α in the homeostasis of cholesterol, fatty acids, bile acids and steroids, as well as in drug metabolism and disposition.

Conclusions

Genetically modified mice with lost (knockout) or gained (transgenic) function are unique and valuable additions to in vitro and wild-type animal models for preclinical investigations. Studies with these mouse models help better understand the in vivo role of specific drug-metabolizing enzyme, transporter or xenobiotic receptor in drug metabolism and pharmacokinetics, and consequent effects on drug efficacy or toxicity. Development of humanized mouse models is of particular interest, which may ultimately overcome species difference that impairs accurate prediction of drug metabolism and disposition in humans. However, whether the level of transgene expression in mice could be translated into humans is undefined in many cases. In addition, the disruption of target genes or introduction of genes of interest can sometimes lead to changes in the expression of other related genes in the genetically modified mice. Mouse genetic backgrounds could also affect the results. One should not overlook the limitations when studying drug metabolism and pharmacokinetics with such genetically modified mouse models.

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References

- Belinsky MG, Dawson PA, Shchaveleva I, Bain LJ, Wang R, Ling V, Chen ZS, Grinberg A, Westphal H, Klein-Szanto A, Lerro A, Kruh GD. Analysis of the in vivo functions of Mrp3. *Mol Pharmacol* 2005;68:160–168. [PubMed: 15814571]
- Bogaards JJ, Bertrand M, Jackson P, Oudshoorn MJ, Weaver RJ, van Bladeren PJ, Walther B. Determining the best animal model for human cytochrome P450 activities: a comparison of mouse, rat, rabbit, dog, micropig, monkey and man. *Xenobiotica* 2000;30:1131–1152. [PubMed: 11307970]
- Bonzo JA, Belanger A, Tukey RH. The role of chrysin and the ah receptor in induction of the human UGT1A1 gene in vitro and in transgenic UGT1 mice. *Hepatology* 2007;45:349–360. [PubMed: 17256720]
- Breedveld P, Pluim D, Cipriani G, Wielinga P, van Tellingen O, Schinkel AH, Schellens JH. The effect of Bcrp1 (Abcg2) on the in vivo pharmacokinetics and brain penetration of imatinib mesylate (Gleevec): implications for the use of breast cancer resistance protein and P-glycoprotein inhibitors to enable the brain penetration of imatinib in patients. *Cancer Res* 2005;65:2577–2582. [PubMed: 15805252]
- Buters JT, Sakai S, Richter T, Pineau T, Alexander DL, Savas U, Doehmer J, Ward JM, Jefcoate CR, Gonzalez FJ. Cytochrome P450 CYP1B1 determines susceptibility to 7, 12-

- dimethylbenz[a]anthracene-induced lymphomas. *Proc Natl Acad Sci U S A* 1999;96:1977–1982. [PubMed: 10051580]
- Buters JT, Tang BK, Pineau T, Gelboin HV, Kimura S, Gonzalez FJ. Role of CYP1A2 in caffeine pharmacokinetics and metabolism: studies using mice deficient in CYP1A2. *Pharmacogenetics* 1996;6:291–296. [PubMed: 8873215]
- Cai H, Nguyen N, Peterkin V, Yang YS, Hotz K, La Placa DB, Chen S, Tukey RH, Stevens JC. A humanized UGT1 mouse model expressing the UGT1A1*28 allele for assessing drug clearance by UGT1A1-dependent glucuronidation. *Drug Metab Dispos* 2010;38:879–886. [PubMed: 20124398]
- Chen C, Stock JL, Liu X, Shi J, Van Deusen JW, DiMattia DA, Dullea RG, de Morais SM. Utility of a novel Oatp1b2 knockout mouse model for evaluating the role of Oatp1b2 in the hepatic uptake of model compounds. *Drug Metab Dispos* 2008;36:1840–1845. [PubMed: 18556442]
- Chen S, Beaton D, Nguyen N, Senekeo-Effenberger K, Brace-Sinnokrak E, Argikar U, Rimmel RP, Trotter J, Barbier O, Ritter JK, Tukey RH. Tissue-specific, inducible, and hormonal control of the human UDP-glucuronosyltransferase-1 (UGT1) locus. *J Biol Chem* 2005;280:37547–37557. [PubMed: 16155002]
- Cheung C, Akiyama TE, Ward JM, Nicol CJ, Feigenbaum L, Vinson C, Gonzalez FJ. Diminished hepatocellular proliferation in mice humanized for the nuclear receptor peroxisome proliferator-activated receptor alpha. *Cancer Res* 2004;64:3849–3854. [PubMed: 15172993]
- Cheung C, Ma X, Krausz KW, Kimura S, Feigenbaum L, Dalton TP, Nebert DW, Idle JR, Gonzalez FJ. Differential metabolism of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in mice humanized for CYP1A1 and CYP1A2. *Chem Res Toxicol* 2005a;18:1471–1478. [PubMed: 16167840]
- Cheung C, Yu AM, Chen CS, Krausz KW, Byrd LG, Feigenbaum L, Edwards RJ, Waxman DJ, Gonzalez FJ. Growth hormone determines sexual dimorphism of hepatic cytochrome P450 3A4 expression in transgenic mice. *J Pharmacol Exp Ther* 2006;316:1328–1334. [PubMed: 16291874]
- Cheung C, Yu AM, Ward JM, Krausz KW, Akiyama TE, Feigenbaum L, Gonzalez FJ. The cyp2e1-humanized transgenic mouse: role of cyp2e1 in acetaminophen hepatotoxicity. *Drug Metab Dispos* 2005b;33:449–457. [PubMed: 15576447]
- Chu XY, Strauss JR, Mariano MA, Li J, Newton DJ, Cai X, Wang RW, Yabut J, Hartley DP, Evans DC, Evers R. Characterization of mice lacking the multidrug resistance protein MRP2 (ABCC2). *J Pharmacol Exp Ther* 2006;317:579–589. [PubMed: 16421286]
- Conklin DJ, Haberkzettel P, Prough RA, Bhatnagar A. Glutathione-S-transferase P protects against endothelial dysfunction induced by exposure to tobacco smoke. *Am J Physiol Heart Circ Physiol* 2009;296:H1586–H1597. [PubMed: 19270193]
- Corchero J, Granvil CP, Akiyama TE, Hayhurst GP, Pimprale S, Feigenbaum L, Idle JR, Gonzalez FJ. The CYP2D6 humanized mouse: effect of the human CYP2D6 transgene and HNF4alpha on the disposition of debrisoquine in the mouse. *Mol Pharmacol* 2001;60:1260–1267. [PubMed: 11723233]
- Dai G, Chou N, He L, Gyamfi MA, Mendy AJ, Slitt AL, Klaassen CD, Wan YJ. Retinoid X receptor alpha Regulates the expression of glutathione s-transferase genes and modulates acetaminophen-glutathione conjugation in mouse liver. *Mol Pharmacol* 2005;68:1590–1596. [PubMed: 16157696]
- Dai G, Wan YJ. Animal models of xenobiotic receptors. *Curr Drug Metab* 2005;6:341–355. [PubMed: 16101573]
- Dalton TP, Dieter MZ, Matlib RS, Childs NL, Shertzer HG, Genter MB, Nebert DW. Targeted knockout of Cyp1a1 gene does not alter hepatic constitutive expression of other genes in the mouse [Ah] battery. *Biochem Biophys Res Commun* 2000;267:184–189. [PubMed: 10623596]
- Derkenne S, Curran CP, Shertzer HG, Dalton TP, Dragin N, Nebert DW. Theophylline pharmacokinetics: comparison of Cyp1a1(–/–) and Cyp1a2(–/–) knockout mice, humanized hCYP1A1_1A2 knock-in mice lacking either the mouse Cyp1a1 or Cyp1a2 gene, and Cyp1(+/-) wild-type mice. *Pharmacogenet Genomics* 2005;15:503–511. [PubMed: 15970798]
- Dragin N, Uno S, Wang B, Dalton TP, Nebert DW. Generation of 'humanized' hCYP1A1_1A2_Cyp1a1/1a2(–/–) mouse line. *Biochem Biophys Res Commun* 2007;359:635–642. [PubMed: 17560947]

- Eraly SA, Vallon V, Vaughn DA, Gangoiti JA, Richter K, Nagle M, Monte JC, Rieg T, Truong DM, Long JM, Barshop BA, Kaler G, Nigam SK. Decreased renal organic anion secretion and plasma accumulation of endogenous organic anions in OAT1 knock-out mice. *J Biol Chem* 2006;281:5072–5083. [PubMed: 16354673]
- Fang C, Behr M, Xie F, Lu S, Doret M, Luo H, Yang W, Aldous K, Ding X, Gu J. Mechanism of chloroform-induced renal toxicity: non-involvement of hepatic cytochrome P450-dependent metabolism. *Toxicol Appl Pharmacol* 2008a;227:48–55. [PubMed: 18031782]
- Fang C, Gu J, Xie F, Behr M, Yang W, Abel ED, Ding X. Deletion of the NADPH-cytochrome P450 reductase gene in cardiomyocytes does not protect mice against doxorubicin-mediated acute cardiac toxicity. *Drug Metab Dispos* 2008b;36:1722–1728. [PubMed: 18463197]
- Felmlee MA, Lon HK, Gonzalez FJ, Yu AM. Cytochrome P450 expression and regulation in CYP3A4/CYP2D6 double transgenic humanized mice. *Drug Metab Dispos* 2008;36:435–441. [PubMed: 18048490]
- Fernandez-Salguero P, Pineau T, Hilbert DM, McPhail T, Lee SS, Kimura S, Nebert DW, Rudikoff S, Ward JM, Gonzalez FJ. Immune system impairment and hepatic fibrosis in mice lacking the dioxin-binding Ah receptor. *Science* 1995;268:722–726. [PubMed: 7732381]
- Finn RD, McLaren AW, Carrie D, Henderson CJ, Wolf CR. Conditional deletion of cytochrome P450 oxidoreductase in the liver and gastrointestinal tract: a new model for studying the functions of the P450 system. *J Pharmacol Exp Ther* 2007;322:40–47. [PubMed: 17435107]
- Foreman JE, Chang SC, Ehresman DJ, Butenhoff JL, Anderson CR, Palkar PS, Kang BH, Gonzalez FJ, Peters JM. Differential hepatic effects of perfluorobutyrate mediated by mouse and human PPAR- α . *Toxicol Sci* 2009;110:204–211. [PubMed: 19359353]
- Fujiwara R, Nguyen N, Chen S, Tukey RH. Developmental hyperbilirubinemia and CNS toxicity in mice humanized with the UDP glucuronosyltransferase 1 (UGT1) locus. *Proc Natl Acad Sci U S A* 2010;107:5024–5029. [PubMed: 20194756]
- Gomez A, Ingelman-Sundberg M. Pharmacoeugenetics: its role in interindividual differences in drug response. *Clin Pharmacol Ther* 2009;85:426–430. [PubMed: 19242404]
- Gonzalez FJ. Role of gene knockout and transgenic mice in the study of xenobiotic metabolism. *Drug Metab Rev* 2003;35:319–335. [PubMed: 14705864]
- Gonzalez FJ, Kimura S. Study of P450 function using gene knockout and transgenic mice. *Arch Biochem Biophys* 2003;409:153–158. [PubMed: 12464254]
- Gonzalez FJ, Peters JM, Cattley RC. Mechanism of action of the nongenotoxic peroxisome proliferators: role of the peroxisome proliferator-activator receptor α . *J Natl Cancer Inst* 1998;90:1702–1709. [PubMed: 9827524]
- Gonzalez FJ, Yu AM. Cytochrome P450 and xenobiotic receptor humanized mice. *Annu Rev Pharmacol Toxicol* 2006;46:41–64. [PubMed: 16402898]
- Granvil CP, Yu AM, Elizondo G, Akiyama TE, Cheung C, Feigenbaum L, Krausz KW, Gonzalez FJ. Expression of the human CYP3A4 gene in the small intestine of transgenic mice: in vitro metabolism and pharmacokinetics of midazolam. *Drug Metab Dispos* 2003;31:548–558. [PubMed: 12695342]
- Gu J, Chen CS, Wei Y, Fang C, Xie F, Kannan K, Yang W, Waxman DJ, Ding X. A mouse model with liver-specific deletion and global suppression of the NADPH-cytochrome P450 reductase gene: characterization and utility for in vivo studies of cyclophosphamide disposition. *J Pharmacol Exp Ther* 2007;321:9–17. [PubMed: 17218484]
- Gu J, Weng Y, Zhang QY, Cui H, Behr M, Wu L, Yang W, Zhang L, Ding X. Liver-specific deletion of the NADPH-cytochrome P450 reductase gene: impact on plasma cholesterol homeostasis and the function and regulation of microsomal cytochrome P450 and heme oxygenase. *J Biol Chem* 2003;278:25895–25901. [PubMed: 12697746]
- Henderson CJ, Otto DM, Carrie D, Magnuson MA, McLaren AW, Rosewell I, Wolf CR. Inactivation of the hepatic cytochrome P450 system by conditional deletion of hepatic cytochrome P450 reductase. *J Biol Chem* 2003a;278:13480–13486. [PubMed: 12566435]
- Henderson CJ, Otto DM, McLaren AW, Carrie D, Wolf CR. Knockout mice in xenobiotic metabolism. *Drug Metab Rev* 2003b;35:385–392. [PubMed: 14705867]

- Henderson CJ, Pass GJ, Wolf CR. The hepatic cytochrome P450 reductase null mouse as a tool to identify a successful candidate entity. *Toxicol Lett* 2006;162:111–117. [PubMed: 16343823]
- Henderson CJ, Smith AG, Ure J, Brown K, Bacon EJ, Wolf CR. Increased skin tumorigenesis in mice lacking pi class glutathione S-transferases. *Proc Natl Acad Sci U S A* 1998;95:5275–5280. [PubMed: 9560266]
- Henderson CJ, Wolf CR. Transgenic analysis of human drug-metabolizing enzymes: preclinical drug development and toxicology. *Mol Interv* 2003;3:331–343. [PubMed: 14993465]
- Henderson CJ, Wolf CR, Kitteringham N, Powell H, Otto D, Park BK. Increased resistance to acetaminophen hepatotoxicity in mice lacking glutathione S-transferase Pi. *Proc Natl Acad Sci U S A* 2000;97:12741–12745. [PubMed: 11058152]
- Huang W, Zhang J, Moore DD. A traditional herbal medicine enhances bilirubin clearance by activating the nuclear receptor CAR. *J Clin Invest* 2004;113:137–143. [PubMed: 14702117]
- Imaoka T, Kusuvara H, Adachi M, Schuetz JD, Takeuchi K, Sugiyama Y. Functional involvement of multidrug resistance-associated protein 4 (MRP4/ABCC4) in the renal elimination of the antiviral drugs adefovir and tenofovir. *Mol Pharmacol* 2007;71:619–627. [PubMed: 17110501]
- Ingelman-Sundberg M, Sim SC, Gomez A, Rodriguez-Antona C. Influence of cytochrome P450 polymorphisms on drug therapies: pharmacogenetic, pharmacoeigenetic and clinical aspects. *Pharmacol Ther* 2007;116:496–526. [PubMed: 18001838]
- Jiang XL, Shen HW, Yu AM. Pinoline may be used as a probe for CYP2D6 activity. *Drug Metab Dispos* 2009;37:443–446. [PubMed: 19095720]
- Jiang Z, Dalton TP, Jin L, Wang B, Tsuneoka Y, Shertzer HG, Dekar R, Nebert DW. Toward the evaluation of function in genetic variability: characterizing human SNP frequencies and establishing BAC-transgenic mice carrying the human CYP1A1_CYP1A2 locus. *Hum Mutat* 2005;25:196–206. [PubMed: 15643613]
- Jonker JW, Buitelaar M, Wagenaar E, Van Der Valk MA, Scheffer GL, Scheper RJ, Plosch T, Kuipers F, Elferink RP, Rosing H, Beijnen JH, Schinkel AH. The breast cancer resistance protein protects against a major chlorophyll-derived dietary phototoxin and protoporphyria. *Proc Natl Acad Sci U S A* 2002;99:15649–15654. [PubMed: 12429862]
- Jonker JW, Merino G, Musters S, van Herwaarden AE, Bolscher E, Wagenaar E, Mesman E, Dale TC, Schinkel AH. The breast cancer resistance protein BCRP (ABCG2) concentrates drugs and carcinogenic xenotoxins into milk. *Nat Med* 2005;11:127–129. [PubMed: 15685169]
- Jonker JW, Wagenaar E, Mol CA, Buitelaar M, Koepsell H, Smit JW, Schinkel AH. Reduced hepatic uptake and intestinal excretion of organic cations in mice with a targeted disruption of the organic cation transporter 1 (Oct1 [Slc22a1]) gene. *Mol Cell Biol* 2001;21:5471–5477. [PubMed: 11463829]
- Jonker JW, Wagenaar E, Van Eijl S, Schinkel AH. Deficiency in the organic cation transporters 1 and 2 (Oct1/Oct2 [Slc22a1/Slc22a2]) in mice abolishes renal secretion of organic cations. *Mol Cell Biol* 2003;23:7902–7908. [PubMed: 14560032]
- Kastner P, Grondona JM, Mark M, Gansmuller A, LeMeur M, Decimo D, Vonesch JL, Dolle P, Chambon P. Genetic analysis of RXR alpha developmental function: convergence of RXR and RAR signaling pathways in heart and eye morphogenesis. *Cell* 1994;78:987–1003. [PubMed: 7923367]
- Kotegawa T, Laurijsens BE, Von Moltke LL, Cotreau MM, Perloff MD, Venkatakrishnan K, Warrington JS, Granda BW, Harmatz JS, Greenblatt DJ. In vitro, pharmacokinetic, and pharmacodynamic interactions of ketoconazole and midazolam in the rat. *J Pharmacol Exp Ther* 2002;302:1228–1237. [PubMed: 12183684]
- Lagas JS, Vlaming ML, Schinkel AH. Pharmacokinetic assessment of multiple ATP-binding cassette transporters: the power of combination knockout mice. *Mol Interv* 2009;9:136–145. [PubMed: 19592674]
- Lee SS, Buters JT, Pineau T, Fernandez-Salguero P, Gonzalez FJ. Role of CYP2E1 in the hepatotoxicity of acetaminophen. *J Biol Chem* 1996;271:12063–12067. [PubMed: 8662637]
- Lee SS, Pineau T, Drago J, Lee EJ, Owens JW, Kroetz DL, Fernandez-Salguero PM, Westphal H, Gonzalez FJ. Targeted disruption of the alpha isoform of the peroxisome proliferator-activated

- receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. *Mol Cell Biol* 1995;15:3012–3022. [PubMed: 7539101]
- Leggas M, Adachi M, Scheffer GL, Sun D, Wielinga P, Du G, Mercer KE, Zhuang Y, Panetta JC, Johnston B, Scheper RJ, Stewart CF, Schuetz JD. Mrp4 confers resistance to topotecan and protects the brain from chemotherapy. *Mol Cell Biol* 2004;24:7612–7621. [PubMed: 15314169]
- Li Y, Cui Y, Hart SN, Klaassen CD, Zhong XB. Dynamic patterns of histone methylation are associated with ontogenic expression of the Cyp3a genes during mouse liver maturation. *Mol Pharmacol* 2009;75:1171–1179. [PubMed: 19188337]
- Liang HC, Li H, McKinnon RA, Duffy JJ, Potter SS, Puga A, Nebert DW. Cyp1a2(–/–) null mutant mice develop normally but show deficient drug metabolism. *Proc Natl Acad Sci U S A* 1996;93:1671–1676. [PubMed: 8643688]
- Liggett SB. Genetically modified mouse models for pharmacogenomic research. *Nat Rev Genet* 2004;5:657–663. [PubMed: 15372087]
- Lofgren S, Baldwin RM, Carleros M, Terelius Y, Fransson-Steen R, Mwinyi J, Waxman DJ, Ingelman-Sundberg M. Regulation of human CYP2C18 and CYP2C19 in transgenic mice: influence of castration, testosterone, and growth hormone. *Drug Metab Dispos* 2009;37:1505–1512. [PubMed: 19339376]
- Lofgren S, Baldwin RM, Hiratsuka M, Lindqvist A, Carlberg A, Sim SC, Schulke M, Snait M, Edenro A, Fransson-Steen R, Terelius Y, Ingelman-Sundberg M. Generation of mice transgenic for human CYP2C18 and CYP2C19: characterization of the sexually dimorphic gene and enzyme expression. *Drug Metab Dispos* 2008;36:955–962. [PubMed: 18276835]
- Lorico A, Rappa G, Finch RA, Yang D, Flavell RA, Sartorelli AC. Disruption of the murine MRP (multidrug resistance protein) gene leads to increased sensitivity to etoposide (VP-16) and increased levels of glutathione. *Cancer Res* 1997;57:5238–5242. [PubMed: 9393741]
- Lu H, Choudhuri S, Ogura K, Csanaky IL, Lei X, Cheng X, Song PZ, Klaassen CD. Characterization of organic anion transporting polypeptide 1b2-null mice: essential role in hepatic uptake/toxicity of phalloidin and microcystin-LR. *Toxicol Sci* 2008;103:35–45. [PubMed: 18296417]
- Ma X, Shah Y, Cheung C, Guo GL, Feigenbaum L, Krausz KW, Idle JR, Gonzalez FJ. The PREgnane X receptor gene-humanized mouse: a model for investigating drug-drug interactions mediated by cytochromes P450 3A. *Drug Metab Dispos* 2007;35:194–200. [PubMed: 17093002]
- Manautou JE, de Waart DR, Kunne C, Zelcer N, Goedken M, Borst P, Elferink RO. Altered disposition of acetaminophen in mice with a disruption of the Mrp3 gene. *Hepatology* 2005;42:1091–1098. [PubMed: 16250050]
- Manis JP. Knock out, knock in, knock down--genetically manipulated mice and the Nobel Prize. *N Engl J Med* 2007;357:2426–2429. [PubMed: 18077807]
- Merino G, Alvarez AI, Pulido MM, Molina AJ, Schinkel AH, Prieto JG. Breast cancer resistance protein (BCRP/ABCG2) transports fluoroquinolone antibiotics and affects their oral availability, pharmacokinetics, and milk secretion. *Drug Metab Dispos* 2006;34:690–695. [PubMed: 16434544]
- Merino G, Jonker JW, Wagenaar E, van Herwaarden AE, Schinkel AH. The breast cancer resistance protein (BCRP/ABCG2) affects pharmacokinetics, hepatobiliary excretion, and milk secretion of the antibiotic nitrofurantoin. *Mol Pharmacol* 2005;67:1758–1764. [PubMed: 15709111]
- Mimura J, Yamashita K, Nakamura K, Morita M, Takagi TN, Nakao K, Ema M, Sogawa K, Yasuda M, Katsuki M, Fujii-Kuriyama Y. Loss of teratogenic response to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in mice lacking the Ah (dioxin) receptor. *Genes Cells* 1997;2:645–654. [PubMed: 9427285]
- Moriguchi T, Motohashi H, Hosoya T, Nakajima O, Takahashi S, Ohsako S, Aoki Y, Nishimura N, Tohyama C, Fujii-Kuriyama Y, Yamamoto M. Distinct response to dioxin in an arylhydrocarbon receptor (AHR)-humanized mouse. *Proc Natl Acad Sci U S A* 2003;100:5652–5657. [PubMed: 12730383]
- Morimura K, Cheung C, Ward JM, Reddy JK, Gonzalez FJ. Differential susceptibility of mice humanized for peroxisome proliferator-activated receptor alpha to Wy-14,643-induced liver tumorigenesis. *Carcinogenesis* 2006;27:1074–1080. [PubMed: 16377806]

- Muruganandan S, Sinal CJ. Mice as clinically relevant models for the study of cytochrome P450-dependent metabolism. *Clin Pharmacol Ther* 2008;83:818–828. [PubMed: 18388875]
- Mutch DM, Crespy V, Clough J, Henderson CJ, Lariani S, Mansourian R, Moulin J, Wolf CR, Williamson G. Hepatic cytochrome P-450 reductase-null mice show reduced transcriptional response to quercetin and reveal physiological homeostasis between jejunum and liver. *Am J Physiol Gastrointest Liver Physiol* 2006;291:G63–G72. [PubMed: 16455785]
- Nguyen N, Bonzo JA, Chen S, Chouinard S, Kelner MJ, Hardiman G, Belanger A, Tukey RH. Disruption of the *ugt1* locus in mice resembles human Crigler-Najjar type I disease. *J Biol Chem* 2008;283:7901–7911. [PubMed: 18180294]
- Otto DM, Henderson CJ, Carrie D, Davey M, Gundersen TE, Blomhoff R, Adams RH, Tickle C, Wolf CR. Identification of novel roles of the cytochrome p450 system in early embryogenesis: effects on vasculogenesis and retinoic Acid homeostasis. *Mol Cell Biol* 2003;23:6103–6116. [PubMed: 12917333]
- Ou Z, Huang M, Zhao L, Xie W. Use of transgenic mice in UDP-glucuronosyltransferase (UGT) studies. *Drug Metab Rev* 2010;42:119–127.
- Pan YZ, Gao W, Yu AM. MicroRNAs regulate CYP3A4 expression via direct and indirect targeting. *Drug Metab Dispos* 2009;37:2112–2117. [PubMed: 19581388]
- Pass GJ, Carrie D, Boylan M, Lorimore S, Wright E, Houston B, Henderson CJ, Wolf CR. Role of hepatic cytochrome p450s in the pharmacokinetics and toxicity of cyclophosphamide: studies with the hepatic cytochrome p450 reductase null mouse. *Cancer Res* 2005;65:4211–4217. [PubMed: 15899812]
- Pineau T, Fernandez-Salguero P, Lee SS, McPhail T, Ward JM, Gonzalez FJ. Neonatal lethality associated with respiratory distress in mice lacking cytochrome P450 1A2. *Proc Natl Acad Sci U S A* 1995;92:5134–5138. [PubMed: 7761462]
- Qian YM, Sun XJ, Tong MH, Li XP, Richa J, Song WC. Targeted disruption of the mouse estrogen sulfotransferase gene reveals a role of estrogen metabolism in intracrine and paracrine estrogen regulation. *Endocrinology* 2001;142:5342–5350. [PubMed: 11713234]
- Rao A, Haywood J, Craddock AL, Belinsky MG, Kruh GD, Dawson PA. The organic solute transporter alpha-beta, Ostalpha-Ostbeta, is essential for intestinal bile acid transport and homeostasis. *Proc Natl Acad Sci U S A* 2008;105:3891–3896. [PubMed: 18292224]
- Rubio-Aliaga I, Frey I, Boll M, Groneberg DA, Eichinger HM, Balling R, Daniel H. Targeted disruption of the peptide transporter *Pept2* gene in mice defines its physiological role in the kidney. *Mol Cell Biol* 2003;23:3247–3252. [PubMed: 12697824]
- Saini SP, Sonoda J, Xu L, Toma D, Uppal H, Mu Y, Ren S, Moore DD, Evans RM, Xie W. A novel constitutive androstane receptor-mediated and CYP3A-independent pathway of bile acid detoxification. *Mol Pharmacol* 2004;65:292–300. [PubMed: 14742670]
- Schinkel AH, Mayer U, Wagenaar E, Mol CA, van Deemter L, Smit JJ, van der Valk MA, Voordouw AC, Spits H, van Tellingen O, Zijlmans JM, Fibbe WE, Borst P. Normal viability and altered pharmacokinetics in mice lacking *mdr1*-type (drug-transporting) P-glycoproteins. *Proc Natl Acad Sci U S A* 1997;94:4028–4033. [PubMed: 9108099]
- Schinkel AH, Smit JJ, van Tellingen O, Beijnen JH, Wagenaar E, van Deemter L, Mol CA, van der Valk MA, Robanus-Maandag EC, te Riele HP, et al. Disruption of the mouse *mdr1a* P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. *Cell* 1994;77:491–502. [PubMed: 7910522]
- Schinkel AH, Wagenaar E, Mol CA, van Deemter L. P-glycoprotein in the blood-brain barrier of mice influences the brain penetration and pharmacological activity of many drugs. *J Clin Invest* 1996;97:2517–2524. [PubMed: 8647944]
- Schinkel AH, Wagenaar E, van Deemter L, Mol CA, Borst P. Absence of the *mdr1a* P-Glycoprotein in mice affects tissue distribution and pharmacokinetics of dexamethasone, digoxin, and cyclosporin A. *J Clin Invest* 1995;96:1698–1705. [PubMed: 7560060]
- Schmidt JV, Su GH, Reddy JK, Simon MC, Bradfield CA. Characterization of a murine *Ahr* null allele: involvement of the *Ah* receptor in hepatic growth and development. *Proc Natl Acad Sci U S A* 1996;93:6731–6736. [PubMed: 8692887]

- Seneko-Effenberger K, Chen S, Brace-Sinnokrak E, Bonzo JA, Yueh MF, Argikar U, Kaeding J, Trotter J, Rimmel RP, Ritter JK, Barbier O, Tukey RH. Expression of the human UGT1 locus in transgenic mice by 4-chloro-6-(2,3-xylydino)-2-pyrimidinylthioacetic acid (WY-14643) and implications on drug metabolism through peroxisome proliferator-activated receptor alpha activation. *Drug Metab Dispos* 2007;35:419–427. [PubMed: 17151188]
- Shen AL, O'Leary KA, Kasper CB. Association of multiple developmental defects and embryonic lethality with loss of microsomal NADPH-cytochrome P450 oxidoreductase. *J Biol Chem* 2002;277:6536–6541. [PubMed: 11742006]
- Shen H, Ocheltree SM, Hu Y, Keep RF, Smith DE. Impact of genetic knockout of PEPT2 on cefadroxil pharmacokinetics, renal tubular reabsorption, and brain penetration in mice. *Drug Metab Dispos* 2007;35:1209–1216. [PubMed: 17452417]
- Shen H, Smith DE, Keep RF, Xiang J, Brosius FC 3rd. Targeted disruption of the PEPT2 gene markedly reduces dipeptide uptake in choroid plexus. *J Biol Chem* 2003;278:4786–4791. [PubMed: 12473671]
- Shen HW, Yu AM. Difference in desipramine metabolic profile between wild-type and CYP2D6-humanized mice. *Drug Metab Lett* 2009;3:234–241. [PubMed: 19995332]
- Staudinger JL, Goodwin B, Jones SA, Hawkins-Brown D, MacKenzie KI, LaTour A, Liu Y, Klaassen CD, Brown KK, Reinhard J, Willson TM, Koller BH, Kliewer SA. The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity. *Proc Natl Acad Sci U S A* 2001;98:3369–3374. [PubMed: 11248085]
- Sucov HM, Dyson E, Gumeringer CL, Price J, Chien KR, Evans RM. RXR alpha mutant mice establish a genetic basis for vitamin A signaling in heart morphogenesis. *Genes Dev* 1994;8:1007–1018. [PubMed: 7926783]
- Sweet DH, Miller DS, Pritchard JB, Fujiwara Y, Beier DR, Nigam SK. Impaired organic anion transport in kidney and choroid plexus of organic anion transporter 3 (Oat3 (Slc22a8)) knockout mice. *J Biol Chem* 2002;277:26934–26943. [PubMed: 12011098]
- Sykes D, Sweet DH, Lowes S, Nigam SK, Pritchard JB, Miller DS. Organic anion transport in choroid plexus from wild-type and organic anion transporter 3 (Slc22a8)-null mice. *Am J Physiol Renal Physiol* 2004;286:F972–F978. [PubMed: 15075193]
- Takenaka K, Morgan JA, Scheffer GL, Adachi M, Stewart CF, Sun D, Leggas M, Ejendal KF, Hrycyna CA, Schuetz JD. Substrate overlap between Mrp4 and Abcg2/Bcrp affects purine analogue drug cytotoxicity and tissue distribution. *Cancer Res* 2007;67:6965–6972. [PubMed: 17638908]
- Tong MH, Jiang H, Liu P, Lawson JA, Brass LF, Song WC. Spontaneous fetal loss caused by placental thrombosis in estrogen sulfotransferase-deficient mice. *Nat Med* 2005;11:153–159. [PubMed: 15685171]
- Tsuda M, Terada T, Mizuno T, Katsura T, Shimakura J, Inui K. Targeted disruption of the multidrug and toxin extrusion 1 (mate1) gene in mice reduces renal secretion of metformin. *Mol Pharmacol* 2009;75:1280–1286. [PubMed: 19332510]
- Tsunoda SM, Velez RL, von Moltke LL, Greenblatt DJ. Differentiation of intestinal and hepatic cytochrome P450 3A activity with use of midazolam as an in vivo probe: effect of ketoconazole. *Clin Pharmacol Ther* 1999;66:461–471. [PubMed: 10579473]
- Uno S, Dalton TP, Dragin N, Curran CP, Derkenne S, Miller ML, Shertzer HG, Gonzalez FJ, Nebert DW. Oral benzo[a]pyrene in Cyp1 knockout mouse lines: CYP1A1 important in detoxication, CYP1B1 metabolism required for immune damage independent of total-body burden and clearance rate. *Mol Pharmacol* 2006;69:1103–1114. [PubMed: 16377763]
- Uno S, Dalton TP, Sinclair PR, Gorman N, Wang B, Smith AG, Miller ML, Shertzer HG, Nebert DW. Cyp1a1(–/–) male mice: protection against high-dose TCDD-induced lethality and wasting syndrome, and resistance to intrahepatocyte lipid accumulation and uroporphyrin. *Toxicol Appl Pharmacol* 2004;196:410–421. [PubMed: 15094312]
- van de Steeg E, van der Kruijsen CM, Wagenaar E, Burggraaff JE, Mesman E, Kenworthy KE, Schinkel AH. Methotrexate pharmacokinetics in transgenic mice with liver-specific expression of human organic anion-transporting polypeptide 1B1 (SLCO1B1). *Drug Metab Dispos* 2009;37:277–281. [PubMed: 19022939]

- van Herwaarden AE, Jonker JW, Wagenaar E, Brinkhuis RF, Schellens JH, Beijnen JH, Schinkel AH. The breast cancer resistance protein (Bcrp1/Abcg2) restricts exposure to the dietary carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine. *Cancer Res* 2003;63:6447–6452. [PubMed: 14559835]
- van Herwaarden AE, Smit JW, Sparidans RW, Wagenaar E, van der Kruijssen CM, Schellens JH, Beijnen JH, Schinkel AH. Midazolam and cyclosporin a metabolism in transgenic mice with liver-specific expression of human CYP3A4. *Drug Metab Dispos* 2005;33:892–895. [PubMed: 15845749]
- van Herwaarden AE, Wagenaar E, van der Kruijssen CM, van Waterschoot RA, Smit JW, Song JY, van der Valk MA, van Tellingen O, van der Hoorn JW, Rosing H, Beijnen JH, Schinkel AH. Knockout of cytochrome P450 3A yields new mouse models for understanding xenobiotic metabolism. *J Clin Invest* 2007;117:3583–3592. [PubMed: 17975676]
- van Waterschoot RA, Rooswinkel RW, Sparidans RW, van Herwaarden AE, Beijnen JH, Schinkel AH. Inhibition and stimulation of intestinal and hepatic CYP3A activity: studies in humanized CYP3A4 transgenic mice using triazolam. *Drug Metab Dispos* 2009;37:2305–2313. [PubMed: 19752211]
- van Waterschoot RA, van Herwaarden AE, Lagas JS, Sparidans RW, Wagenaar E, van der Kruijssen CM, Goldstein JA, Zeldin DC, Beijnen JH, Schinkel AH. Midazolam metabolism in cytochrome P450 3A knockout mice can be attributed to up-regulated CYP2C enzymes. *Mol Pharmacol* 2008;73:1029–1036. [PubMed: 18156313]
- Vlaming ML, Mohrmann K, Wagenaar E, de Waart DR, Elferink RP, Lagas JS, van Tellingen O, Vainchtein LD, Rosing H, Beijnen JH, Schellens JH, Schinkel AH. Carcinogen and anticancer drug transport by Mrp2 in vivo: studies using Mrp2 (Abcc2) knockout mice. *J Pharmacol Exp Ther* 2006;318:319–327. [PubMed: 16611851]
- Wan YJ, An D, Cai Y, Repa JJ, Hung-Po Chen T, Flores M, Postic C, Magnuson MA, Chen J, Chien KR, French S, Mangelsdorf DJ, Sucov HM. Hepatocyte-specific mutation establishes retinoid X receptor alpha as a heterodimeric integrator of multiple physiological processes in the liver. *Mol Cell Biol* 2000;20:4436–4444. [PubMed: 10825207]
- Wang R, Lam P, Liu L, Forrest D, Yousef IM, Mignault D, Phillips MJ, Ling V. Severe cholestasis induced by cholic acid feeding in knockout mice of sister of P-glycoprotein. *Hepatology* 2003;38:1489–1499. [PubMed: 14647060]
- Wang R, Salem M, Yousef IM, Tuchweber B, Lam P, Childs SJ, Helgason CD, Ackerley C, Phillips MJ, Ling V. Targeted inactivation of sister of P-glycoprotein gene (spgp) in mice results in nonprogressive but persistent intrahepatic cholestasis. *Proc Natl Acad Sci U S A* 2001;98:2011–2016. [PubMed: 11172067]
- Wei P, Zhang J, Egan-Hafley M, Liang S, Moore DD. The nuclear receptor CAR mediates specific xenobiotic induction of drug metabolism. *Nature* 2000;407:920–923. [PubMed: 11057673]
- Weng Y, Fang C, Turesky RJ, Behr M, Kaminsky LS, Ding X. Determination of the role of target tissue metabolism in lung carcinogenesis using conditional cytochrome P450 reductase-null mice. *Cancer Res* 2007;67:7825–7832. [PubMed: 17699788]
- Wijnholds J, Evers R, van Leusden MR, Mol CA, Zaman GJ, Mayer U, Beijnen JH, van der Valk M, Krimpenfort P, Borst P. Increased sensitivity to anticancer drugs and decreased inflammatory response in mice lacking the multidrug resistance-associated protein. *Nat Med* 1997;3:1275–1279. [PubMed: 9359705]
- Wu C, Jiang XL, Shen HW, Yu AM. Effects of CYP2D6 status on harmaline metabolism, pharmacokinetics and pharmacodynamics, and a pharmacogenetics-based pharmacokinetic model. *Biochem Pharmacol* 2009;78:617–624. [PubMed: 19445902]
- Wu L, Gu J, Cui H, Zhang QY, Behr M, Fang C, Weng Y, Kluetzman K, Swiatek PJ, Yang W, Kaminsky L, Ding X. Transgenic mice with a hypomorphic NADPH-cytochrome P450 reductase gene: effects on development, reproduction, and microsomal cytochrome P450. *J Pharmacol Exp Ther* 2005;312:35–43. [PubMed: 15328377]
- Wu L, Gu J, Weng Y, Kluetzman K, Swiatek P, Behr M, Zhang QY, Zhuo X, Xie Q, Ding X. Conditional knockout of the mouse NADPH-cytochrome p450 reductase gene. *Genesis* 2003;36:177–181. [PubMed: 12929087]

- Wu Y, Zhang X, Bardag-Gorce F, Robel RC, Aguilo J, Chen L, Zeng Y, Hwang K, French SW, Lu SC, Wan YJ. Retinoid X receptor alpha regulates glutathione homeostasis and xenobiotic detoxification processes in mouse liver. *Mol Pharmacol* 2004;65:550–557. [PubMed: 14978233]
- Xia CQ, Milton MN, Gan LS. Evaluation of drug-transporter interactions using in vitro and in vivo models. *Curr Drug Metab* 2007;8:341–363. [PubMed: 17504223]
- 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]
- Yu AM. Small interfering RNA in drug metabolism and transport. *Curr Drug Metab* 2007;8:700–708. [PubMed: 17979658]
- Yu AM. Role of microRNAs in the regulation of drug metabolism and disposition. *Expert Opin Drug Metab Toxicol* 2009;5:1513–1528. [PubMed: 19785514]
- Yu AM, Fukamachi K, Krausz KW, Cheung C, Gonzalez FJ. Potential role for human cytochrome P450 3A4 in estradiol homeostasis. *Endocrinology* 2005;146:2911–2919. [PubMed: 15817670]
- Yu AM, Granvil CP, Haining RL, Krausz KW, Corchero J, Kupfer A, Idle JR, Gonzalez FJ. The relative contribution of monoamine oxidase and cytochrome p450 isozymes to the metabolic deamination of the trace amine tryptamine. *J Pharmacol Exp Ther* 2003a;304:539–546. [PubMed: 12538805]
- Yu AM, Idle JR, Byrd LG, Krausz KW, Kupfer A, Gonzalez FJ. Regeneration of serotonin from 5-methoxytryptamine by polymorphic human CYP2D6. *Pharmacogenetics* 2003b;13:173–181. [PubMed: 12618595]
- Yu AM, Idle JR, Gonzalez FJ. Polymorphic cytochrome P450 2D6: humanized mouse model and endogenous substrates. *Drug Metab Rev* 2004;36:243–277. [PubMed: 15237854]
- Yu AM, Idle JR, Herraiz T, Kupfer A, Gonzalez FJ. Screening for endogenous substrates reveals that CYP2D6 is a 5-methoxyindolethylamine O-demethylase. *Pharmacogenetics* 2003c;13:307–319. [PubMed: 12777961]
- Zaher H, zu Schwabedissen HE, Tirona RG, Cox ML, Obert LA, Agrawal N, Palandra J, Stock JL, Kim RB, Ware JA. Targeted disruption of murine organic anion-transporting polypeptide 1b2 (Oatp1b2/Slco1b2) significantly alters disposition of prototypical drug substrates pravastatin and rifampin. *Mol Pharmacol* 2008;74:320–329. [PubMed: 18413659]
- Zelcer N, van de Wetering K, de Waart R, Scheffer GL, Marschall HU, Wielinga PR, Kuil A, Kunne C, Smith A, van der Valk M, Wijnholds J, Elferink RO, Borst P. Mice lacking Mrp3 (Abcc3) have normal bile salt transport, but altered hepatic transport of endogenous glucuronides. *J Hepatol* 2006;44:768–775. [PubMed: 16225954]
- 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]
- Zhang QY, Fang C, Zhang J, Dunbar D, Kaminsky L, Ding X. An intestinal epithelium-specific cytochrome P450 (P450) reductase-knockout mouse model: direct evidence for a role of intestinal p450s in first-pass clearance of oral nifedipine. *Drug Metab Dispos* 2009;37:651–657. [PubMed: 19056912]
- Zhang QY, Gu J, Su T, Cui H, Zhang X, D'Agostino J, Zhuo X, Yang W, Swiatek PJ, Ding X. Generation and characterization of a transgenic mouse model with hepatic expression of human CYP2A6. *Biochem Biophys Res Commun* 2005;338:318–324. [PubMed: 16126166]
- Zhou S, Morris JJ, Barnes Y, Lan L, Schuetz JD, Sorrentino BP. Bcrp1 gene expression is required for normal numbers of side population stem cells in mice, and confers relative protection to mitoxantrone in hematopoietic cells in vivo. *Proc Natl Acad Sci U S A* 2002;99:12339–12344. [PubMed: 12218177]
- Zhou X, Zhuo X, Xie F, Kluetzman K, Shu YZ, Humphreys WG, Ding X. Role of CYP2A5 in the clearance of nicotine and cotinine: insights from studies on a Cyp2a5-null mouse model. *J Pharmacol Exp Ther* 2009;332:578–587. [PubMed: 19923441]
- Zwart R, Verhaagh S, Buitelaar M, Popp-Snijders C, Barlow DP. Impaired activity of the extraneuronal monoamine transporter system known as uptake-2 in Orct3/Slc22a3-deficient mice. *Mol Cell Biol* 2001;21:4188–4196. [PubMed: 11390648]

Figure 1.

Studies with wild-type and Tg-CYP2D6 mice revealed a significant ($P < 0.05$; two-way ANOVA) impact of CYP2D6 status on (a) harmaline pharmacokinetics (5 and 15 mg/kg, i.p.; $N = 3$ at each time point), and (b) drug effects on mouse marble-burying behavior (0–10 mg/kg, i.p.; $N = 14$ in each group) (Wu et al., 2009).

Figure 2.

Sexual dimorphism and developmental expression of human *CYP3A4* transgene, as well as murine P450s. (a) Expression of the *CYP3A4* transgene and murine Cyp3a and Cyp2b is dependent on gender and age [2–16 week (W)] in mouse livers (Yu et al., 2005). (b) Hepatic CYP3A4 and murine P450 expression profiles are changed in mice following growth hormone (GH) infusion, compared to the SHAM control (Cheung et al., 2006). HLM, human liver microsomes.

Figure 3. Sharp difference in ivermectin (p.o.) toxicity between *Mdr1a*-null and wild-type mice (Schinkel et al., 1994).

Figure 4.

Differential induction of CYP3A4/Cyp3a by pregnenolone-16 α -carbonitrile (PCN) and rifampicin (RIF) in wild-type and *PXR*-humanized mice (Ma et al., 2007). Mice treated with drug vehicle were used as control (CON).

Table 1

Drug-metabolizing enzyme genetically modified mouse models.

Gene	Category	Example applications	Reference
<i>Cyp1a1</i>	Knockout	Gene expression and function	(Dalton et al., 2000)
<i>Cyp1a2</i>	Knockout	Potential physiological role of <i>Cyp1a2</i> Metabolism of xenobiotics	(Pineau et al., 1995) (Liang et al., 1996)
<i>Cyp1a1/1a2</i> <i>CYP1A1/1A2</i>	Knockout Humanized	Assess the risk of environmental toxicant	(Dragin et al., 2007)
<i>Cyp1b1</i>	Knockout	Evaluate the role of <i>Cyp1b1</i> in DMBA carcinogenesis	(Buters et al., 1999)
<i>Cyp2a5</i> <i>CYP2A6</i>	Knockout Transgenic	Role of <i>Cyp2a5</i> in clearance of nicotine and cotinine Function of <i>CYP2A6</i> in coumarin metabolism	(Zhou et al., 2009) (Zhang et al., 2005)
<i>CYP2C18/</i> <i>2C19</i>	Transgenic	Gene expression and function in drug metabolism	(Lofgren et al., 2008; Lofgren et al., 2009)
<i>CYP2D6</i>	Transgenic/ humanized	Debrisoquine metabolism and disposition Indolealkylamine metabolic pharmacogenetics	(Corchero et al., 2001) (Yu et al., 2003c)
<i>Cyp2e1</i> <i>CYP2E1</i>	Knockout Humanized	Acetaminophen toxicity	(Lee et al., 1996) (Cheung et al., 2005b)
<i>CYP3A4</i>	Transgenic	Intestinal metabolism of midazolam, drug-drug metabolism, and physiological regulation	(Granvil et al., 2003) (van Herwaarden et al., 2005)
<i>Cyp3a</i> <i>CYP3A4</i>	Knockout Humanized	Docetaxel metabolism and pharmacokinetics	(van Herwaarden et al., 2007)
<i>Cpr</i>	Knockout	Drug metabolism and toxicity	(Shen et al., 2002; Otto et al., 2003) (Gu et al., 2003; Henderson et al., 2003a; Wu et al., 2003)
<i>Gstp</i>	Knockout	DMBA carcinogenesis Acetaminophen toxicity	(Henderson et al., 1998) (Henderson et al., 2000)
<i>Sult1e1</i>	Knockout	Physiological and pathological role of <i>Sult1e1</i>	(Qian et al., 2001; Tong et al., 2005)
<i>Ugt1</i> <i>UGT1</i>	Knockout Transgenic Humanized	Tissue distribution, induction and hormonal regulation Physiological role in bilirubin homeostasis	(Nguyen et al., 2008) (Chen et al., 2005) (Cai et al., 2010; Fujiwara et al., 2010)

Table 2

Effects of ketoconazole on midazolam pharmacokinetics ($AUC_{\text{(ketoconazole)}}/AUC_{\text{(control)}}$) in humans and animal models.

	Intravenous administration	Oral administration	Reference
Humans	5	16	(Tsunoda et al., 1999)
Rats	2	5	(Kotegawa et al., 2002)
Wild-type mice	n.a.	3	(Granvil et al., 2003)
Tg-CYP3A4 mice	n.a.	8	(Granvil et al., 2003)

Table 3

Drug transporter genetically modified mouse models.

Gene	Category	Example applications	References
<i>Mdr1a (Abcb1a)</i>	Knockout	Drug absorption, distribution and elimination, including brain penetration	(Schinkel et al., 1994; Schinkel et al., 1995; Schinkel et al., 1996)
<i>Mdr1a/1b (Abcb1a/1b)</i>	Knockout	Drug distribution	(Schinkel et al., 1997)
<i>Bsep (Abcb11)</i>	Knockout	Bile acid distribution and excretion	(Wang et al., 2001; Wang et al., 2003)
<i>Mrp1 (Abcc1)</i>	Knockout	Disposition of xenobiotic drugs and endogenous leukotrienes	(Wijnholds et al., 1997) (Lorico et al., 1997)
<i>Mrp2 (Abcc2)</i>	Knockout	Glutathione excretion, bile flow and drug disposition	(Vlaming et al., 2006) (Chu et al., 2006)
<i>Mrp3 (Abcc3)</i>	Knockout	Acetaminophen disposition; biliary excretion	(Belinsky et al., 2005) (Zelcer et al., 2006)
<i>Mrp4 (Abcc4)</i>	Knockout	Drug resistance and brain penetration.	(Leggas et al., 2004)
<i>Bcrp1 (Abcg2)</i>	Knockout	Drug absorption, distribution and elimination, including the secretion of compounds into milk	(Zhou et al., 2002) (Jonker et al., 2002)
<i>Osta</i>	Knockout	Bile acid disposition	(Rao et al., 2008)
<i>Pept2 (Slc15a2)</i>	Knockout	Role of Pept2 in drug distribution and excretion	(Rubio-Aliaga et al., 2003) (Shen et al., 2003; Shen et al., 2007)
<i>Mate1 (Slc47a1)</i>	Knockout	Renal secretion of metformin	(Tsuda et al., 2009)
<i>Oct1 (Slc22a1)</i>	Knockout	Hepatic uptake and intestinal disposition of organic cation drugs	(Jonker et al., 2001)
<i>Oct2 (Slc22a2)</i> <i>Oct1/2 (Slc22a1/2)</i>	Knockout	Renal excretion of drugs	(Jonker et al., 2003)
<i>Oct3 (Slc22a3)</i>	Knockout	Role of Oct3 in drug distribution	(Zwart et al., 2001)
<i>Oat1 (Slc22a6)</i>	Knockout	Excretion of organic anions and drugs	(Eraly et al., 2006)
<i>Oat3 (Slc22a8)</i>	Knockout	Uptake of organic anions and drugs	(Sweet et al., 2002) (Sykes et al., 2004)
<i>OATP1B1 (SLCO1B1)</i>	Transgenic	Hepatic uptake of mitoxantrone	(van de Steeg et al., 2009)
<i>Oatp1b2 (Slco1b2)</i>	Knockout	Drug disposition and hepatic uptake	(Zaher et al., 2008) (Lu et al., 2008) (Chen et al., 2008)

Table 4

Xenobiotic receptor genetically modified mouse models.

Gene	Category	Example applications	References
<i>Ahr</i>	Knockout	Role of Ahr in gene regulation and liver function	(Fernandez-Salguero et al., 1995) (Schmidt et al., 1996) (Mimura et al., 1997)
<i>AHR</i>	Humanized	Species difference in AHR-mediated gene regulation	(Moriguchi et al., 2003)
<i>Car (Nr1i3)</i>	Knockout	Role of Car in gene regulation and liver toxicity	(Wei et al., 2000)
<i>CAR (NR1I3)</i>	Humanized Transgenic	Species differences in CAR-mediated gene regulation Gene regulation and bile acid detoxification	(Zhang et al., 2002; Huang et al., 2004) (Saini et al., 2004)
<i>Ppara (Nr1c1)</i>	Knockout	Gene regulation and peroxisome proliferation	(Lee et al., 1995)
<i>PPARα (NR1C1)</i>	Humanized	Species difference in PPARα-mediated gene regulation	(Cheung et al., 2004)
<i>Pxr (Nr1i2)</i>	Knockout	Role of Pxr in regulation of drug metabolism and disposition	(Xie et al., 2000) (Staudinger et al., 2001)
<i>PXR (NR1I2)</i>	Humanized	Species difference in PXR-mediated gene regulation	(Staudinger et al., 2001) (Ma et al., 2007)
<i>Rxra (Nr2b1)</i>	Knockout	Physiological role of Rxra (embryonic lethality)	(Kastner et al., 1994; Sucov et al., 1994)
<i>Rxra (hepatocyte)</i>	Knockout	Regulation of endobiotics and xenobiotics metabolism	(Wan et al., 2000)

Table 5

Comparison of the responses to peroxisome proliferators (e.g., Wy-14,643) in wild-type, *Ppara*-knockout, *PPARα*-humanized mice and humans.

Response to peroxisome proliferators	Wild-type mice	<i>Ppara</i> -null mice	<i>PPARα</i> -humanized mice	Humans
PPARα expression	Murine Ppara	None	Human PPARα	Human PPARα
Increase in peroxisomes	Yes	No	No	No
Induction of enzymes in fatty acid oxidation, synthesis, transport	Yes	No	Yes	No
Induction of cell cycle genes	Yes	No	No	No
Cell proliferation	Yes	No	No	No
Hypolipidemia	Yes	No	Yes	Yes
Increased risk of cancer	Yes	No	No	No