

Minireview

Transcriptional Regulation of CYP2D6 Expression

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ABSTRACT

CYP2D6-mediated drug metabolism exhibits large interindividual variability. Although genetic variations in the CYP2D6 gene are well known contributors to the variability, the sources of CYP2D6 variability in individuals of the same genotype remain unexplained. Accumulating data indicate that transcriptional regulation of CYP2D6 may account for part of CYP2D6 variability. Yet, our understanding of factors governing transcriptional regulation of CYP2D6 is limited. Recently, mechanistic studies of increased CYP2D6-mediated drug metabolism in pregnancy revealed two

transcription factors, small heterodimer partner (SHP) and Krüppel-like factor 9, as a transcriptional repressor and an activator, respectively, of CYP2D6. Chemicals that increase SHP expression (e.g., retinoids and activators of farnesoid X receptor) were shown to downregulate CYP2D6 expression in the humanized mice as well as in human hepatocytes. This review summarizes the series of studies on the transcriptional regulation of CYP2D6 expression, potentially providing a basis to better understand the large interindividual variability in CYP2D6-mediated drug metabolism.

Introduction

Cytochrome P450 (P450) 2D6 is a major drug-metabolizing enzyme expressed in the liver and extrahepatic organs (such as brain and intestine). CYP2D6 mediates hepatic metabolism of approximately 20% of marketed drugs (e.g., codeine, amitriptyline, fluvoxamine, risperidone, fluoxetine, aripiprazole, paroxetine, and dextromethorphan) (Yu et al., 2004; Zanger et al., 2004). Substrates of CYP2D6 also include neurotoxins such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, which is implicated in the development of Parkinson's disease (Hiroi et al., 1998); British Caucasian subjects carrying nonfunctional CYP2D6 alleles exhibit increased susceptibility to Parkinson's disease (Smith et al., 1992; Lu et al., 2014). In addition to the role as a xenobiotic-metabolizing enzyme, multiple biologic roles of CYP2D6 in the brain have been suggested. For example, CYP2D6 catalyzes production of endogenous neurotransmitters, such as dopamine and serotonin, from respective precursors (Hiroi et al., 1998; Yu et al., 2003). This catalytic activity of CYP2D6 may contribute in part to certain personality traits (e.g., anxiety and impulsivity) (Bertilsson et al., 1989; Gan et al., 2004; Gonzalez et al., 2008) by modulating blood perfusion rates to the thalamus (Kirchheiner et al., 2011).

The rate of CYP2D6-mediated drug metabolism exhibits large variability among individuals. For example, the metabolic rates of a

CYP2D6 probe drug (dextromethorphan) in 99 different human hepatic microsomes vary by ~60-fold (Hart et al., 2008). Furthermore, urinary metabolic ratio (i.e., ratio between metabolite and parent drug) of dextromethorphan ranged over 5 orders of magnitude in 660 subjects (Gaedigk et al., 2008). Of note, the much-greater variability observed in urinary metabolic ratio was shown to be due in part to differential urinary pH, which can impact urinary excretion of parent drugs and metabolites (Labbe et al., 2000; Ozdemir et al., 2004). Based on the CYP2D6 activity levels, one of the following four CYP2D6 phenotypes can be assigned to an individual: poor metabolizer (PM), intermediate metabolizer (IM), extensive metabolizer, and ultrarapid metabolizer. In the United States, the frequencies of the four phenotypes are 2, 3, 92, and 5%, respectively (Gaedigk et al., 2016). CYP2D6 phenotype frequencies vary in different ethnic groups, and have been reviewed previously (Gaedigk et al., 2016).

CYP2D6, located on chromosome 22q13, is highly polymorphic. Many of its genetic polymorphisms are associated with altered CYP2D6 activities in individuals, contributing to large interindividual variability in CYP2D6 activity levels. Over 100 different genetic polymorphisms in the CYP2D6 gene have been identified since the first discovery of CYP2D6 genetic polymorphisms that are responsible for the PM phenotype (Kagimoto et al., 1990) (<http://www.cypalleles.ki.se>). Some of these polymorphisms are CYP2D6 null alleles that lead to minimal expression of CYP2D6 protein or expression of nonfunctional CYP2D6 proteins, and subjects carrying these alleles exhibit the PM phenotype. Similarly, the presence of CYP2D6 genetic polymorphisms associated with decreased enzyme activity has been implicated in the IM phenotype, and multiple copies of wild-type CYP2D6 gene have been

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ABBREVIATIONS: atRA, *all trans* RA; C/EBP, CCAAT/enhancer-binding protein; FXR, farnesoid X receptor; GW4064, 3-(2,6-dichlorophenyl)-4-(3'-carboxy-2-chlorostilben-4-yl)oxymethyl-5-isopropylisoxazole; HNF, hepatocyte nuclear factor; IM, intermediate metabolizer; KLF, Krüppel-like factor; P450, cytochrome P450; PM, poor metabolizer; RA, retinoic acid; SHP, small heterodimer partner; SNP, single nucleotide polymorphism; tg-CYP2D6, CYP2D6-humanized.

implicated in the ultrarapid metabolizer phenotype (Zanger et al., 2004). Overall, the mean CYP2D6 activity increases with higher CYP2D6 activity score (i.e., a semiquantitative and collective representation of CYP2D6 genotypes) (Gaedigk et al., 2008). These results indicate important roles of CYP2D6 genetic polymorphisms in determining CYP2D6 activity level such that the information on CYP2D6 genotypes can be used to estimate the CYP2D6 phenotype in an individual. PM subjects can be reliably identified based on the information on CYP2D6 genotypes, because CYP2D6 activity levels in subjects carrying the null alleles are distinctively low (Gaedigk et al., 2008). Of note, however, CYP2D6 activity levels in non-PM subjects exhibit wide variability so that they cannot be predicted reliably based on the genotype information alone (Gaedigk et al., 2008); individuals with the same CYP2D6 genotype exhibit wide variability in CYP2D6 activity levels. For example, the urinary metabolic ratio of dextromethorphan in individuals carrying the wild-type CYP2D6 alleles varied over 2 orders of magnitude (Gaedigk et al., 2008). A similar finding was obtained for debrisoquine, whose urinary metabolic ratio is not influenced by urinary pH (Sachse et al., 1997; Ozdemir et al., 2004). This suggests that CYP2D6 variability in non-PM subjects is potentially multifactorial, and in addition to the known genetic polymorphisms of CYP2D6, other factors potentially contribute to the variability.

Studies have shown that CYP2D6 activities positively correlate with its mRNA expression levels (Rodriguez-Antona et al., 2001; Zanger et al., 2001; Yang et al., 2010; Temesvari et al., 2012). For example, in 398 human liver tissues that include both PM and non-PM tissues, CYP2D6 mRNA expression and activity levels were correlated with a correlation coefficient (r) value of 0.53 (after adjustment for age and sex) (Yang et al., 2010). In another study, CYP2D6 mRNA expression and activity levels exhibited a stronger correlation ($r = 0.95$) in over 130 non-PM human liver tissues (Temesvari et al., 2012). Interestingly, the extent of correlation was similar to that of CYP3A4 ($r = 0.55$ and 0.90 in the former and latter study, respectively). CYP3A4 is a drug-metabolizing enzyme whose expression and activity are governed at the level of gene transcription via actions of multiple transcription factors (Goodwin et al., 2002a,b). The fact that both CYP2D6 and CYP3A4 exhibit similar extent of correlation between mRNA and activity levels (in human liver tissues) raises the possibility that CYP2D6 expression and activity may also be determined at the level of CYP2D6 transcription, and differential transcriptional regulation of CYP2D6 may contribute to large interindividual variability in CYP2D6-mediated drug metabolism. In this review, we summarize our current understanding of factors involved in transcriptional regulation of CYP2D6 expression to provide a mechanistic basis to gauge their potential contribution to interindividual variability in drug metabolism.

Genetic Polymorphisms of CYP2D6 that Modulate CYP2D6 Expression

Although a majority of genetic polymorphisms identified to date are located in the exonic or intronic region of the CYP2D6 gene, there have been sporadic reports of the presence of single nucleotide polymorphisms (SNPs) within regulatory regions of CYP2D6 and their roles in differential expression of CYP2D6. For example, carriers of $-1584C>G$ (rs1080985) polymorphism (first discovered in the carriers of CYP2D6*2) exhibited increased metabolism of CYP2D6 substrates (i.e., dextromethorphan and thioridazine) (Dorado et al., 2009; Llerena et al., 2013). CYP2D6 protein levels in the liver tissues of $-1584 C/C$ carriers were $\sim 50\%$ of those of either $-1584 C/G$ or G/G carriers (Zanger et al., 2001; Dorado et al., 2009; Llerena et al., 2013). The underlying molecular mechanisms for the finding and the role of $-1584C>G$ in transcriptional regulation of CYP2D6 remain unclear.

A systematic approach to identify SNPs associated with altered P450 expression and/or activity (using 398 human liver tissues and SNP arrays) revealed that three long-range polymorphisms of CYP2D6—namely, rs8138080 (126 kb downstream of CYP2D6), rs17478227, and rs5751247 (~ 127 and ~ 106 kb upstream of CYP2D6, respectively)—are associated with lower CYP2D6 activity as well as the mRNA levels (Yang et al., 2010). It is unknown whether the long-range polymorphisms are functional; however, they are in strong linkage disequilibrium with CYP2D6*4 (rs3892097, rs1065852) and CYP2D6*10 (rs1065852), which result in nonfunctional or decreased-function enzymes, respectively (Yang et al., 2010), raising the possibility that the decreased CYP2D6 activity of CYP2D6*4 and *10 alleles may be partly responsible for the lower CYP2D6 activity in the liver tissues carrying the long-range polymorphisms.

Most recently, studies have found another SNP, rs5758550 (A>G), that is associated with enhanced CYP2D6 expression (Wang et al., 2014, 2015). SNP rs5758550 is located ~ 115 kb downstream of the CYP2D6 gene, and is in linkage with CYP2D6*2. Results from *in vitro* promoter reporter assays demonstrated that rs5758550G is associated with (2-fold) higher CYP2D6 promoter activity as compared with rs5758550A (Wang et al., 2015). In HepG2 cells, deletion of the region surrounding rs5758550 (using CRISPR (clustered regularly interspaced short palindromic repeats)-Cas9 technology) led to >2 -fold decreases in CYP2D6 mRNA level (Wang et al., 2015), suggesting an important regulatory role of rs5758550 in basal CYP2D6 expression. Together, these results suggest that SNPs in upstream or downstream regulatory regions of the CYP2D6 gene may alter CYP2D6 expression and, thus, its activity. Systematic approaches appear necessary to identify other promoter or enhancer SNPs that may play a role in the transcriptional regulation of CYP2D6 expression and to elucidate their roles in interindividual variability in CYP2D6-mediated drug metabolism.

Transcription Factors Involved in the Regulation of Basal CYP2D6 Expression

To date, only a small number of transcription factors have been identified to be involved in the transcriptional regulation of CYP2D6. These transcription factors include hepatocyte nuclear factor (HNF) 4α and CCAAT/enhancer-binding protein (C/EBP) α .

HNF4 α is a member of the nuclear receptor family and a global regulator of genes involved in liver development or liver-specific functions (Gonzalez, 2008). Whole-body deletion of HNF4 α results in embryonic lethality in mice (Chen et al., 1994). On the other hand, mice without hepatic HNF4 α (as a result of organ-specific, conditional targeted gene deletion) are viable but exhibit disrupted lipid homeostasis (Hayhurst et al., 2001), demonstrating a key role of HNF4 α in maintenance of normal hepatic function. Also, HNF4 α is known to regulate expression of drug-metabolizing enzymes, including CYP2D6. A systematic study using over 400 human liver tissues showed that HNF4 α mRNA expression significantly correlated with microsomal CYP2D6 enzyme activity (Yang et al., 2010). In HepG2 cells, ectopically expressed HNF4 α enhanced promoter activity of CYP2D6 by binding to a response element (i.e., $-53/-41$) in the CYP2D6 promoter (Cairns et al., 1996). HNF4 α knockdown (by using antisense targeting) led to significant decreases in CYP2D6 expression in human hepatocytes, indicating that HNF4 α is required for the constitutive expression of CYP2D6 (Jover et al., 2001). Similarly, liver-specific deletion of HNF4 α led to (greater than 50%) decreases in CYP2D6 expression and activity in mice (Corchero et al., 2001). Together, these results indicate key roles of HNF4 α in the regulation of constitutive CYP2D6 expression in liver.

Numerous genetic polymorphisms have been reported for HNF4 α , and some have been shown to modulate CYP2D6 expression. G60D

polymorphism confers lower DNA binding activity of HNF4 α , and is associated with decreased hepatic CYP2D6 expression (Lee et al., 2008). Human pharmacokinetic study of **tolterodine** (a CYP2D6 substrate) demonstrated that HNF4 α G60D and CYP2D6 genotypes together account for a significant proportion of the variation in tolterodine area under the curve and maximal plasma concentration after an oral dose ($C_{\max}$) ($r^2 = 0.81$ and 0.59 , respectively; $p < 0.01$), and the relative contribution by the HNF4 α genotype to tolterodine pharmacokinetic variability is approximately 25% of that by CYP2D6 genotypes (Jiang et al., 2013). HNF4 α G60D allele frequency is very low [at most 1.3%, observed in Korean populations; almost non-existent in Caucasians and African Americans (Lee et al., 2008)], and thus its overall contribution to CYP2D6 variability in non-Asian populations may be low. Other rare missense SNPs in HNF4 α have been reported that lead to maturity-onset diabetes of the young or hyperinsulinemic hypoglycemia (reviewed by Colclough et al., 2013). Structural studies and in vitro experiments have shown that some of these disease-linked mutations are linked to decreased HNF4 α binding to DNA and, subsequently, reduced HNF4 α transactivation of target gene promoters (Lu et al., 2008; Chandra et al., 2013). Whether these **HNF4 α polymorphisms** are associated with altered HNF4 α transactivation of CYP2D6 promoter and thus differential CYP2D6 expression and activity remains unclear.

Studies on functional SNPs in HNF4 α regulatory regions (i.e., upstream, 5'-, or 3'-untranslated region) have been sporadic. Rs11574744 is an SNP in the 3'-untranslated region of HNF4 α (minor allele frequency of 3.4%) and located in putative binding sites of multiple microRNAs (Wirsing et al., 2011; Ramamoorthy et al., 2012). Subjects carrying rs11574744 (both homozygous and heterozygous) exhibited a lower CYP2D6 metabolic ratio (of dextromethorphan) than those with wild-type HNF4 α , but the difference did not reach statistical significance ($p = 0.10$) (Ramamoorthy et al., 2012), suggesting that its clinical implication may be minor.

C/EBP α is a transcription factor expressed in many different tissues, including the placenta, liver, and small intestine (Schrem et al., 2004). Biologic functions of C/EBP α are diverse and include nutrient metabolism, cell cycle control, and liver regeneration (Schrem et al., 2004). C/EBP α is expressed at later stages of liver development, and overexpression of **C/EBP α in HepG2 cells increased the mRNA levels of CYP2D6** as well as other drug-metabolizing enzymes, such as CYP2B6 and CYP2C9 (Jover et al., 1998). On the other hand, knockdown of C/EBP α expression (by using small interfering RNA) led to decreased expression of CYP2D6 in HepG2 cells (Matsunaga et al., 2012), suggesting that C/EBP α is a transcriptional regulator involved in basal CYP2D6 expression. C/EBP α binding site was identified within -1231/-1220 of CYP2D6 via in silico promoter analysis, promoter reporter assays, and chromatin immunoprecipitation assays in HepG2 cells (Matsunaga et al., 2012). These results indicate that **C/EBP α** is a transcriptional activator of CYP2D6 expression. Its regulatory role in CYP2D6 expression in in vivo systems or in human liver tissues remains to be verified.

Transcriptional Regulation of CYP2D6 during Pregnancy

CYP2D6 was considered to be a noninducible gene; prototypical inducers of drug-metabolizing enzymes (e.g., rifampin or phenobarbital) do not induce CYP2D6 expression. Interestingly, accumulating clinical data indicate that **CYP2D6-mediated drug metabolism is enhanced during pregnancy**, presenting a rare case of CYP2D6 induction. The apparent induction of CYP2D6 activity during pregnancy was first observed in 1983; the peak plasma concentrations obtained after an oral dose of metoprolol [a CYP2D6 probe substrate; oral bioavailability ~80% (Regardh et al., 1981)] **in pregnant women were only 20–40% of**

that after delivery (Hogstedt et al., 1983). A subsequent study showed that systemic clearance of metoprolol estimated after an intravenous dose was **26–97% higher at term pregnancy as compared** with after delivery (Hogstedt et al., 1985). Later studies using other CYP2D6 substrates [e.g., dextromethorphan (Wadelius et al., 1997; Tracy et al., 2005), clonidine (Buchanan et al., 2009; Claessens et al., 2010), and paroxetine (Ververs et al., 2009)] showed similar increases in CYP2D6-mediated drug metabolism during pregnancy. For example, the plasma metabolic ratio of dextromethorphan (i.e., dextromethorphan/dextrorphan) at 2 hours after an oral dosing was decreased (by 53%) during pregnancy (Wadelius et al., 1997), and the urinary metabolic ratio of dextromethorphan in a cumulative 24-hour urine sample was decreased by 26–48% (Tracy et al., 2005). **Together, these studies demonstrated significant increases in CYP2D6-mediated drug metabolism in pregnant women.**

Notably, the increases in CYP2D6-mediated drug metabolism were not observed in CYP2D6 IM or PM subjects. For example, whereas steady-state plasma concentrations of paroxetine decreased in CYP2D6 extensive metabolizer subjects (indicative of CYP2D6 induction), such changes were not shown in IM or PM subjects (Ververs et al., 2009). Similarly, increases in dextromethorphan metabolism in pregnant women (as compared with postpartum women) were not observed in CYP2D6 PM subjects (Wadelius et al., 1997). **One potential explanation of these findings is increased transcriptional activation of CYP2D6 during pregnancy.**

The mechanism underlying CYP2D6 induction during pregnancy was unknown, in part due to a lack of an appropriate animal model that can reproduce the phenotype. For example, the expression of CYP2D6 orthologs in rodents (i.e., rat CYP2D2 and mouse Cyp2d22) does not increase during pregnancy (Dickmann et al., 2008; Koh et al., 2011). This apparent interspecies difference could be due to large divergence in the upstream regulatory sequences of P450 genes between rodents and humans (Wilson et al., 2008). CYP2D6-humanized (tg-CYP2D6) mouse genome harbors the human CYP2D6 gene plus 2.5 kb of upstream regulatory region of CYP2D6 (Corchero et al., 2001; Hayhurst et al., 2001). In tg-CYP2D6 mice, pregnancy led to 2- to 3-fold increases in CYP2D6 expression (at mRNA and protein levels) as well as in CYP2D6 activity as compared with the prepregnancy or postpartum period (Koh et al., 2014a), presenting the first animal model for the study of CYP2D6 regulation during pregnancy.

Previous studies have suggested potential roles of female hormones (i.e., estradiol and progesterone) in altered expression of P450 (such as CYP3A4) in pregnancy (Choi et al., 2013). However, these hormones (at high concentrations attained at term pregnancy) did not affect CYP2D6 expression in human hepatocytes (Choi et al., 2013). Consistent with the results, urinary metabolic ratios of CYP2D6 substrates (sparteine or dextromethorphan) were not affected by the use of oral contraceptives (Bock et al., 1994; Tamminga et al., 1999), and the CYP2D6 mRNA and protein levels in 300 human liver tissues did not differ by sex (Zanger et al., 2005). Together, these results suggest insignificant roles of female hormones, if any, in CYP2D6 induction during pregnancy.

HNF4 α is a key regulator of basal CYP2D6 expression, but hepatic HNF4 α expression (at mRNA and protein levels) did not differ among different gestational time points in tg-CYP2D6 mice (Koh et al., 2014a). Interestingly, however, HNF4 α activity reflected in the extent of HNF4 α recruitment to the CYP2D6 promoter exhibited significant increases at term pregnancy as compared with the prepregnancy or postpartum period. The pivotal role of HNF4 α in CYP2D6 induction during pregnancy was further verified in mice in which HNF4 α is conditionally knocked down in the liver; CYP2D6 induction during pregnancy was abrogated in these mice (Koh et al., 2014a).

HNF4 α activity is known to be modulated by its interaction with other transcription factors (Gonzalez, 2008), and altered expression of these transcription factor can affect HNF4 α activity. For example, enhanced

expression of peroxisome proliferator-activated receptor γ coactivator 1 α (a coactivator of HNF4 α) by glucagon leads to elevated expression of HNF4 α target genes (Yoon et al., 2001). cDNA microarray experiments using liver tissues of tg-CYP2D6 mice collected at different gestational time points revealed one downregulated [i.e., small heterodimer partner (SHP)] and seven upregulated transcription factors including Krüppel-like factor 9 (KLF9), in the livers of pregnant mice (as compared with those of nonpregnant mice) (Koh et al., 2014a,b). SHP is a nuclear receptor that does not have a DNA-binding domain, and behaves as a corepressor by physically interacting with various transcription factors (including HNF4 α) and inhibiting their activities (Zhou et al., 2010). In HepG2 cells, SHP overexpression suppressed HNF4 α transactivation of the CYP2D6 promoter (Koh et al., 2014a). In tg-CYP2D6 mice, SHP knockdown (using small interfering RNA) led to a significant increase in CYP2D6 expression. These studies in vitro and in vivo systems demonstrated a role of SHP as a repressor of CYP2D6 expression.

HNF4 α is involved in regulation of a large number of hepatic genes (Gonzalez, 2008). Despite the role of HNF4 α as a master regulator of hepatic genes, HNF4 α appeared to regulate expression of its target genes in a gene-specific manner during pregnancy. For example, results in tg-CYP2D6 mice showed that expression of other target genes of HNF4 α (e.g., ApoC2) did not increase during pregnancy (Koh et al., 2014a). These results suggest a potential role of promoter contexts in modulating HNF4 α transactivation of its target genes and the presence of additional regulatory factors potentially involved in CYP2D6 regulation during pregnancy, such as KLF9. KLF9 mediates various biologic functions, including proliferation of keratinocytes or progesterone signaling in the reproductive system (Zhang et al., 2003; Spohl et al., 2012), and its role in the liver is not well understood. In tg-CYP2D6 mice, KLF9 was among one of seven transcription factors whose expression was upregulated during pregnancy (Koh et al., 2014b). In HepG2 cells, KLF9 (but not other upregulated transcription factors) weakly activated CYP2D6 promoter by itself, and more importantly, KLF9 significantly enhanced HNF4 α transactivation of the CYP2D6 promoter (Koh et al., 2014b). Although the detailed molecular mechanisms remain unclear, the proximal promoter region (−22/−14) of CYP2D6 that harbors the KLF9 binding site was found to be critical in the potentiation of HNF4 α transactivation of the CYP2D6 promoter (Koh et al., 2014b), suggesting that the KLF9 action involves direct binding of KLF9 to the CYP2D6 promoter. Concurrent changes in SHP and KLF9 expression (i.e., decreased SHP and increased KLF9) activated CYP2D6 promoter in a synergistic manner in HepG2 cells, suggesting potential roles of multiple transcription factors (and their functional interplay) in differential regulation of HNF4 α target genes during pregnancy.

What triggers KLF9 induction during pregnancy remains unclear, but currently available evidence suggests potential involvement of cortisol in KLF9 and CYP2D6 regulation in pregnancy. The plasma concentrations of free cortisol are 1.5- to 2-fold higher at term pregnancy as compared with the prepregnancy levels, and cortisol increases CYP2D6 expression in human hepatocytes (Farooq et al., 2016). While it remains to be examined whether KLF9 plays a key role in the cortisol action on CYP2D6 expression in liver tissues, cortisol was previously shown to increase KLF9 expression in keratinocytes and hippocampal neurons (Bagamasbad et al., 2012; Spohl et al., 2012).

Together, our studies to elucidate mechanisms underlying CYP2D6 induction during pregnancy revealed novel transcription factors involved in transcriptional regulation of CYP2D6 expression (i.e., SHP and KLF9). Better understanding of the roles of these transcription factors in the regulation of CYP2D6 expression may provide a mechanistic basis to identify factors contributing to interindividual variability in CYP2D6-mediated drug metabolism and improve drug therapy for CYP2D6 substrates.

Modulators of CYP2D6 Transcription

Farnesoid X Receptor Agonists. SHP is a representative target gene of a nuclear receptor farnesoid X receptor (FXR). FXR is a bile acid sensor and is activated by endogenous bile acids. Upon activation, FXR transactivates the SHP promoter by binding to FXR response elements in the proximal promoter region (Chanda et al., 2008). SHP (as a corepressor) in turn inhibits the activity of other transcription factors (such as HNF4 α) and modulates expression of genes involved in bile acid homeostasis (Li and Chiang, 2014). SHP expression in Fxr-null mice was only ~20% of that in the wild-type mice, indicating that FXR is critical in governing basal SHP expression (Sinal et al., 2000). Synthetic FXR agonists [such as 3-(2,6-dichlorophenyl)-4-(3'-carboxy-2-chlorostilben-4-yl)oxymethyl-5-isopropylisoxazole (GW4064)] or endogenous bile acids enhance SHP expression by activating FXR.

In primary human hepatocytes, GW4064 treatment (for 48 hours) led to increased SHP and decreased CYP2D6 expression and activity (Pan et al., 2015). A similar finding was observed in tg-CYP2D6 mice treated with GW4064 (10 mg/kg/day, intraperitoneally administered for 5 days). CYP2D6 repression by GW4064 was abrogated when Shp was deleted in tg-CYP2D6 mice, indicating essential roles of SHP in CYP2D6 repression by GW4064 (Pan et al., 2015). Importantly, these results provide proof-of-concept evidence for the roles of SHP modulators in altering CYP2D6 expression, and suggest the possibility that other modulators of SHP may also affect CYP2D6 expression.

Recent studies revealed that, in addition to maintaining bile acid homeostasis, FXR plays a key regulatory role in glucose and lipid homeostasis (Ma et al., 2006; Zhang et al., 2006; Thomas et al., 2008). Fxr-null mice develop severe fatty liver, associated with elevated serum glucose and impaired glucose and insulin tolerance (Ma et al., 2006), and FXR activation (by using GW4064) improves hyperglycemia and hyperlipidemia in diabetic mice (Zhang et al., 2006). Numerous FXR agonists are under development as novel therapeutics for metabolic diseases (such as nonalcoholic steatohepatitis and diabetes) and are at different stages of preclinical and clinical studies (Thomas et al., 2008). The possibility of potential drug-drug interactions between these FXR agonists and CYP2D6 substrates (through enhanced SHP expression) remains to be examined clinically.

Estrogen. Estrogen is the major component in oral contraceptives and hormone replacement therapy. Estrogen regulates growth and differentiation as well as multiple physiologic functions by activating the estrogen receptor. Although estrogen at physiologic concentrations does not seem to have significant effects on CYP2D6-mediated drug metabolism (Bock et al., 1994; Tamminga et al., 1999; Zanger et al., 2005), intrahepatic cholestasis that often accompanies the therapeutic use of estrogen may alter CYP2D6 expression. Intrahepatic cholestasis has been reported in women on oral contraceptives or (postmenopausal) hormone replacement therapy (Schreiber and Simon, 1983), or in men taking estrogens for the treatment of prostate cancer (Kontturi and Sotaniemi, 1969). Estrogen-induced cholestasis also occurs during pregnancy, and intrahepatic cholestasis of pregnancy is the most common liver disease during pregnancy (Reyes, 1997; Riely and Bacq, 2004).

Considering that cholestasis leads to FXR activation and subsequent upregulation of SHP (Goodwin et al., 2000), the effects of estrogen-induced cholestasis on CYP2D6 expression were examined in tg-CYP2D6 mice. Mice administered high-dose ethinylestradiol (10 mg/kg/day; subcutaneous injection for 5 days) exhibited increased SHP expression accompanied by 2- to 3-fold decreases in CYP2D6 expression and activity (Pan and Jeong, 2015). In these mice, recruitments of SHP and HNF4 α to the CYP2D6 promoter were increased and decreased, respectively, suggesting that enhanced SHP

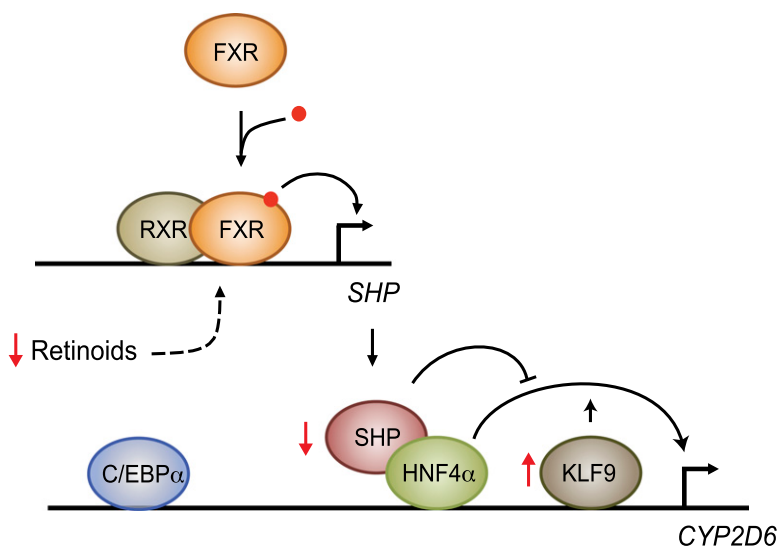


Fig. 1. Potential factors affecting CYP2D6 transcription. Transcriptional activators of CYP2D6 that are known so far include HNF4 α , C/EBP α , and KLF9. FXR activation by bile acids or synthetic FXR agonists leads to upregulation of SHP, a transcriptional repressor of CYP2D6 expression. Retinoids induce SHP expression in liver tissues by as-yet-unknown mechanisms. During pregnancy, hepatic retinoid levels decrease, and this is accompanied by reduced SHP expression. Concurrently, KLF9 expression increases. These changes potentially explain CYP2D6 induction observed in pregnant women. Red arrows represent directional changes in its hepatic levels or expression during pregnancy.

expression represses HNF4 α transactivation of CYP2D6 promoter in ethinylestradiol-induced cholestasis.

Bile acids are major products of cholesterol catabolism in the liver and are major components of bile. Primary bile acids (cholic acid and chenodeoxycholic acid) are synthesized in human liver and are mainly conjugated with **taurine or glycine** before excretion into bile canaliculi and then the small intestine. Once in the intestine, the conjugated primary bile acids are deconjugated and converted into secondary bile acids (e.g., deoxycholic acid and lithocholic acid) by gut microbiota. Most bile acids are reabsorbed from the intestine, conjugated in the liver, and re-excreted into bile (i.e., enterohepatic circulation). Human hepatic bile acids are mainly (>95%) composed of conjugated or unconjugated cholic acid, chenodeoxycholic acid, and deoxycholic acid (Garcia-Canaveras et al., 2012), the most potent bile acid species in the activation of FXR (Wang et al., 1999; Lew et al., 2004). In human liver tissues, concentrations of bile acids exhibit large interindividual variations (i.e., coefficient of variation greater than 100% for major bile acids) (Garcia-Canaveras et al., 2012). Potential roles of bile acids in the regulation of CYP2D6 expression and interindividual variability in CYP2D6-mediated drug metabolism remain to be defined.

Retinoids. Retinoids are derivatives of vitamin A and play a key role in physiologic processes, including vision, **epithelial differentiation, embryogenesis, and reproduction** (D'Ambrosio et al., 2011; Napoli, 2012). The liver is a major organ for retinoid storage and metabolism. Retinoids are stored in hepatic stellate cells in the form of retinyl ester, which is then converted to bioactive retinoic acid (RA) in hepatocytes. (Shirakami et al., 2012). Among three stereoisomeric forms of RA [*all trans* RA (atRA), 9-*cis* RA, and 13-*cis* RA], atRA is the major RA that exerts biologic activities via binding to its cognate nuclear receptor (i.e., retinoic acid receptor). 9-*cis* RA and 13-*cis* RA can be converted to atRA in the body (Chen and Juchau, 1998). RAs are also used as pharmacological agents for the treatment of cancers or skin diseases (Baldwin et al., 2013; Veal et al., 2013; Chen et al., 2014).

The mechanistic studies for CYP2D6 induction during pregnancy revealed that decreased SHP expression at term pregnancy is potentially responsible for the phenomenon (Koh et al., 2014a). A previous study reported that atRA induces SHP expression in HepG2 cells (Cai et al., 2010), and this is potentially responsible for hyperlipidemia often seen in patients treated with high-dose RA for cancer treatment. More importantly, this raises the possibility that altered retinoid homeostasis during pregnancy may underlie **decreased SHP expression**. Indeed, in

tg-CYP2D6 mice, hepatic atRA levels were significantly decreased at term pregnancy as compared with prepregnancy (Koh et al., 2014a). Also, in nonpregnant tg-CYP2D6 mice, atRA (5 mg/kg/day, intraperitoneally administered for 5 days) significantly increased hepatic SHP expression and decreased CYP2D6 mRNA and activity levels (Koh et al., 2014a), suggesting that lower hepatic retinoids may be responsible for SHP downregulation during pregnancy. Similar changes in SHP and CYP2D6 expression were observed in human hepatocytes upon atRA treatment (data not shown; Won et al., manuscript in preparation), indicating that CYP2D6 regulation by atRA is likely conserved in humans. The molecular mechanisms underlying how retinoids upregulate SHP expression are currently unclear. In healthy adults, hepatic levels of hepatic vitamin A exhibit ~300-fold interindividual variability (Ukleja et al., 2002), and whether this plays a role in interindividual variability in CYP2D6-mediated drug metabolism remains to be determined.

Conclusion

Results from recent studies revealed novel transcriptional regulators of CYP2D6 (i.e., SHP and KLF9), and upstream regulators of these transcription factors (including retinoids and FXR activators) have been shown to modulate CYP2D6 expression in *in vitro* and *in vivo* settings (Fig. 1). Together, these studies advance our current understanding of transcriptional regulation of CYP2D6, providing a basis to predict potential drug-drug or drug-disease interactions involving CYP2D6 substrates, and to identify factors contributing to interindividual variability in CYP2D6-mediated drug metabolism.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Pan, Ning, Jeong.

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