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CYP2D6 protein level is the major contributor to inter-individual variability in CYP2D6-mediated drug metabolism in healthy human liver tissue

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Abstract

CYP2D6 genetic polymorphisms are considered a major contributor to the large inter-individual variability in *CYP2D6*-mediated drug metabolism, but fail to explain significant portion of the variability. The aim of this study was to assess the ability of *CYP2D6* activity score (AS) estimated from *CYP2D6* genotype to predict *CYP2D6* expression and enzyme activity. The *CYP2D6* gene region was sequenced in 115 healthy human liver tissue samples to determine their *CYP2D6* AS. Additionally, *CYP2D6* enzyme activity, protein, and mRNA levels were estimated. *CYP2D6* AS explained 23% of the inter-individual variability in *CYP2D6* activity, but only 7.5% in tissues assigned AS 1-2. *CYP2D6* protein level was found to be the major determinant of *CYP2D6* activity, explaining 59% of variability. These findings suggest that while *CYP2D6* AS is a good predictor of poor metabolizer phenotype, additional non-genetic factors may govern the rate of *CYP2D6*-mediated metabolism in those without the poor metabolizer phenotype.

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Author Contributions

M.N., J.D., L.R., and H.J. wrote the manuscript; M.N., J.D., and H.J. designed the research; M.N. performed the research; M.N., J.D., L.R., and H.J. analyzed the data.

Keywords

CYP2D6; liver tissues; genetic polymorphisms; variability

Introduction

CYP2D6 is a major drug-metabolizing enzyme responsible for eliminating over 20% of clinically used drugs.¹ Substrates of CYP2D6 include tricyclic antidepressants (e.g. desipramine), selective serotonin receptor inhibitors (e.g., paroxetine), β -blockers (e.g., metoprolol), opioid analgesics (e.g., codeine) and anti-cancer agents (e.g., tamoxifen).¹ Notably, CYP2D6-mediated drug metabolism exhibits large inter-individual variability. Urinary metabolic ratio (MR; a ratio of parent drug amount over metabolite amount) estimated after administration of a CYP2D6 substrate (e.g., dextromethorphan) ranges over several orders of magnitude in different individuals.² Based on the results from urinary MR, an individual can be classified as ultrarapid metabolizer (UM), normal metabolizer (NM), intermediate metabolizer (IM) and poor metabolizer (PM) of CYP2D6.² CYP2D6 phenotype typically shows bimodal or trimodal distribution in European and US population, with PMs accounting for 5–10% of the populations.^{2, 3}

Genetic polymorphisms of *CYP2D6* are considered a major contributor to inter-individual variability in CYP2D6 activity. To date, over 100 *CYP2D6* allelic variations have been identified.⁴ These *CYP2D6* variants can be characterized by the presence of single nucleotide polymorphisms (SNPs), gene hybrids with the pseudogene *CYP2D7*, gene deletion, or gene duplications/multiplications.⁵ The outcomes can range from normal (e.g., *CYP2D6**2), decreased (e.g., *CYP2D6**10), increased (e.g., *CYP2D6**1×N), or no (e.g., *CYP2D6**5) function of the CYP2D6 enzyme. Considering the large number and complexity of *CYP2D6* genetic polymorphisms, the CYP2D6 activity score (AS) system was introduced as a simplified tool to estimate the contribution of *CYP2D6* genotypes to clinical phenotype.² The previous study has shown a strong relationship between CYP2D6 AS and clinically observed MR of a CYP2D6 substrate, demonstrating that mean CYP2D6 activity increases with higher CYP2D6 AS.²

Interestingly, while individuals with an AS of 0 (i.e., both *CYP2D6* alleles are non-functional) were consistently found to exhibit the PM phenotype, substantial inter-individual variability in CYP2D6 activity was observed in non-PM populations. For example, CYP2D6 activity (when determined by measuring urinary MR of dextromethorphan) exhibited significant overlaps among different groups of AS>0.² Furthermore, large inter-individual variability in CYP2D6 activity, ranging three orders of magnitude, was observed in wild-type (*wt*) carriers of *CYP2D6*.² Of note, a distant enhancer SNP (rs5758550) linked to *CYP2D6**2 is known to modulate the enhancer activity for *CYP2D6* expression,⁶ and differential urinary pH can alter urinary excretion of dextromethorphan and its metabolite (leading to variable urinary MR).⁷ The extent of their contribution to differential CYP2D6 expression and activity is unclear. Together, the ability of *CYP2D6* genetic polymorphisms to predict variability in CYP2D6-mediated drug metabolism remains to be further defined.

The goal of this study was to assess the ability of genotype-derived CYP2D6 activity scores to predict CYP2D6 expression and enzyme activity in the liver. To this end, we used high-throughput DNA sequencing to thoroughly identify *CYP2D6* variants, and systematically evaluated the utility of CYP2D6 AS for the prediction of in vitro metabolic activity (and enzyme expression) in over 100 healthy human liver tissue samples.

Results

Human liver tissue

One hundred and fifteen liver tissue samples were included in the study (mean age 49 years; 70 males and 45 females). While age and sex are known not to affect CYP2D6-mediated drug metabolism,^{8–10} these factors (including the source of liver tissues) were included in data analysis as potential covariates to ensure the robustness of the analysis. Ethnicity information was available for 50 of the samples (41 Caucasian, 3 African American, 5 Asian, and 1 Hispanic).

Variability in CYP2D6 activity explained by CYP2D6 AS

CYP2D6 activity levels in the liver tissues were estimated by using a CYP2D6 probe drug, dextromethorphan. S9 fractions were used to minimize variability introduced during tissue preparation and processing.¹¹ Dextromethorphan formation rate in the human liver S9 fractions exhibited large inter-individual variability, ranging from 0.23 to 74 pmol dextromethorphan/min/mg S9 protein (median 3.4 pmol/min/mg S9 protein) (Figure 1a). The distribution of CYP2D6 activity followed log-normal distribution.

Next, genetic polymorphisms of *CYP2D6* gene were identified. The majority (n=94; 82%) of the tissues had two copies of *CYP2D6*. Eleven liver samples had one copy, and one sample had 0 copies. The remaining nine samples had a copy number of >2 when a probe targeting intron 6 of *CYP2D6* was used (Figure 1b).

The *CYP2D6* gene region (9.4-kb total; from 4-kb upstream to 1-kb downstream) was sequenced by using high-throughput DNA sequencing complemented with Sanger sequencing. Eighteen *CYP2D6* allelic variants (including three rare variants *22, *23, and *108) and subsequently 38 *CYP2D6* diplotypes were identified. We did not identify any novel nonsynonymous SNPs in *CYP2D6* in this cohort of tissues. *CYP2D6* alleles were assigned based on established *CYP* allele nomenclature, and the frequency of detected alleles is shown in Table 1. CYP2D6 activity levels varied widely in the samples of different *CYP2D6* genotypes (Figure 1b). Also, large variation in CYP2D6 activity was noted in the samples of the same genotype (e.g., *CYP2D6**1/*1) (Figure 1b). To evaluate the utility of AS in predicting CYP2D6 activity levels in liver tissue, AS was calculated for each liver sample. Median dextromethorphan formation rate in AS=0 samples was 1.1 pmol/min/mg S9 protein (Table 2). The median CYP2D6 activity levels in S9 fractions increased with AS (Table 2; Figure 1c, $p<0.0001$). However, large inter-individual variability in CYP2D6 activity levels was observed within each AS category (Figure 1a). Considering that a distant enhancer SNP (rs5758550) linked to *CYP2D6**2 can modulate the enhancer activity for *CYP2D6* expression and thus CYP2D6 activity,⁶ the SNP was detected in the samples of

AS=2 and its association with CYP2D6 activity was examined. CYP2D6 activity level did not differ between rs5758550A and G genotypes ($p=0.7$; data not shown).

The extent of inter-individual variability in CYP2D6 activity that can be explained by CYP2D6 AS was estimated by using linear regression analysis with adjustment for the covariates age, sex, and liver source. Overall, 23% of variability in CYP2D6 activity was explained by AS after adjustment for covariates. In the samples exhibiting AS of 1 to 2 (~80% of the samples), AS accounted for 7.5% of the variability in CYP2D6 activity.

Variability in CYP2D6 activity explained by differential expression of CYP2D6

To further identify the sources of CYP2D6 variability, CYP2D6 protein expression in liver S9 fraction was measured by using semi-quantitative western blots. In tissues with detectable CYP2D6 protein levels, the protein expression exhibited log-normal distribution (Figure 2a). Eleven liver samples exhibited CYP2D6 protein levels below the detection limit (equivalent to 0.5 in Figure 2a); nine were carriers of two no-function *CYP2D6* alleles (*CYP2D6**4, *5 and *6). Interestingly, CYP2D6 protein was not detectable in two samples that carried only one no-function allele (*CYP2D6**1/*4 and *4/*4I) for as-yet-unknown reasons. The mean CYP2D6 activity level of these eleven samples was 1.2 pmol dextrorphan production/min/mg S9 protein. Overall, CYP2D6 activity was highly correlated with its protein expression (Figure 2b, $p<0.0001$), consistent with previous reports.^{12–14} Results from regression analysis of all liver samples indicated that 59% of the variability in CYP2D6 activity could be explained by differential CYP2D6 protein expression when adjusted for sex, age and liver source.

To examine whether genetic polymorphisms have any impact on the association between CYP2D6 protein expression and activity, we further examined the associations separately for each AS group. The association was examined only in the AS groups of 1, 1.5, and 2 as the other groups had very small sample sizes (i.e., $n<10$). Association between CYP2D6 activity and protein levels was strongest in AS=2 group, and were less robust in the samples with a lower AS (Figure 2c). To determine whether genetic heterogeneity may diminish the correlation, association between CYP2D6 activity and protein levels was examined separately for the carriers of common *CYP2D6* variants. Within the AS=1 group, the association between CYP2D6 activity and protein levels in (the most frequent) *CYP2D6**4/*wt* carriers was stronger compared to that for the whole AS=1 group (Figure 2c; adjusted $r^2=0.54$ vs. 0.33, for *4/*wt* and the whole AS=1 groups, respectively).

To determine the extent of variability in CYP2D6 activity level that could be explained by differential transcription of the gene, we examined CYP2D6 mRNA expression by quantitative real time (qRT)-PCR. The distribution of CYP2D6 mRNA levels was generally log-normal (Figure 3a), and one tissue (the only *CYP2D6**5/*5 carrier in our study) had no amplification, from a *CYP2D6* gene deletion.

Results from the regression analysis showed that CYP2D6 activity levels are significantly correlated with the mRNA levels in tissues of AS>0 (Figure 3b, $p<0.0001$), with the adjusted r^2 value of 0.25 ($r^2 = 0.21$ when all 115 samples were analyzed). Considering that the amount of variability in CYP2D6 activity explained by differential mRNA levels is much

smaller than that observed with CYP2D6 protein levels, the sources of disconnect between CYP2D6 mRNA and activity levels were explored. Specifically, the relationship between CYP2D6 protein and mRNA levels in the liver tissues was examined. CYP2D6 protein level was significantly associated with its mRNA expression (Figure 3c; adjusted $r^2 = 0.11$; $p < 0.0001$). Considering that certain genetic polymorphisms in *CYP2D6* that are expected to cause the disconnect between mRNA and protein levels (e.g., *CYP2D6**4 allele causing a frameshift in protein translation¹⁵ or *CYP2D6**41 favoring the production of a mRNA splice variant lacking exon 6¹⁶), correlation between CYP2D6 mRNA and protein levels was examined in the absence of such variants. The mRNA levels of CYP2D6 in carriers of the *CYP2D6**1 and *2 alleles (i.e., *wt* carriers) did not differ from *CYP2D6**4 or *41 carriers (Figure 3d), whereas CYP2D6 protein levels significantly decreased in *4 carriers and showed a trend toward decreased levels ($p = 0.06$) in *41 carriers when compared with *wt* carriers. In the carriers of *CYP2D6**4 (frameshift), *6 (frameshift), *41 (splicing defect) and *36 (*CYP2D7* gene hybrid) alleles (i.e., open circles in Figure 3e), CYP2D6 protein and mRNA levels did not correlate ($p = 0.54$). In the remaining samples, the correlation between CYP2D6 mRNA and protein levels improved to the adjusted r^2 value of 0.17 (from $r^2 = 0.11$) (Figure 3e). This level of correlation was similar to that in the carriers of *wt* only ($n = 37$; $r^2 = 0.17$; data not shown).

Discussion

This is the first study to our knowledge that quantitatively assessed the ability of *CYP2D6* genetic polymorphisms and AS to predict CYP2D6 expression and activity levels in human liver tissue. A systematic approach was undertaken to detect nearly all *CYP2D6* genotypes (including rare variants) and measure mRNA, protein, and activity levels of CYP2D6 in over 100 snap-frozen healthy human liver tissues. This allowed examination of CYP2D6 genotype-phenotype relationship in the absence of potential confounding factors that can affect the CYP2D6 phenotype *in vivo*, such as urinary pH.

CYP2D6 activity in the samples exhibited large inter-individual variability and apparent log-normal distribution, consistent with the previous results for CYP2D6 activity or protein levels in hepatic microsomes.^{17, 18} The frequencies of different genotypes and copy number variation of *CYP2D6* observed in this study were comparable to the reported values in those of European descent or US population.^{19, 20} Also, the distribution of CYP2D6 AS in our liver cohort was comparable to the previous reported in a population of European ancestry.¹⁹ Consistent with clinical observations using dextromethorphan urinary MR or metoprolol AUC as markers of CYP2D6 activity levels,^{2, 21} a significant positive correlation between AS and CYP2D6 activity was observed. However, CYP2D6 activity levels within each AS category varied widely (over two orders of magnitude), and extensive overlap among different AS categories were observed especially in the samples with AS > 0. Overall, AS explained 23% of the inter-individual variability in CYP2D6 activity. In the tissue samples with an AS of 1–2 (conventionally associated with EM phenotype; >80% of samples), the portion of CYP2D6 variability in activity explained by AS shrunk to 7.5%. No-function alleles of *CYP2D6* (i.e., *CYP2D6**4, *5, *6, and *12) alone accounted for 14% of the variability in CYP2D6 activity, suggesting that the overall *CYP2D6* genotype-phenotype association may be driven primarily by the effect of no-function *CYP2D6* alleles in the

tissues. The extent of overall association between *CYP2D6* AS and enzyme activity observed in our samples is much smaller as compared to the clinically reported studies where 50–60% of variability in urinary MR (of dextromethorphan) is explained by *CYP2D6* genotypes and AS.² Different magnitude of variability (i.e., urinary MR and hepatic *CYP2D6* activity varying over six and three orders of magnitude, respectively) may in part explain the difference. MR (dextromethorphan/dextrorphan amount ratio) reflects changes in the amount of both the parent drug and metabolite. This can potentially magnify the distinction between PM and non-PM subjects, leading to larger *CYP2D6* variability in urinary MR than in liver tissues, as well as a greater extent of *CYP2D6* variability explained by *CYP2D6* AS and genotypes.

In liver samples with AS>0, *CYP2D6* activity was highly correlated with its protein level, suggesting that *CYP2D6* protein expression governs its activity levels in the liver, consistent with the previous reports.^{13, 14, 22} Nearly 60% of the variability in *CYP2D6* activity can be explained by differential *CYP2D6* protein expression in the tissues. Multiple factors may account for the remaining variability. First, while dextromethorphan O-demethylation (to form dextrorphan) is mediated primarily by *CYP2D6*, other CYPs are known to mediate this biotransformation at a slower rate.^{23, 24} Consistent with this notion, we found that a small amount of dextrorphan was formed in liver tissues carrying only no-function *CYP2D6* alleles (i.e., AS=0). Second, prior medication use of liver tissue donors may have impacted *CYP2D6* enzyme activity. Many drugs including paroxetine (a commonly used antidepressant) are mechanism-based inhibitors of *CYP2D6* and can decrease enzyme activity without affecting protein levels.^{25, 26} The list of medications used by donors was not available for the liver tissues used in this study. Third, the presence of glucuronidation cofactors in S9 fractions (used in this study) may have enabled dextrorphan glucuronidation to occur to varying extents. However, the basal concentration of UDP glucuronic acid (the cofactor for glucuronidation reaction) in our S9 fraction is estimated to be ~5 μM ,²⁷ and since the k_m values of UDP glucuronic acid for the typical substrates of glucuronidation enzymes are >100 μM ,²⁷ the impact of dextrorphan glucuronidation on estimation of *CYP2D6* enzyme activity should be minimal. Fourth, the *CYP2D6* antibody used in this study to measure protein quantity in human liver S9 fractions may not detect certain *CYP2D6* variants. The epitope for the antibody used in our study has not been mapped, and thus it is not known whether the antibody has similar binding affinity to *CYP2D6* proteins from variant *CYP2D6* alleles. Fifth, differential expression and/or activity of CYP partners including cytochrome P450 oxidoreductase (POR)²⁸ and cytochrome b5²⁹ may in part contribute to inter-individual variability in *CYP2D6* activity, but their levels were not measured in this study.

To test whether *CYP2D6* expression at mRNA level can serve as a predictor of *CYP2D6* activity in the liver tissues, we examined the association between *CYP2D6* mRNA and activity levels. *CYP2D6* mRNA expression in AS>0 samples accounted for about 25% of *CYP2D6* activity, which is consistent with previously reported correlations between *CYP2D6* microsomal activity and *CYP2D6* mRNA levels in human liver tissue.⁹ Interestingly, *CYP2D6* mRNA levels correlated worse with protein levels (adjusted r^2 of 0.11) than enzyme activity, indicating mRNA is likely a poor predictor of *CYP2D6* activity *in vivo*. Further, this finding likely reflects the dynamic nature of the transcription and

translation processes. For example, the estimated half-life of CYP2D6 mRNA is 4.8 hours³⁰ while CYP2D6 protein half-life is approximately 51–70 hours.^{31, 32} Due to the multiple steps involved in the regulation of gene expression in dynamic biological processes, the correlation between CYP protein and mRNA levels is expected to be imperfect even when gene expression is governed entirely at the transcriptional level.³³ Human liver tissues used in this study likely provide a snapshot view of this dynamic biological process. Of note, accumulating evidence suggests that factors modulating CYP2D6 transcription are capable of regulating CYP2D6 expression and thus the enzyme activity.^{9, 13, 17, 34, 35}

Pharmacological or pathological induction of small heterodimer partner, the transcriptional repressor of CYP2D6 expression, was shown to decrease CYP2D6 expression in CYP2D6-humanized transgenic mice.^{36–38} How much these factors (that modulate CYP2D6 transcription) contribute to inter-individual variability in CYP2D6 expression remains to be determined.

Our findings support the continued use of genotyped-derived CYP2D6 AS to predict poor metabolizers when administering drugs that are CYP2D6 substrates. We detected substantial variability in the CYP2D6 enzyme activity of samples with an AS > 0 (Figure 1 and Table 2). This, coupled with the relatively poor correlation we observed between AS and CYP2D6 activity, indicate that AS alone may not be sufficient to estimate a patient's CYP2D6 metabolizer status beyond that of a poor metabolizer. Our cohort only contained one sample with an AS > 3, and its CYP2D6 activity was within the ultrarapid metabolizer range; however, due to the small sample size, we are unable to conclude whether or not AS > 3 is a reliable estimate of UM status. While our findings suggest that CYP2D6 protein levels are, by far, the strongest predictor of enzyme activity, obtaining liver tissue samples are not practical in the clinical setting. Thus, additional factors such as concomitant CYP2D6 substrate use and patient-specific health conditions that affect CYP2D6 expression (e.g. pregnancy³⁹) must also be considered when attempting to predict a patient's metabolizer status.

To improve the predictive ability of the genotype-derived AS, additional research is needed to better characterize the functional effects of less common *CYP2D6* polymorphisms on enzyme activity *in vivo*. For example, the two samples with the lowest CYP2D6 activity and protein expression in our cohort had *10/*23 and *4/*108 genotypes. CYP2D6.23 was previously shown to exhibit the enzyme activity comparable to the wild-type CYP2D6 (using tamoxifen as a CYP2D6 substrate),⁴⁰ and enzyme activity of CYP2D6.108 is unknown. A value of 1 was assigned to each, *23 and *108, to calculate AS of 1.5 and 1, for these samples, respectively. However, our data suggest that they may be either decreased or no-function alleles (due to altered CYP2D6 expression and/or activity). Such potential misclassifications would clearly affect AS accuracy, and better functional characterization of *CYP2D6* variants would likely make the genotype-derived AS more reliable.

In conclusion, we identified CYP2D6 protein level as the major predictor enzyme activity in healthy human livers. Our results suggest that CYP2D6 AS is a reasonable predictor of CYP2D6 activity for PMs, but not necessarily for NMs. Moreover, we found that CYP2D6 mRNA levels possess a similar predictive value to AS. Further research is needed to

determine the extent of contribution by differential CYP2D6 transcription to inter-individual variability in CYP2D6-mediated drug metabolism.

Methods

Human liver tissue

Frozen normal human liver tissue samples without gross pathology were obtained from liver tissue and cell distribution system (LTCDS, funded by NIH Contract # HHSN276201200017C) and Corning Inc. Mean ages of donors were 49.4 ± 16.1 from Liver Tissue Cell Distribution System (LTCDS) (n=65; 32 males and 33 females) and 49.4 ± 14.7 for Corning Inc. tissues (n=50; 38 males and 12 females).

CYP2D6 genotyping

Genomic DNA was extracted from human liver tissues using Gentra Puregene tissue kit (Qiagen). To ensure accuracy in detecting *CYP2D6* CNV, two Taqman assays targeting intron 6 or exon 9 of *CYP2D6* gene, Hs00010001_cn and Hs04502391_cn (ThermoFisher), were used. Discrepancy between results from two assays (observed for 2 samples) was deemed caused by the presence of *CYP2D6**36 allele and multiple copies of *CYP2D6**4 allele which contain CYP2D7 gene conversion at exon 9, subsequently interfering with exon 9 CNV assay.

SNPs in CYP2D6 were examined using Ampliseq™ panels for target capture followed by Ion Torrent™ sequencing. The exon regions that could not be covered by the Ampliseq™ panel were sequenced using Sanger sequencing. Sequences were obtained from 4-kb upstream of the transcriptional start site to 1-kb downstream of the *CYP2D6* gene (hg19 chr22:42521501-42530883). Raw data from Ion Torrent™ sequencing was filtered for quality control and aligned to hg19 genome assembly. SNP-calling was completed using GATK (<https://software.broadinstitute.org/gatk/>). All the SNPs were further filtered and curated manually based on sequencing quality and Hardy-Weinberg equilibrium. Sanger sequencing results for *CYP2D6* were aligned to hg19 chr22:42525025-42525235 using novoSNP software.⁴¹ *CYP2D6* genotype assignment was based on *CYP2D6* allele nomenclature⁴ and previously published literature.⁵ CYP2D6 AS was assigned to each sample as previously described.²

Measurement of CYP2D6 activity

Frozen human liver tissue (~150 mg) was used to prepare hepatic S9 fraction as previously described.³⁶ CYP2D6 activities in S9 fractions were estimated using the formation rate of dextrophan (from dextromethorphan) as a marker. The concentrations of dextrophan in the reaction were measured by using liquid chromatography tandem mass spectrometry.

Semi-quantitative western blotting

CYP2D6 relative protein expression in S9 fraction was determined by semi-quantitative western blotting using mouse monoclonal Gentest™ anti-human CYP2D6 antibody (catalog # 458246, Corning).

RNA isolation and qRT-PCR

Total RNA was extracted using TRIzol® reagent (ThermoFisher) from human liver tissue samples. RNA quality was examined by agarose gel electrophoresis. Samples without two sharp bands of ribosomal RNA were excluded from the study. qRT-PCR was performed as previously described³⁶ using the following probes: IDT (catalog # Hs.PT.56a.45336286.g; targeting exon1-1 region; detecting all *CYP2D6* variants) and ThermoFisher (catalog # Hs00164385_m1, targeting exon2-3 region of *CYP2D6* variant 1, NM_000106.5; detecting most *CYP2D6* variants) for *CYP2D6*; ThermoFisher (catalog # Hs02800695_m1) for *HPRT1*.

Statistical analysis

Statistical analysis was performed using SAS version 9.4 (Cary, NC). All variables of interest (predictors and outcomes) were assessed for normality; non-normal distributions were normalized using log transformations. To determine basic predictor-outcome associations, a series of unadjusted linear regression analyses were conducted first. Based on these analyses, linear regressions whereby each predictor was examined in relation to the outcome after adjusting for age, sex, and source of liver were conducted.

More detailed information is available in Supplemental Information.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Study Highlights

- What is the current knowledge on the topic?
CYP2D6 genetic polymorphisms have been considered a major contributor to large inter-individual variability in *CYP2D6*-mediated drug metabolism *in vivo*.
- What question did this study address?
How well *CYP2D6* AS and genotypes serve as predictors of *CYP2D6* liver tissues for most human liver tissue has been unclear.
- What does this study add to our knowledge?
While the literature reports that *CYP2D6* AS and genotypes are good predictors of PM and UM status, our data indicate that these factors are poor predictors of *CYP2D6* activity for the majority of human liver tissue samples.
- How might this change clinical pharmacology or translational science?
CYP2D6 genotyping for clinical dosing guidance may be most useful to identify patients who are poor metabolizers. For patients with an AS>0, additional predictors may be needed to accurately predict *CYP2D6* metabolizer status *in vivo*.

Figure 1.

CYP2D6 AS explains a minor portion of variability in CYP2D6 activity level in human liver tissues. (a), CYP2D6 activity levels in human hepatic S9 fractions were measured by using a CYP2D6 probe drug, dextromethorphan. Data shown are distribution of dextromethorphan (DX) formation rate (n=115). Different colors represent samples of different AS. (b), CYP2D6 activity in tissues of different CYP2D6 genotypes is shown in the ascending order of mean values. (c), Correlation between CYP2D6 AS and CYP2D6 activity (in S9 fraction) of 115 liver tissues is shown. The line is the unadjusted linear regression line between Log CYP2D6 activity and *CYP2D6* AS.

Figure 2.

CYP2D6 protein amount governs CYP2D6 activity levels in human liver tissues. (a), Distribution of CYP2D6 protein levels (n=104). Not shown are 11 liver tissues whose CYP2D6 protein levels were below detection limit (i.e., relative CYP2D6 protein level of 0.5). (b), Overall correlation between CYP2D6 activity and protein expression in liver tissues with detectable CYP2D6 protein levels (n=104) is shown. The line denotes unadjusted linear regression of Log CYP2D6 activity vs. Log protein expression. CYP2D6 activity is expressed as dextrorphan (DX) formation rate in S9 fractions. (c), Correlation

between CYP2D6 activity and protein expression stratified by AS is shown. The lines are unadjusted linear regression lines between Log CYP2D6 activity and Log CYP2D6 protein expression for each AS category. r^2 value is the square of adjusted correlation coefficient for all samples in the group. For AS=1 group, r^2 value is 0.54 for *CYP2D6*4/wt* carriers.

Figure 3.

Differential CYP2D6 mRNA levels in part explain the variability in CYP2D6 protein expression in human liver tissues. (a), Distribution of CYP2D6 mRNA levels in liver tissues (n=114). Not shown is the tissue with gene deletion (i.e. *CYP2D6**5/*5; no CYP2D6 mRNA amplification). (b), Overall correlation between CYP2D6 activity, measured as dextromethorphan (DX) formation rate, and mRNA levels in liver tissue samples of AS>0 is shown. r^2 value is the square of adjusted correlation coefficient. (c), Overall correlation between CYP2D6 protein and mRNA levels in tissues with detectable CYP2D6 protein

levels (n=104) is shown. r^2 value is the square of adjusted correlation coefficient. (d), CYP2D6 mRNA (left) and protein (right) expression in CYP2D6 *wt/wt*, **4* (i.e., **4/*4*, **4/wt*) and **41/wt* carriers is shown. Welch's t-test was conducted between *wt* group and **4* group (or **41* group). **, $p < 0.01$. (e), Correlation between CYP2D6 protein and mRNA levels is shown. Open circles represent the carriers of **4*, **6*, **41*, **4x2* and **36x2* alleles (n=41) that were excluded from regression analysis. Filled dots represent the rest of samples (n=63) that were included in regression analysis, and corresponding r^2 value and linear regression line (Log CYP2D6 protein vs. Log CYP2D6 mRNA) are shown.

Table 1Frequency of *CYP2D6* alleles detected in human liver tissues.

Genotype	Frequency	Average frequency in European descendent ¹⁹
*1	0.387	0.383
*2	0.178	0.278
*4	0.139	0.182
*5	0.057	0.029
*6	0.03	0.009
*10	0.030	0.024
*12	0.004	0.0001
*17	0.030	0.004
*22	0.004	NA
*23	0.004	NA
*41	0.070	0.074
*45	0.017	NA
*108	0.004	NA
*1×N	0.004	0.008
*2×N	0.017	0.012
*4×N	0.009	0.002
*10×N	0.009	0.0005
*36×N	0.004	NA

NA: not available, not examined by most studies.

Table 2

Distribution of *CYP2D6* AS and corresponding *CYP2D6* enzyme activity.

AS	<i>CYP2D6</i> activity median (pmol DX/min/mg protein)	Number of samples with low <i>CYP2D6</i> activity (<1 pmol DX ^b /min/mg protein)	Number of samples with medium <i>CYP2D6</i> activity (1–10 pmol DX/min/mg protein)	Number of samples with high <i>CYP2D6</i> activity (>10 pmol DX/min/mg protein)	Number of samples
0	1.1 (0.54–2.28) ^a	4	5		9
0.5	1.8 (0.55–9.2)	3	5		8
1	2.7 (0.23–14)	5	25	2	32
1.5	3.9 (0.14–26)	4	13	6	23
2	5.1 (0.7–60)	1	26	11	38
>2	17 (2.8–74)		2	3	5

^aNumber in parenthesis represents the range of values

^bDextrothorphan