Farnesoid X Receptor Agonist Represses Cytochrome P450 2D6 Expression by Upregulating Small Heterodimer Partner

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ABSTRACT

Cytochrome P450 2D6 (CYP2D6) is a major drug-metabolizing enzyme responsible for eliminating approximately 20% of marketed drugs. Studies have shown that differential transcriptional regulation of CYP2D6 may contribute to large interindividual variability in CYP2D6-mediated drug metabolism. However, the factors governing CYP2D6 transcription are largely unknown. We previously demonstrated small heterodimer partner (SHP) as a novel transcriptional repressor of CYP2D6 expression. SHP is a representative target gene of the farnesoid X receptor (FXR). The objective of this study is to investigate whether an agonist of FXR, 3-(2,6-dichlorophenyl)-4-(3-carboxy-2-chlorostilben-4-yl)oxymethyl-5-isopropylisoxazole (GW4064), alters CYP2D6 expression and activity. In CYP2D6-humanized transgenic mice, GW4064 decreased hepatic CYP2D6 expression and activity (by 2-fold) while increasing SHP expression (by 2-fold) and SHP recruitment to the CYP2D6 promoter. CYP2D6 repression by GW4064 was abrogated in Shp(−/−)-CYP2D6 mice, indicating a critical role of SHP in CYP2D6 regulation by GW4064. Also, GW4064 decreased CYP2D6 expression (by 2-fold) in primary human hepatocytes, suggesting that the results obtained in CYP2D6-humanized transgenic mice can be translated to humans. This proof of concept study provides evidence for CYP2D6 regulation by an inducer of SHP expression, namely, the FXR agonist GW4064.

INTRODUCTION

Cytochrome P450 2D6 (CYP2D6) is a major drug-metabolizing enzyme responsible for eliminating approximately 20% of clinically used medications. CYP2D6-mediated drug metabolism is known to exhibit large interindividual variability (Sachse et al., 1997; Zanger et al., 2001), in which the population is divided into four phenotype categories ranging from poor metabolizer (PM) to ultrarapid metabolizer (HOU et al., 1991; Dahl et al., 1992; Sachse et al., 1997; Zanger et al., 2001; Bertilsson et al., 2002). This interindividual variability is in part explained by genetic polymorphisms in the CYP2D6 gene. For example, polymorphisms associated with low or minimal expression of CYP2D6 protein (e.g., due to frameshift mutation) or the expression of nonfunctional CYP2D6 proteins lead to the PM phenotype. On the other hand, individuals with multiple copies of the CYP2D6 gene present the ultrarapid metabolizer phenotype. Of note, these individuals comprise only a small portion (~10%) of the population (Hou et al., 1991; Dahl et al., 1992; Sachse et al., 1997; Zanger et al., 2001; Bertilsson et al., 2002). Of note, in a non-PM population, urinary metabolic ratios of dextromethorphan/dextromethorphan exhibit significant overlaps among individuals of different functional gene doses for CYP2D6 (Gaedigk et al., 2008), suggesting that CYP2D6 genotypes alone do not fully explain the large interindividual variability in CYP2D6 activity. The sources of CYP2D6 variability in the population remain unclear.

Previous studies have shown that the mRNA expression and activity levels of CYP2D6 are well correlated with each other (Carcillo et al., 2003; Temesvari et al., 2012), and the correlation coefficient ranges from 0.85 to 0.91. Such a high correlation between mRNA and enzyme activity levels was also observed for CYP3A4 (Temesvari et al., 2012), whose activity level is known to be governed by the transcriptional regulation of the gene. These results suggest that differential transcriptional regulation of CYP2D6 may contribute to the large interindividual variability in CYP2D6 activity. However, factors controlling transcriptional regulation of CYP2D6 expression remain poorly understood. We have recently demonstrated that small heterodimer partner (SHP) suppresses hepatocyte nuclear receptor 4α (HNF4α)-mediated transactivation of the CYP2D6 promoter and thus represses hepatic CYP2D6 expression (Koh et al., 2014a). Also, knockdown of SHP expression (by using small interfering RNA) in CYP2D6-humanized transgenic (Tg-CYP2D6) mice led to enhanced hepatic CYP2D6 expression (Koh et al., 2014a). What remains unknown is whether modulators of SHP expression alter hepatic CYP2D6 expression such that the large interindividual variability in CYP2D6 activity may be explained by differential SHP expression and/or activity.

SHP is a representative target gene of the farnesoid X receptor (FXR), a bile acid sensor (Parks et al., 1999). When hepatic concentrations of bile acids are high (e.g., in cholestasis), the ligand-activated FXR transactivates the SHP promoter (Goodwin et al., 2000). SHP in turn suppresses the expression of genes involved in bile acid synthesis and...
up take in the liver (Wang et al., 2002; Ellis et al., 2003; Nishimaki-Mogami et al., 2004; Miao et al., 2009; Li and Chiang, 2014), protecting the liver from the toxicity of excess bile acids. The role of SHP (and FXR) in bile acid homeostasis has been extensively characterized by using selective agonists of FXR, such as GW4064 (Maloney et al., 2000). For example, in rats and human hepatocytes, GW4064 increased SHP expression and thus led to decreased expression of SHP target genes involved in bile acid homeostasis (e.g., CYP7A1 and CYP8B1) (Liu et al., 2003). In the present study, we aimed to verify the role of SHP in the regulation of CYP2D6 expression by examining how a known regulator of SHP expression, GW4064, alters CYP2D6 expression. We provide evidence that GW4064 represses CYP2D6 expression in an SHP-dependent manner.

Materials and Methods

Animals. CYP2D6-humanized transgenic (Tg-CYP2D6) and Shp-null mice were previously described (Corchero et al., 2011; Park et al., 2011). Tg-CYP2D6 mice harbor the CYP2D6 gene along with its ~2.5 kilobase promoter region in the mouse genome (Corchero et al., 2001). Both Tg-CYP2D6 and Shp-null mice were on the C57BL/6 background. Adult male mice (8 weeks of age; 20–25 g body weight) were used for the experiments. GW4064 (10 mg/kg) or vehicle (olive oil) was injected intraperitoneally in mice daily for 5 days (n = 4–5 per group). Mice were sacrificed on the sixth day, and liver tissues were collected. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Illinois at Chicago.

Chemicals and Reagents. Debrisoquine, 4-hydroxydebrisoquin, and para- xanthine were purchased from Biomol (Plymouth Meeting, PA). GW4064, [3-(2- chloro-4-(3,2-dichlorophenyl)-5-(1-methylthyl)-4-isoxazolyl)methoxy]phenyl)ethenyl)benzoic acid] was purchased from Sigma-Aldrich (St. Louis, MO).

Primary Human Hepatocytes. Freshly isolated human hepatocytes, derived from three donors, were obtained from the Liver Tissue Cell Distribution System (Pittsburgh, PA); funded by National Institutes of Health Contract #HHSN276201200017C). Briefly, hepatocytes were shipped over-night in cold preservation media. Upon receipt, the media was replaced with serum-free Williams’ E media (without phenol red) containing 0.1 μM dexamethasone, 10 μg/ml gentamicin, 15 mM HEPES, 2 μM L-glutamine, and 1% insulin-transferrin-sodium selenite media supplement. Cells were allowed to recover from shipping for 10 hours at 37°C in an atmosphere containing 5% CO2. After recovery, the hepatocytes were treated with vehicle control (dimethylsulfoxide) or GW4064 (1 μM) for 48 hours. Cell lysates were collected to prepare RNAs and S9 fractions.

Western Blot. Western blot was performed as described previously (Koh et al., 2014a). CYP2D6 and SHP protein expression levels were determined by using the respective antibodies (CYP2D6, catalog #458246, BD Gentest, Franklin Lakes, NJ; SHP, sc-30169, Santa Cruz Biotechnology, Dallas, TX).

Determination of CYP2D6 Activity. Hepatic S9 fractions were prepared as described previously (Felmlée et al., 2008; Koh et al., 2014a). S9 fractions were incubated with debrisoquine (a CYP2D6 probe substrate) based on the report that mouse endogenous CYP2D6 play minor roles in debrisoquine hydroxylation (Koh et al., 2014a). The concentration of 4-hydroxydebrisoquine was measured using the respective antibodies (CYP2D6, catalog #458246, BD Gentest, Franklin Lakes, NJ; SHP, sc-30169, Santa Cruz Biotechnology, Dallas, TX).

Chromatin Immunoprecipitation Assays. Liver samples were subjected to chromatin immunoprecipitation (ChIP) assays as described previously (Koh et al., 2014a,b). Briefly, livers were finely minced and incubated in phosphate-buffered saline (PBS) containing 1% formaldehyde at room temperature for 15 minutes, and glycine was added to stop the cross-linking reaction. Cell pellets were resuspended in hypotonic buffer (15 mM HEPES, 60 mM KCl, 2 mM EDTA, 0.5% bovine serum albumin, 0.15 mM spermine, 0.5 mM spermidine, and 0.32 M sucrose, pH 8.0) and lysed by homogenization. Nuclei were pelleted and resuspended in nuclei lysis buffer (50 mM Tris-HCl, 2 mM EDTA, and 1% SDS, pH 8.0). The samples were sonicated to shear DNA to a length ranging from 100 to 500 base pairs. After centrifuge, the chromatin samples were immunoprecipitated using magnetic beads coated with 2-μg antibody (HNF4α, sc-6556x; RNA polymerase II, sc-899x; SHP, sc-30169, Santa Cruz) or immunoglobulin G (normal goat IgG, sc-2028; normal rabbit IgG, sc-2027, Santa Cruz) at 4°C overnight. The immune complex on the magnetic beads was collected and extensively washed, and the bound chromatin was eluted. Genomic DNA was purified by phenol chloroform extraction followed by Wizard SV gel and the PCR clean-up system (Promega, Madison, WI). qRT-PCR was performed using the following probes for Cyp8b1: 5′-AAGGCAGG-CAACATGGAGA-3′ (forward) and 5′-CAATGCAAGGTCTGCCCC-3′ (reverse). CYP2D6 probes were described previously (Koh et al., 2014a).

Statistical Analysis. Values were reported as mean ± S.E.M. Statistical differences between the two groups were determined by using Student’s t test.

Results

FXR Agonist GW4064 Represses CYP2D6 Expression and Activity in Tg-CYP2D6 Mice. To determine whether GW4064 alters CYP2D6 expression and activity in vivo, GW4064 or vehicle control was intraperitoneally administered to Tg-CYP2D6 mice for 5 days, and hepatic CYP2D6 mRNA and protein levels were measured by qRT-PCR and Western blot, respectively. Cyp8b1, a gene known to be downregulated by SHP (Inoue et al., 2006), was used as a positive control. The results showed that GW4064 significantly decreased both mRNA and protein expression levels of CYP2D6 by ~2-fold (Fig. 1A; Fig. 1B). CYP2D6 activity, determined by measuring the debrisoquine hydroxylation rate in the hepatic S9 fraction (Koh et al., 2014a), was also found to be decreased by ~2-fold upon GW4064 treatment (Fig. 1C). GW4064 increased SHP expression ~2-fold (Fig. 1A; Fig. 1B), which is consistent with previous results (Goodwin et al., 2000; Li et al., 2010).

We previously showed that SHP represses HNF4α transactivation of the CYP2D6 promoter (Koh et al., 2014a). To determine whether GW4064 alters CYP2D6 promoter activity in mice, ChIP assays were performed using mouse liver tissues. Liver tissues were collected from GW4064 (or vehicle)-treated mice and subjected to ChIP using antibodies against SHP, HNF4α, or RNA polymerase II (Pol II) (a marker of transcription initiation). The protein-bound DNA was analyzed by using a primer set that can detect the HNF4α response element at ~55/-43 of CYP2D6 (Caïrs et al., 1996). As a positive and negative control, recruitment of the transcription factors to the Cyp8b1 promoter or a downstream region of CYP2D6 (+3913/+4368), respectively, was examined. The results demonstrated increased recruitment of SHP and decreased recruitment of HNF4α and Pol II to the CYP2D6 promoter (Fig. 2A). Similar trends were observed for SHP, HNF4α, and Pol II recruitment to the Cyp8b1 promoter region [which harbors the HNF4α response element (Inoue et al., 2006)] (Fig. 2B). Recruitment of the transcription factors to the downstream region of CYP2D6 was minimal and not affected by GW4064 (data not shown). Together, these results suggest that the repressive effect of GW4064 on CYP2D6 is potentially mediated by enhanced SHP expression.

CYP2D6 Repression by GW4064 Is Abrogated in Shp(−/−). CYP2D6 Mice. To examine the essentiality of SHP in CYP2D6 repression by GW4064, Tg-CYP2D6 mice were crossed with Shp-null
mice, mice of Shp(+/+)CYP2D6 or Shp(−/−);CYP2D6 genotype were generated, and CYP2D6 repression by GW4064 or vehicle was compared between the mice of different genotypes. None of the Shp(+/+)CYP2D6 or Shp(−/−);CYP2D6 mice exhibited any prominent phenotypes, and all grew normally. Western blot results showed that SHP protein expression was abolished in Shp(−/−);CYP2D6 mice (data not shown). In the vehicle-treated mice, the basal mRNA expression levels of Cyp8b1 were higher in Shp(−/−);CYP2D6 as compared with Shp(+/+)CYP2D6 mice (Fig. 3A; *P = 0.0004), whereas the basal CYP2D6 expression did not differ between the mice of different genotypes (Fig. 3A; P = 0.16). GW4064 treatment led to decreased expression of CYP2D6 and Cyp8b1 in Shp(+/+)CYP2D6 mice (Fig. 3A), which is similar to the results from Tg-CYP2D6 mice (Fig. 1A). These repressive effects of GW4064 on CYP2D6 and Cyp8b1 expression

Mice, mice of Shp(+/-);CYP2D6 or Shp(-/-);CYP2D6 genotype were generated, and CYP2D6 repression by GW4064 or vehicle was compared between the mice of different genotypes. None of the Shp(+/-);CYP2D6 or Shp(-/-);CYP2D6 mice exhibited any prominent phenotypes, and all grew normally. Western blot results showed that SHP protein expression was abolished in Shp(-/-);CYP2D6 mice (data not shown). In the vehicle-treated mice, the basal mRNA expression levels of Cyp8b1 were higher in Shp(-/-);CYP2D6 as compared with Shp(+/-);CYP2D6 mice (Fig. 3A; *P = 0.0004), whereas the basal CYP2D6 expression did not differ between the mice of different genotypes (Fig. 3A; P = 0.16). GW4064 treatment led to decreased expression of CYP2D6 and Cyp8b1 in Shp(+/-);CYP2D6 mice (Fig. 3A), which is similar to the results from Tg-CYP2D6 mice (Fig. 1A). These repressive effects of GW4064 on CYP2D6 and Cyp8b1 expression

**Fig. 1.** GW4064 represses CYP2D6 expression in Tg-CYP2D6 mice. Tg-CYP2D6 mice were administered with GW4064 (10 mg/kg) or vehicle (olive oil) intraperitoneally daily for 5 days (n = 4 per group). (A) CYP2D6, Shp, and Cyp8b1 mRNA expression was determined by using qRT-PCR. (B) CYP2D6 and SHP protein expression levels were determined by Western blot. The image of the Western blot (right) and the quantified band intensities (after normalization by β-actin (left) are shown. (C) S9 fractions were prepared from the liver tissues of Tg-CYP2D6 mice treated with GW4064 or vehicle control, and CYP2D6 phenotyping was performed using debrisoquine (200 μM). Data shown are 4-hydroxydebrisoquin production rates (in pmol/min per mg protein). Values are presented as mean ± S.E.M. (n = 4). *P < 0.05 and **P < 0.01 versus vehicle treatment.

**Fig. 2.** GW4064 represses HNF4α transactivation of the CYP2D6 promoter. Recruitment of HNF4α, SHP, and RNA polymerase II onto (A) CYP2D6 promoter and (B) Cyp8b1 promoter were analyzed by ChIP assay in the livers of mice treated with GW4064 (10 mg/kg) or vehicle (olive oil). Values are presented as mean ± S.E.M. (n = 4). *P < 0.05 and **P < 0.01 versus vehicle treatment.
were abrogated in Shp(-/-);CYP2D6 mice (Fig. 3A), suggesting that GW4064 represses CYP2D6 transcription through SHP. The protein expression level of CYP2D6 was consistent with the decreased mRNA levels of CYP2D6 by GW4064 (Fig. 3B). Similarly, the decreased CYP2D6 activity levels (as determined by the debrisoquine hydroxylation rate) upon GW4064 treatment was abrogated in Shp(-/-);CYP2D6 mice (Fig. 3C).

To determine whether Shp deletion leads to altered GW4064 effects on the HNF4α transactivation of the CYP2D6 promoter, ChIP assays were performed in the mouse liver tissues. In Shp(+/-);CYP2D6 mice, GW4064 decreased the recruitment of HNF4α and Pol II while increasing SHP recruitment to the CYP2D6 promoter (Fig. 4A), as in Tg-CYP2D6 mice (Fig. 2). These changes in transcription factor recruitment disappeared in Shp(-/-);CYP2D6 mice (Fig. 4A). Similar results were observed in the transcription factor recruitment to the Cyp8b1 promoter (Fig. 4B). Together, these results suggest an essential role of SHP in CYP2D6 repression by GW4064.

SHP Represses CYP2D6 Expression in Primary Human Hepatocytes. To determine whether GW4064 effects on CYP2D6 expression obtained in mice can be translated to humans, primary human hepatocytes were treated with GW4064 or vehicle for 48 hours, and CYP2D6 expression and activity were examined. The results showed that GW4064 treatment decreased CYP2D6 mRNA expression by 1.5- to 2-fold (Fig. 5A) while enhancing SHP expression (Fig. 5B) in human hepatocytes. The mRNA expression levels of positive control gene CYP8B1 were also increased in GW4064-treated human hepatocytes (Fig. 5C). In one batch of human hepatocytes (i.e., human hepatocyte 2), CYP2D6 activity levels were measured by using debrisoquine as a probe drug for CYP2D6. CYP2D6 activity in GW4064-treated cells was significantly lower (Fig. 5D) but to a small extent (~20%), as expected from the long degradation half-life of the CYP2D6 protein (i.e., 51 hours) (Venkatakrishnan and Obach, 2005). Together, these results indicate that as in Tg-CYP2D6 mice, GW4064 represses CYP2D6 expression in human hepatocytes.

Discussion

Previously, we have identified SHP as a novel regulator of CYP2D6 transcription. SHP represses HNF4α transactivation of the CYP2D6 promoter (Koh et al., 2014a). Because SHP is a representative target gene of FXR, in this study, we examined whether upregulation of SHP by a selective FXR agonist (GW4064) alters CYP2D6 expression. Our data showed that GW4064 represses CYP2D6 expression in Tg-CYP2D6 mice as well as human hepatocytes.

Results from this study demonstrated a key role of SHP in CYP2D6 repression by GW4064. The decrease in CYP2D6 expression in GW4064-treated mice was accompanied by increases in SHP expression as well as SHP recruitment to the CYP2D6 promoter. The essential role of SHP in GW4064 action on CYP2D6 expression was further verified in Shp(-/-);CYP2D6 mice in that CYP2D6 repression by GW4064 was abrogated in the mice. Considering that SHP induction is a class action of FXR agonists, these results suggest that FXR activation by other drugs or diseases (e.g., cholestasis) is also expected to repress CYP2D6 expression. Indeed, we found that treatment of human hepatocytes with cholic acid (a major bile acid elevated in cholestasis) led to a significant decrease in CYP2D6 expression (data not shown). Cholestasis is often triggered by drugs, such as rifampicin, erythromycin, ethinylestradiol, and oxyphenicilins (Zhang et al., 2014). Although it remains to be determined whether cholestasis represses CYP2D6-mediated drug metabolism in humans, the results from this study provide a mechanistic basis for the possibility. Additionally, this study provides evidence that supports important roles of SHP in the regulation of CYP2D6 expression and that differential SHP expression and/or activity may potentially contribute to interindividual variability in CYP2D6-mediated drug

Fig. 3. CYP2D6 repression by GW4064 is abrogated in Shp(-/-);CYP2D6 mice. Shp(+/-);CYP2D6 or Shp(-/-);CYP2D6 mice were injected with GW4064 (10 mg/kg) or vehicle (olive oil) intraperitoneally daily for 5 days (n = 5 per group). (A) CYP2D6 and Cyp8b1 mRNA expression levels were measured by using qRT-PCR and normalized by those in vehicle-treated Shp(+/-);CYP2D6 mice. (B) CYP2D6 protein expression level was measured by Western blot. The image of Western blot (right) and the quantified band intensities (CYP2D6/β-actin) (left) are shown after normalization by CYP2D6 expression in the vehicle-treated mice of the respective genotype. (C) S9 from the mice was incubated with debrisoquine (200 μM), and 4-hydroxydebrisoquine concentrations were measured by using liquid chromatography–tandem mass spectrometry. Data shown are 4-hydroxydebrisoquin production rates (in pmol/min per mg protein). Values are presented as mean ± S.E.M. (n = 5). *P < 0.05; **P < 0.01; n.s., not statistically significant.
metabolism in humans. Whether or to what extent different SHP modulators affect hepatic CYP2D6 expression and activity remains to be examined.

Results from our previous study in Tg-CYP2D6 mice showed that CYP2D6 expression was increased in mice with SHP knocked down (by using small interfering RNA) (Koh et al., 2014a), indicating that decreased SHP expression leads to CYP2D6 induction. Interestingly, however, the basal expression levels of CYP2D6 in Shp(−/−);CYP2D6 mice did not differ from those in Shp(+/+);CYP2D6 mice (Fig. 3A).

Previous studies have shown that gene knockout in mice can lead to multiple compensational changes in gene expression (Picciotto and Wickman, 1998), and altered expression of transcription factors in Shp(−/−) mice may compensate for the loss of SHP in regulating CYP2D6 expression. For example, NR0B1 is a nuclear receptor that lacks the DNA-binding domain similarly to SHP (Benoit et al., 2006), and it is known to repress HNF4α transactivation of a hepatic gene (Nedumaran et al., 2009). Our qRT-PCR experiment, however, revealed that NR0B1 mRNA expression is undetectable in mouse liver tissues (data not shown). Also, SHP-interacting leucine zipper protein (SMILE) [initially identified as an SHP-interacting protein (Xie et al., 2008)] has been shown to repress HNF4α transactivation of target genes in the absence of SHP (Xie et al., 2009). Our results showed, however, that the mRNA expression levels of SMILE did not differ between Shp(−/−);CYP2D6 and Shp(+/+);CYP2D6 male mice (data not shown; in female mice, SMILE mRNA expression was even lower in Shp(−/−);CYP2D6 mice as compared with Shp(+/+);CYP2D6 mice). Together, these results suggest that basal CYP2D6 expression in Shp(−/−);CYP2D6 mice may be governed by as-yet-unknown factors.

**Fig. 5.** GW4064 represses CYP2D6 expression and activity in primary human hepatocytes. Primary human hepatocytes (HHs) from three different donors were treated with GW4064 (1 μM) or vehicle control (DMSO) for 48 hours. (A–C) CYP2D6, SHP, and CYP8B1 mRNA expression was determined by qRT-PCR. (D) HH2 was incubated with debrisoquine (200 μM), and 4-hydroxydebrisoquine concentrations in the culture media were measured by using liquid chromatography–tandem mass spectrometry. Data shown are 4-hydroxydebrisoquin production rates (in pmol/min per mg protein). Values are presented as mean ± S.E.M. and calculated from three independent experiments. *P < 0.05 and **P < 0.01 versus vehicle treatment.
Many FXR agonists are currently under development for different hepatic or metabolic diseases, including primary biliary cirrhosis, nonalcoholic steatohepatitis, and diabetes (Thomas et al., 2008). For example, obeticholic acid (i.e., INT-747), a potent selective FXR agonist, is in phase III trials for primary biliary cirrhosis (Pellicciari et al., 2005). Also, the hepatoprotective effects of GW4064 and its analogs have been shown in cholestatic rats and mice with gallstones (Liu et al., 2003; Moschetta et al., 2004; Akwabi-Ameyaw et al., 2008; Bass et al., 2011; Porez et al., 2012). Our results suggest that drug–drug interactions between CYP2D6 substrates and FXR agonists may occur if these FXR agonists are approved and clinically used. Considering the long degradation half-lives of the CYP2D6 protein (Venkataraman and Obach, 2005), it remains difficult to quantitatively predict the clinical outcome of these interactions based on the results from human hepatocytes. On the other hand, the results from Tg-CYP2D6 mice suggest that FXR activation could lead to 2-fold decreases in CYP2D6 activity. The time course and magnitude of this potential drug–drug interaction remain to be examined.

In conclusion, we showed that the FXR agonist GW4064 represses CYP2D6 expression through inducing SHP expression. This suggests that potential drug–drug interactions may occur between CYP2D6 substrates and FXR agonists that are currently under development for hepatic and metabolic disorders. Our results also provide a mechanistic basis to identify potential factors (e.g., bile acids) that may contribute to the interindividual variability in CYP2D6 activity.

Authorship Contributions

Participated in research design: Pan, Jeong.
Conducted experiments: Pan.
Contributed new reagents or analytic tools: Lee.
Performed data analysis: Pan, Jeong.
Wrote or contributed to the writing of the manuscript: Pan, Lee, Jeong.

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