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Minireview

Leveraging in Vitro Models for Clinically Relevant Rare *CYP2D6* Variants in Pharmacogenomics

Sydney Stern, Paula L. Hyland, Michael Pacanowski, and Robert N. Schuck

Center for Drug Evaluation and Research, Office of Translational Science, Office of Clinical Pharmacology, US Food and Drug Administration, Silver Spring, Maryland

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ABSTRACT

Cytochrome P450 2D6 (CYP2D6) is responsible for the metabolism of up to 20% of small-molecule drugs and therefore, may impact the safety and efficacy of medicines in broad therapeutic areas. *CYP2D6* is highly polymorphic, and the frequency of variants can differ across racial and ethnic populations, significantly affecting enzymatic function and drug metabolism. However, rare variants of *CYP2D6* present a unique challenge for academia, industry, and regulatory agencies alike due to the lack of feasibility of characterizing their clinical relevance in clinical trials, particularly in variants that exhibit population-specific frequencies in racial and ethnic groups that are poorly represented in clinical trials. Despite significant advancement in pharmacogenomics, the substrate specificity and related clinical relevance of these *CYP2D6* rare variants remain largely unclear, and further efforts are warranted to characterize the burden of these variants on adverse drug reactions and drug efficacy. Thus, cell-based in vitro systems can be used to inform substrate-specific effects and the overall relevance of a rare variant. Liver microsomes, cell-based expression systems, ex vivo primary samples, and purified variant protein have all been used with various substrates to potentially predict the clinical impact of new substrates. In this review, we identify rare variants of *CYP2D6* that demonstrate differences across races in prevalence and thus are often unassessed in clinical trials. Accordingly, we examine current pharmacogenomic in vitro models used to analyze the functional impact of these rare variants in a substrate-specific manner.

SIGNIFICANCE STATEMENT

Variants of CYP2D6 play a clinically relevant role in drug metabolism, leading to potential safety and efficacy concerns. Although the influence of prevalent variants is often well characterized, rare variants are traditionally not included in clinical trials. This review captures the clinical relevance of rare variants in *CYP2D6* by highlighting in vitro models that analyze their impact on the metabolism of CYP2D6 substrates.

Introduction

Pharmacogenomics (PGx) is the study of variation of DNA and RNA characteristics as related to drug response (https://www.fda.gov/media/71389/download). Myriad examples of genetic variants in drug-metabolizing enzymes are associated with changes in drug pharmacokinetics and thus may have downstream effects on drug efficacy and/or safety. For example, some adverse drug reactions are caused, at least in part, by genetic variations leading to overexposure of a medication that exceeds therapeutic range (Zanger and Schwab, 2013; Micaglio et al., 2021). Similarly, genetic variations may lead to lower drug exposures that are below the therapeutic range, potentially compromising efficacy. Thus, the identification and evaluation of genetic factors that predispose patients to adverse drug reactions or preemptively recognize inadequate responders has the potential to improve patient care, and in some cases, pharmacogenomic testing may be recommended or required to guide prescribing.

Cytochrome P450s (P450s) are the major metabolic system for phase I metabolism, responsible for approximately 80% of oxidative metabolism and 50% of overall elimination of drugs (Zhao et al., 2021). The activity of each P450 enzyme can be classified into genotype-inferred phenotypes—poor metabolizer (PM), intermediate metabolizer (IM), normal metabolizer (NM), or ultra-rapid metabolizer—by the combination of alleles. Different alleles are typically denoted with asterisk (*) nomenclature, where each haplotype is assigned an asterisk number (e.g., *1) (https://www.pharmvar.org/documents/Allele_Designation_and_Evidence_Level_Criteria_V2.1.pdf). P450s are known to be highly polymorphic with multiple variant alleles; thus, detection of these alleles can predict metabolic phenotypes and potentially be used to guide drug therapy (van der Weide and Steijns, 1999). However, different variants may have different effects on the metabolic function of the P450 enzyme, ranging from a complete loss of function (LOF), resulting from a premature stop codon, splicing defects, deletions, missense mutations, and/or frameshift (Zanger and Schwab, 2013), to gain of function.

Although some variants are relatively common and can be characterized in clinical trials of individual drugs, others are rare or only prevalent

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ABBREVIATIONS: AMP, Association for Molecular Pathology; CPIC, Clinical Pharmacogenetics Implementation Consortium; FDA, Food and Drug Administration; IM, intermediate metabolizer; LOF, loss of function; NM, normal metabolizer; P450, cytochrome P450; PBPK, physiologically based pharmacokinetic modeling; PGx, pharmacogenomics; PK, pharmacokinetic; PM, poor metabolizer; WT, wild type.

in populations that are frequently underrepresented in clinical trials, making clinical assessments infeasible. Therefore, precisely defining the functional effect of a variant is the first step in determining if the variant may have clinically significant effects on the metabolism of certain drugs. For instance, variants that lead to complete LOF are absent of enzymatic capacity and associated with a PM phenotype for all substrates. Thus, results of clinical studies for common variants associated with PM phenotypes can be confidently extrapolated to rare LOF variants. However, for reduced-function variants, the magnitude of effect may depend on the underlying nature of the variant and may be substrate specific. Therefore, findings from clinical studies of common reduced-function variants cannot be ubiquitously extrapolated to rare variants. In vitro systems used to assess the metabolism of drugs by individual P450s have drastically improved our ability to predict drug-drug interactions in clinical studies and may also help predict the clinical impact of rare genetic variants by determining their functional impact. However, due to differences between in vitro systems as well as the anticipated substrate-specific effects on different drugs, it is critical to assess functional effects of reduced-function variants across multiple systems and multiple substrates to potentially predict clinical effects for new substrates.

CYP2D6 is one of the most highly polymorphic enzymes in the P450 family and is responsible for metabolizing approximately 20%–25% of drugs (Taylor et al., 2020). More than 170 allelic variants and subvariants exist, and the frequency of variants can significantly differ based on race and ethnicity (Sistonen et al., 2005; https://files.cpicpgx.org/data/report/current/frequency/CYP2D6_frequency_table.xlsx). Currently, over 70 different Food and Drug Administration (FDA)-approved drugs have *CYP2D6* PGx-based information within their FDA-approved drug labeling; of these, 43 drugs have actionable PGx recommendations, and four drugs require or recommend *CYP2D6* testing before being prescribed (Table 1) (https://www.pharmgkb.org/gene/PA128/labelAnnotation). In addition, approximately six Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for 38 drugs focus on CYP2D6 interactions (https://cpicpgx.org/guidelines/). Although the functional impact of certain variants of *CYP2D6*, such as *CYP2D6*2*, is well characterized, there are numerous rare variants whose clinical implications have yet to be fully understood due to their low population prevalence. Thus, gaining insight into the function of rare *CYP2D6* variants is critical to understanding whether they may have clinically significant effects and should be used to guide care of patients, thus allowing a precision medicine approach to be applied to patients with these rare variants.

Rare *CYP2D6* Variants and Clinical Implications

Although clinical pharmacogenetic studies are a valuable source of information for optimizing drug dosing in individuals with polymorphic drug-metabolizing enzymes such as *CYP2D6*, the low prevalence and population-specific differences in frequency of rare variants often result in limited representation of these variants in clinical trials with limited diversity. Consequently, in vitro systems may provide valuable information to support extrapolating clinical findings and characterizing the impact of these rare variants. Currently, two main approaches to demonstrate P450 substrate metabolic profiles include liquid chromatography–mass spectrometry for metabolite detection and fluorometric methods that measure the formation of quantifiable metabolites using fluorogenic substrates (Donato et al., 2004; Chen et al., 2007). Multiple in vitro models can be used to understand variant function and substrate specificity, such as patient-derived samples, liver microsomes, cell-based expression systems, and purified variant protein in combination with high-performance liquid chromatography, immunoblotting, and other quantitative methods. Additionally, liquid chromatography–mass spectrometry is considered the gold standard bioanalytical assay for metabolite detection and thus serves as a common technique to characterize enzymatic function by evaluating the metabolism of substrates such as tamoxifen, debrisoquine, dextromethorphan, bufuralol, and codeine.

Curated databases have developed catalogs for identified alleles and potential clinical implications to store relevant in vitro, in vivo, and clinical data in a centralized repository to uncover trends in genotype-phenotype and optimize drug therapy. Several groups have sought to organize the existing functional evidence of rare *CYP2D6* variants and their impact on substrates. The Pharmacogene Variant (PharmVar) Consortium established a haplotype and allele repository for 139 different star variants. CPIC developed an activity score–based classification system where designated genotype activity scores are used to determine phenotype classification, where 0 is considered a PM, 0.25–1 is an IM, 1.25–2.25 is an NM, and >2.25 is considered an ultra-rapid metabolizer (Caudle et al., 2020; Bousman et al., 2023). The clinical functional status is available for numerous star alleles based on varying levels of evidence available for each variant (Relling and Klein, 2011; Gaedigk et al., 2018). However, limited data are available for numerous rare *CYP2D6* variants, and a systematic comparison of the available data has not been published to date.

This review evaluates and summarizes the available literature on rare *CYP2D6* variants that may have a clinically significant impact on drug metabolism. We defined "rare" variants as variants that have an overall low prevalence (minor allele frequency of <5%) in the American population. We first identified variants by their population prevalence and categorization by the Association for Molecular Pathology (AMP) as tier 1 (recommendation for a minimum panel of variant alleles) or tier 2 (an extended panel of variant alleles) variants that should be considered when designing studies to evaluate the influence of *CYP2D6* (Pratt et al., 2021). From that list, we then prioritized variants that have a predominance in populations underrepresented in clinical trials conducted in the United States, such as Asian, Hispanics or Latino, and African American populations, and limited our review to reduced-function variants where the magnitude of effect is currently not well characterized. Tier 1 and 2 alleles known to have complete LOF were excluded from this review. For the genetic variant information, we used a modification of the Human Genome Variation Society recommendations for complete variant description or nomenclature, which includes the coding reference sequence, followed by the variant description (e.g., base change and position), and then the predicted consequences in parentheses. Because the coding reference (NM_000106.6) for CYP2D6 (Nofziger et al., 2020; Gaedigk et al., 2021; https://www.pharmvar.org/gene/CYP2D6) does not change throughout the manuscript, we cited the variant description (base change/position in the coding sequence from the ATG start) and protein change if applicable.

CYP2D6*9. CYP2D6*9 is an AMP tier 1 variant categorized as reduced function with a CPIC-designated activity score of 0.25 and an IM phenotype (https://files.cpicpgx.org/data/report/current/allele_function_reference/CYP2D6_allele_functionality_reference.xlsx). *CYP2D6*9* is a result of c.841-843del p.(Lys281del); the reported population-specific allele frequency observed in African or Asian populations is <0.5%, 2.8% in European populations, and 1.6% in Hispanic or Latino populations (Nofziger et al., 2020; Kane, 2021). Due to the overall rarity of this variant, data conclusively evaluating its functional activity and substrate specificity are limited. However, in vitro models, such as COS7 cells, primary human hepatocytes and purified microsomes, and HEK293T cells, have been used to evaluate the metabolic impact of CYP2D6*9.

Although research specifically focused on a single rare variant is limited, investigations have been conducted across many *CYP2D6* alleles.

TABLE 1

FDA-approved drugs with CYP2D6 genotype/phenotype considerations specified in the prescribing information

Drug	Indication	Phenotype of Concern	Prescribing Information
Amitriptyline	Depression	PM, IM, UM	Monitor TCA plasma levels
Amoxapine	Depression	PM, IM, UM	Monitor TCA plasma levels
Aripiprazole	Schizophrenia and bipolar disorder	PM	Dose modification
Aripiprazole lauroxil	Schizophrenia	PM	Dose modification
Atomoxetine	ADHD	PM	Adjust titration interval and dose modification
Brexpiprazole	Major depressive disorder and schizophrenia	PM	Dose modification
Carvedilol	Mild to severe chronic heart failure	PM	Informative
Cevimeline	Sjogren syndrome	PM	Use with caution
Clomipramine	Obsessive-compulsion disorder	PM	Informative
Clozapine	Treatment-resistant schizophrenia and reducing suicidal behavior in patients with schizophrenia or schizoaffective disorder	PM	Dose modification
Codeine Sulfate	Relief of mild to moderate severe pain	UM	Use with Caution
Eliglustat	Gaucher disease	PM, IM, NM, UM	Dose modification based on phenotype
Darifenacin	Overactive bladder	UM, PM	Informative
Desipramine	Depression	PM	Informative
Deutetrabenazine	Chorea associated with Huntington disease	PM	Dose modification
Dextromethorphan/quinidine	Pseudobulbar affect	PM	Determine risk for toxicity
dextromethorphan hydrobromide and bupropion hydrochloride	Major depressive disorder	PM	Dose modification
Donepezil	Alzheimer disease	UM, PM	Informative
Doxepin	Depression, anxiety, and sleep disorders	PM	Informative
Fesoterodine	Overactive bladder	PM	Informative
Flibanserin	Premenopausal women who acquire generalized hypoactive sexual desire disorder	PM	Monitor for adverse events
Fluoxetine/olanzapine	Depressive episodes associated with bipolar I disorder in adults and treatment resistant depression	PM	Informative
Fluvoxamine	Obsession and compulsions in patients with OCD	PM	Use with caution
Gefitinib	Metastatic non–small cell lung cancer whose tumor have EGFR exon 19 deletions or exon 21 (L858R) substitution mutations	PM	Monitor for adverse reaction
Iloperidone	Schizophrenia	PM	Dose modification
Imipramine	Depression and childhood enuresis	PM	Informative
Iofexidine	Opioid withdrawal symptoms	PM	Monitor for adverse events
Metoclopramide	Gastroesophageal reflux	PM	Dose modification
Mirabegron	Overactive bladder	PM	Informative
Nortriptyline	Major depressive disorder and other psychiatric disorders	PM	Informative
Oliceridine	Management of acute severe pain	PM	Monitor for adverse events
Perphenazine	Schizophrenia and severe nausea and vomiting	PM	Informative
Pimozide	Tourette syndrome	PM	Dose modification
Pitolisant	Excessive daytime sleepiness in adult patients with narcolepsy	PM	Dose modification
Propafenone	Atrial fibrillation who do not have structural heart disease, paroxysmal supraventricular tachycardiaa associated with disabling symptoms in patients who do not have structural heart disease, and life-threatening ventricular arrhythmias	PM	Informative
Protriptyline	Depression	PM	Informative
Tamoxifen	Estrogen receptor–positive metastatic breast cancer, early-stage estrogen receptor–positive breast cancer	PM	Informative
Tamsulosin	Signs and symptoms of benign prostatic hyperplasia	PM	Use with Caution
Tetrabenazine	Huntington disease	PM, NM	Dose modification based on PM status
Thioridazine	Schizophrenia	PM	Contraindicated in PM
Tolterodine	Overactive bladder	PM	Informative
Tramadol	Management of moderate to moderately severe chronic pain	UM	Warning
Trimipramine	Depression	PM	Informative
Valbenazine	Tardive dyskinesia	PM	Dose modification
Venlafaxine	Major depressive disorder, generalized anxiety disorder, social anxiety disorder, panic disorder	PM	Informative
Viloxazine	Attention deficit hyperactivity disorder	PM	Informative
Vortioxetine	Major depressive disorder	PM	Dose modification

ADHD, attention-deficit/hyperactivity disorder; EGFR, epidermal growth factor receptor; OCD, obsessive-compulsive disorder; SNRI, serotonin norepinephrine reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant; UM, ultra-rapid metabolizer.

For example, Muroi et al. (2014) evaluated 49 *CYP2D6* allelic variants, including CYP2D6*9 transiently expressed in COS-7 cells compared with wild type (WT), to determine variant impact on tamoxifen metabolism in vitro. The Michaelis-Menten kinetics for N-desmethyltamoxifen 4-hydroxylation showed that CYP2D6*9 had a statistically significant reduction in the Vmax and, consequently, a 63% reduction in intrinsic clearance (Table 2). Additionally, the *CYP2D6*9* variant expressed significantly less CYP2D6 protein compared with WT. However, no loading control was used to normalize variation in protein loading; thus, direct comparison of protein expression is challenging. Similarly, CYP2D6*9-mediated bufuralol 1'-hydroxylation and dextromethorphan O-demethylation led to approximately 70% and 62% reductions in CYP2D6 activity, respectively, compared with WT (Muroi et al., 2014).

To further understand the association between variant and metabolic activation of the antimalarial drug primaquine, in vitro enzymatic analyses were performed using protein lysates isolated from HEK293FT cells transiently transfected with WT and 49 *CYP2D6* variants and then exposed to primaquine (Saito et al., 2018). Although previously reported by Muroi et al. (2014) that COS-7 cells transiently expressing *CYP2D6*9* had reduced CYP2D6 protein, in contrast, Saito et al. (2018) demonstrated that *CYP2D6*9* had similar protein expression to WT CYP2D6 in HEK293T cells. Differences in the loading and subsequent normalization of protein could account for the discrepancy between studies. Consistent with prior studies, the intrinsic clearance of primaquine was significantly reduced (40%) compared with WT (Table 2).

CYP2D6*9, together with other variants of CYP2D6, was assessed in a study that analyzed protein levels and bufuralol hydroxylation activity using protein purified from liver microsomes from 39 Japanese and 44 White subjects (Shimada et al., 2001). Of the 44 White subjects, a single subject had a 3-bp deletion (2701–2703), resulting in the deletion of a single amino acid at position 281 and the *CYP2D6*9* genotype. This subject had reduced CYP2D6 protein expression and 5.6 times less bufuralol 1'-hydroxylation activity than WT (Table 2).

Clinical research has also assessed the association between metoprolol concentrations and CYP2D6 variant to evaluate the differences in plasma concentration-time curves and oral clearance (Zineh et al., 2004). Although overall concurrence between in vitro models and clinical research suggests that the CYP2D6*9 variant has reduced functional activity, the activity is only moderately reduced (activity score of 0.75) in metoprolol metabolism (Zineh et al., 2004). Variants with activity scores between 0.5–0.75 were grouped as IM, which demonstrated that metoprolol had a longer half-life and higher area under the curve compared with EMs but that the magnitude of function may vary in a substrate-dependent manner. As expected with reduced activity scores that correspond to IMs, there was a correlation between CYP2D6*9 genotype-phenotype and differences in metoprolol pharmacokinetics, particularly regarding the metabolism and clearance.

Taken together, these in vitro and clinical studies support that CYP2D6*9 exhibits reduced activity, potentially driven in part by reduced protein expression, and has a wide impact on its substrates. Although it is difficult to directly compare substrates and differing models, the impact of *CYP2D6*9* on metabolic activity throughout cited literature ranged from 18%–40% of WT (Fig. 1A), indicating that different in vitro models consistently show significantly reduced metabolic activity.

CYP2D6*10. CYP2D6*10 is an AMP tier 1 variant with an activity score of 0.25 and IM phenotype (Kane, 2021; Pratt et al., 2021). *CYP2D6*10* is characterized by a combination of missense substitutions in exon 1, which lead to a key substitution of cytosine-to-thymine c.100C>T p. (Pro34Ser), c. 408G>C (no change), and a nonspecified modification c.1457G>Cp.(Ser486Thr) (Hicks et al., 2014). The population-specific reported allele frequency in European populations is <2%, 4%–6% in African populations, and 9%–44% in Asian populations, with c.100C>T p.(Pro34Ser) as the predominant substitution (Bagheri et al., 2015; Fedorinov et al., 2018). The magnitude of effect of the reduced enzymatic activity is often substrate specific (van der Lee et al., 2021). Although there is a relatively large amount of literature on this variant, substrate-specific characterization demonstrates high variability, making the implementation of clinical recommendations challenging.

Enzymatic activity of this variant has been investigated in a diverse array of in vitro models as well as in clinical studies, highlighting the substrate-specific nature. To uncover the enzymatic differences in CYP2D6*10 based on substrate, different models have been developed, such as purified CYP2D6*10 protein expressed from baculovirus-mediated insect cells and reconstituted with lipid and cytochrome P450 reductase (Yu et al., 2002). Studies have shown that the CYP2D6*10-mediated reaction rate for dextromethorphan O-demethylation and N-demethylation, codeine O-demethylation, and fluoxetine N-demethylation is reduced compared with the WT-mediated reaction rate for dextromethorphan, codeine, and fluoxetine (Table 2) (Yu et al., 2002). Compared with other reduced-function variants, such as *CYP2D6*17*, the overall enzyme catalytic activity for three substrates (codeine, dextromethorphan, and fluoxetine) were decreased the most in the *CYP2D6*10* variant, consistent with the severely reduced phenotype corresponding to the activity score.

Similarly, investigation into the substrate-dependent catalytic efficiency using an identical model of microsomes prepared from insect cells containing baculovirus-expressing CYP2D6*10 with NADPH-cytochrome P450 reductase indicated an overall decrease in the catalytic efficiency following the treatment of codeine, dextromethorphan, debrisoquine, bufuralol, atomoxetine, nortiptyline, tramadol, and (s)-fluoxetine (Shen et al., 2007). Specifically, CYP2D6*10-mediated bufuralol hydroxylation had higher affinity than WT. However, CY2D6*10-mediated affinity of (S)-fluoxetine was reduced, and the affinity for dextromethorphan and nortriptyline was enhanced, demonstrating preferential binding affinity based on substrate. The authors speculate that the reduced intrinsic clearance was related to the substantial reduction in Vmax rather than difference in Km values (Table 2). Further investigation using enzyme inhibition studies for dextromethorphan o-demethylase or atomoxetine 4-hydroxylase activity found a mixed effect on the enzyme inhibition affinity for (S)-fluoxetine, imipramine, and (S)-norfluoxetine, as demonstrated by the inhibition ratios that ranged from 0.32–1.51 compared with the ratios for quinidine and thioridazine, which were 2.3 and 19, respectively (Shen et al., 2007).

Additionally, CYP2D6*10 and other variants were transiently expressed in COS-7 cells, and the enzymatic activity following bufuralol and dextromethorphan was characterized (Sakuyama et al., 2008). Results remain consistent across models, suggesting that the apparent Km value for bufuralol 1'-hydroxylation was significantly higher and the intrinsic clearance value was significantly lower than WT (Sakuyama et al., 2008). Likewise, the dextromethorphan O-demethylation activity was significantly reduced, and CYP2D6*10 expressed ∼65% less protein than WT. Although trends were consistent, the magnitude of effect varied by source of enzyme, model, and laboratory technique.

The clinical implication of the functional activity of rare *CYP2D6* variants is often challenging to obtain within prospective clinical trials; therefore, observational studies enable investigations into the associations between CYP2D6 genotype/phenotype, pharmacokinetics (PK), and treatment outcomes. In the case of *CYP2D6*10*, the high prevalence in individuals of East Asian ancestry may increase the power of observational studies to demonstrate a genotype effect. For example, Xu et al. (2008) found that serum 4-hydroxy-tamoxifen concentrations

TABLE 2

CYPD6 rare variants and model results

Variant Alleles	Genetic Changes	Amino Acid Substitution	cDNA Transfection in COS-1 or COS-7	cDNA Transfected in HEK293T	Baculovirus-Mediated Insect Cells	Patient Samples and Clinical Data
CYP2D6*9	c.841-843delAAG	p.(Lys281del)	Dextromethorphan Vmax was significantly reduced from 0.13 pmol/min per pmol of CYP2D6 compared with WT (0.45 pmol/min per pmol CYP2D6). Additionally, dextromethorphan intrinsic clearance was reduced from 0.0081 μL/min per nmol of CYP2D6 to 0.0030 μL/min per nmol of CYP2D6 when CYP2D6*9 was evaluated (Muroi et al., 2014)	The Km was increased (20.2 μM vs 15.7 μM), whereas the Vmax (1.06 area ratio/min per pmol CYP2D6 vs 1.39 area ratio/min per pmol) was reduced compared with WT (Saito et al., 2018)	N/A	Liver samples from 44 White subjects found that a subject with a CYP2D6*9 genotype had 9.2 pmol/mg protein compared with WT 13.0 pmol/mg. Additionally, the Km value of the 1'-hydroxylation was slightly higher, and Vmax was reduced compared with the WT (Shimada et al., 2001)
CYP2D6*10	*c.100C>T*, c.1457G>C	*p.(Pro34Ser)*, p.(Ser486Thr)	Decreased enzyme activity following bufuralol and dextromethorphan treatment (Sakuyama et al., 2008)	N/A	Clearance of dextromethorphan O-demethylation and fluoxetine N-demethylation declined by 50-fold and 100-fold (Yu et al., 2002). CYP2D6*10-mediated bufuralol hydroxylation had an affinity of 12–50 μM and intrinsic clearance value of 0.05–0.24 μL/min per pmol P450. Additionally, the $Km_{CYP2D6*10}/Km_{CYP2D6*1}$ ratio was 0.5 for (S)-fluoxetine; 1 to 2 for atomoxetine, bufuralol, codeine, debrisoquine, and tramadol; and >3 for dextromethorphan and nortriptyline (Shen et al., 2007)	Mean serum 4-OH-tamoxifen concentrations were 1.3 times lower in Chinese women with breast cancer homozygous for CYP2D6*10 (Xu et al., 2008)
CYP2D6*14B	*c.505G>A*, c.886C>T, c.1457G>C	*p.(Gly169Arg)*, p.(Arg296Cys), p.(Ser486Thr)	Km was reduced from 9.2 μM in WT to 18.9 μM with the CYP2D6*14 variant. Furthermore, the reduced metabolic capacity goes from 0.2 μL/min per pmol to 0.07 μL/min per pmol (Sakuyama et al., 2008) CYP2D6*14 exhibited <15% n-desmethyltamoxifen 4-hydroxylation, and ∼36% bufuralol 1'-hydroxylation and dextromethorphan O-demethylation compared to WT (Muroi et al., 2014)	N/A	N/A	A single subject homozygous for *14 was found to be a PM and not an IM, marking the first report of this phenotype (Cai et al., 2007)
CYP2D6*17	*c.320C>T*, c.886C>T, c.1457G>C	*p.(Thr107Ile)*, p.(Arg296Cys), p.(Ser486Thr)	CYP2D6*17 exhibited 20% bufuralol hydroxylase activity compared with WT (Oscarson et al., 1997) N-desmethyltamoxifen showed 10%	N/A	The reaction rate for DXM metabolism of the DXM O-demethylation and N-demethylation decreased 10-fold, and the estimated intrinsic clearance of these	10-fold higher parent-to-metabolite ratios of debrisoquine and dextromethorphan in homozygous Tanzanians (Wennerholm et al., 2002)

TABLE 2 *continued*

Variant Alleles	Genetic Changes	Amino Acid Substitution	cDNA Transfection in COS-1 or COS-7	cDNA Transfected in HEK293T	Baculovirus-Mediated Insect Cells	Patient Samples and Clinical Data
			hydroxylation activity of WT (Muroi et al., 2014)		metabolites was decreased 6% and 33% (Yu et al., 2002). The estimated intrinsic clearance for fluoxetine N-demethylated metabolite, norfluoxetine, decreased approximately four-fold and another two-fold with the c.320C>T p.(Thr107Ile) substitution (Yu et al., 2002). The c.320C>T p.(Thr107Ile) substitution led to a six-fold decrease in codeine metabolism	
CYP2D6*29	c.406G>A+c.408G>C, c.886C>T, c.1012G>A, c.1457G>C	p.(Val136Met), p.(Arg296Cys), p.(Val338Met), p.(Ser486Thr)	The catalytic activity of hydroxylation of bufuralol and debrisoquine was reduced to 23% and 63% compared with WT (Wennerholm et al., 2001). The estimated intrinsic clearance of dextromethorphan in COS-7 cells was 47%, Vmax was reduced (40%), and a higher affinity was observed compared with CYP2D6*2. Additionally, the estimated intrinsic clearance of bufuralol is 64%, reduced Vmax (36%), and reduced affinity compared with CYP2D6*2 (Gaedigk et al., 2002).	N/A	N/A	Tanzanian subjects homozygous for CYP2D6*29 displayed higher metabolic ratios for dextromethorphan and metoprolol (Wennerholm et al., 2002). The single patient with CYP2D6*29 genotype demonstrated reduced clearance (4.67 mL/min) compared with WT (5.7 mL/min) (Grimsrud et al., 2019)
CYP2D6*49	c.100C>T, *c.358G>A*, c.1457G>C	p.(Pro34Ser), *p.(Phe120Ile)*, p.(Ser486Thr)	O-demethylation was significantly increased to 323 μM compared with WT (10 μM) (Matsunaga et al., 2009)			

DXM, dextromethorphan.
*Italicized genetic changes (nomenclature NM_000106.6 at the ATG start site) and amino acid substitutions, if applicable, are specific to the variant alleles and used for classification.

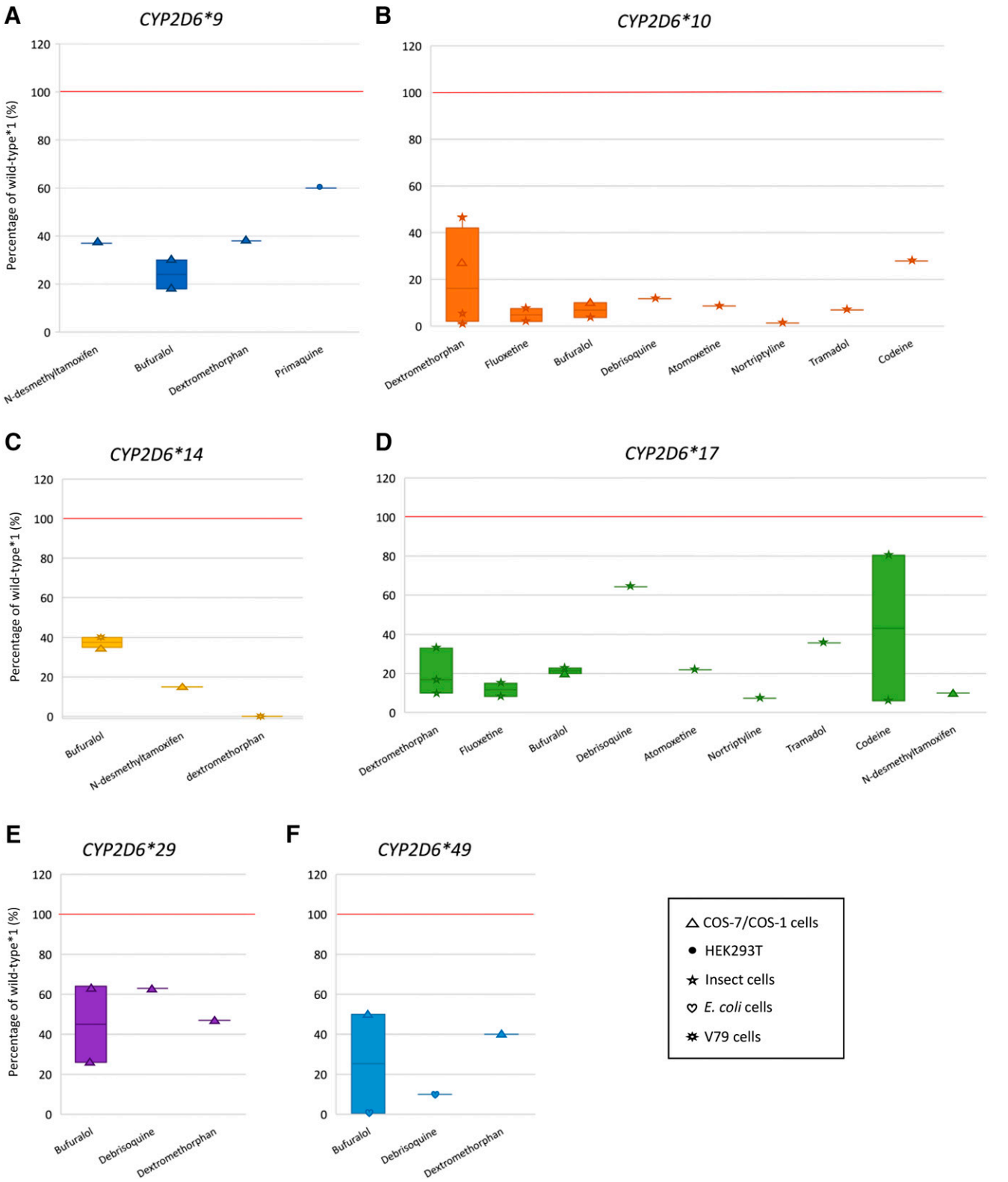

Fig. 1. CYP2D6 activity measured by published intrinsic clearance (Vmax/Km; pmol/pmol P450/min) based on the substrate in a reaction for each variant relative to WT (CYP2D6*1). Substrates include bufuralol, dextromethorphan, codeine, debrisoquine, fluoxetine, atomoxetine, and N-desmethyltamoxifen and nortriptyline, tramadol, and primaquine. Undetectable levels of substrates were omitted from the graph. Models included COS-1, COS-7, V79, HEK293T cells, *E. coli* cells, and baculovirus-mediated insect cells.

were 1.3 times lower in Chinese women with breast cancer who were homozygous for *CYP2D6*10*, and subsequently, tamoxifen-treated women had lower disease-free survival than Chinese women who expressed WT *CYP2D6* (Table 2). A similar study demonstrated that women with metastatic breast cancer who are homozygous for *CYP2D6*10* had lower steady-state concentrations of 4-hydro-N-desmethyltamoxifen, the primary and active metabolite of tamoxifen, known as endoxifen, and 4-hydroxy-tamoxifen compared with WT and

thus may not benefit from treatment to the same extent (Lim et al., 2007). It is challenging to draw conclusions on PK-pharmacodynamic relationships based on retrospective and cohort data; however, additional clinical studies and in vitro models specifically evaluating the relationship between CYP2D6*10 enzymatic function and tamoxifen metabolism may be able to provide additional insight on the role of CYP2D6*10-mediated tamoxifen PK.

Although clinical data for most rare variants are very scarce due to the high prevalence of *CYP2D6*10* in East Asian populations, such as Thai, Chinese, Japanese, and Indian, clinical studies have generated foundational evidence to suggest a clinically relevant interaction between *CYP2D6*10* and tamoxifen metabolism. This rare variant demonstrates how the combination of in vitro models and observational studies aids in knowledge that cannot be feasibly obtained within clinical trials. Although direct comparison across different models and with different substrates has its limitations, overall, the in vitro models consistently demonstrate substantially reduced function of CYP2D6*10, although the wide range of magnitude of effect on enzyme function (1%–47% of WT) highlights substrate-specific effects (Fig. 1B). Further research into which in vitro models closely mimic the substrate-specific effects in humans, using tamoxifen as a substrate, may pave the way for comparing other substrates in the future.

CYP2D6*14. CYP2D6*14 is an AMP tier 2 rare variant with a reduced activity score of 0.5 and IM phenotype that has been relatively well characterized considering its extremely low prevalence (Kane, 2021). Multiple genetic variants [i.e., c.1457G>C p.(Ser486Thr), c.886C>T p.(Arg296Cys), and c.505G>A p.(Gly169Arg)] are associated with the encoded CYP2D6*14, where c.505G>A p.(Gly169Arg) is a definitive substitution necessary for *CYP2D6*14* (Daly et al., 1996; Wang et al., 1999). Historically, *CYP2D6*14* was classified as *CYP2D6*14A* and *CYP2D6*14B*, distinguished by functional activity and expression levels. The c.505G>A p.(Gly169Arg) and c.100C>T p.(Pro34Ser) substitutions in combination are often present in the no-function *CYP2D6*14A* allele. However, current revision of the characterization by PharmGKB has led to the change in nomenclature from *CYP2D6*14A* to **114* and *CYP2D6*14B* to **14*. Thus, we focused on *CYP2D6*14B* results. This variant is found in 0.3% of the Asian population, approximately 1.8% of the Chinese population, and an unknown percentage of the European and African populations (Kane, 2021). There are discrepancies in population prevalence and activity score, ranging from 0.25 to 0.5 depending on the specific studies and whether the variant **14A* and **14B* are distinguished.

Transiently expressed CYP2D6*14 in COS-7 cells was used to evaluate the enzymatic activity following bufuralol and dextromethorphan treatment (Sakuyama et al., 2008). The findings suggest that CYP2D6*14B demonstrated a significantly higher apparent Km value and significantly lower intrinsic clearance for bufuralol 1'-hydroxylation compared with WT (Sakuyama et al., 2008). Interestingly, CYP2D6*14B protein expression via western blot showed similar CYP2D6 protein expression as WT. The authors note that **14A* harbored c.100C>T p.(Pro34Ser), c.505G>A p.(Gly169Arg), c.886C>T p.(Arg296Cys), and c.1457G>C p.(Ser486Thr) substitutions, leading to undetectable protein levels, whereas c.505G>A p.(Gly169Arg), c.886C>T p.(Arg296Cys), and c.1457G>C p.(Ser486Thr) substitutions are commonly seen within the *CYP2D6*14B* variant. Specifically, *CYP2D6*14B* harboring c.505G>A p.(Gly169Arg), c.886C>T p.(Arg296Cys), and 1457G>C p.(Ser486Thr) substitutions had higher activity, attributed mainly to the absence of the c.100C>T p.(Pro34Ser) substitution. Compared with WT, the CYP2D6*14B variant had significantly reduced metabolic activity defined by the IM status.

Similarly, Muroi et al. (2014) found that both *14A and *14B had undetectable kinetic parameters, likely due to the variants catalyzing an undetectable amount of metabolite or a lower substrate concentration in COS-7 cells transiently expressing CYP2D6*14A and CYP2D6*14B. However, when a higher substrate concentration (80 μM) of N-desmethyltamoxifen was used, CYP2D6*14B exhibited reduced activity, approximately <15% of WT. The authors suggest that the addition of the c.100C>T p.(Pro34Ser) substitution led to the decreased N-desmethyltamoxifen 4-hydroxylation activity. In addition, the dextromethorphan O-demethylation and bufuralol 1'-hydroxylation was significantly reduced compared with WT.

To identify the unique single amino acid substitution that impacts the metabolic activity, Shiraishi et al. (2002) found that the combination of c.505G>A p.(Gly169Arg) and c.100C>T p.(Pro34Ser) in the *CYP2D6*14B* variant resulted in alterations in the tertiary structure of CYP2D6. Furthermore, the metabolic activity decreased due to translation of a protein that was 50 amino acids shorter than the WT CYP2D6 protein expressed in V79 cells (Table 2) (Shiraishi et al., 2002). However, a prior investigation indicated that c.505G>A p.(Gly169Arg)-substituted CYP2D6 led to approximately 40% of the WT activity, supporting that this specific substitution could be a potentially central mechanism for CYP2D6*14 functional activity (Shiraishi et al., 2002). However, Shiraishi et al. (2002) found that c.505G>A p.(Gly169Arg) or c.100C>T p.(Pro34Ser) substitution alone did not fully eliminate activity of bufuralol 1'-hydroxylation and dextromethorphan O-demethylation in transfected V79 cells. It was not until the impact of simultaneous substitution of c.505G>A p.(Gly169Arg) and c.100C>T p.(Pro34Ser) was assayed that the enzymatic activity of bufuralol 1'-hydroxylation and dextromethorphan O-demethylation was shown to be completely abolished. This is similar to findings from Wang et al. (1999), demonstrating that CYP2D6 activity cannot be eliminated by a single substitution alone and that a combination of various substitutions is necessary to abolish the activity of CYP2D6.

Overall, the reduced activity of the CYP2D6*14 variant is well supported throughout literature. However, the magnitude of the effect of the reduction in activity was variable based on substrate, and the specific variants contributing to functional deficiency remain unclear. Variation in metabolic activity ranges from 0% to 40% of WT depending on the substrate and model (Fig. 1C), indicating that although the magnitude is variable, a potentially clinically significant reduction in activity was consistently observed. Discerning whether the complexity of catalytic incompetence or the lack of functional protein contributes to the phenotype of *CYP2D6*14* is critical in advancing our understanding of the structure-function relationship for these variants and other similar variants. Additional site-directed mutagenesis studies in multiple in vitro models may provide a comprehensive analysis of the role of each substitution in *CYP2D6*14*.

CYP2D6*17. *CYP2D6*17* is an AMP tier 1 variant with an activity score of 0.5, corresponding to an IM and characterized by multiple missense variants in exon 2, leading to a key nucleotide cytosine-to-thymine modification, c.320C>T p.(Thr107Ile), and other nonspecific modifications, including c.886C>T p.(Arg296Cys) and c.1457G>C p.(Ser486Thr) (Saghafi et al., 2018). *CYP2D6*17* has a reported population-specific allele frequency of 17%–19% in African populations, <0.5% in European and Asian populations, and 2.3% in Hispanic or Latino populations (Pratt et al., 2021). CYP2D6*17 is known for having a high degree of enzymatic variability depending on the substrate, and thus it remains unclear how this functional activity reduction impacts clinically relevant drug metabolism across the spectrum of CYP2D6 substrates.

In vitro models enable the ability to examine the role of different single nucleotide polymorphisms within *CYP2D6*17* and appreciate the associated phenotype in a substrate-specific manner. One such model used four different *CYP2D6* cDNAs [WT, c.320C>T p.(Thr107Ile),

c.886C>T p.(Arg296Cys) plus c.1457G>C p.(Ser486Thr), and c.886C>T p.(Arg296Cys)+c.1457G>C p.(Ser486Thr)+ c.320C>T p.(Thr107Ile)], corresponding to the *17 variant expressed in COS-1 cells to demonstrate that CYP2D6*17 exhibited 20% hydroxylase activity for bufuralol metabolism compared with WT (Oscarson et al., 1997). Likewise, yeast cells expressing three amino acid substitutions [c.320C>T p.(Thr107Ile), c.886C>T p.(Arg296Cys), and c.1457G>C p.(Ser486Thr)], corresponding to *CYP2D6*17*, were introduced sequentially and in various combinations. Overall, the CYP2D6*17 enzyme had five-fold higher affinity for bufuralol compared with WT. The c.320C>T p.(Thr107Ile) variant in combination with c.886C>T p.(Arg296Cys) characterized the reduced affinity of bufuralol, whereas the c.320C>T p.(Thr107Ile) variant alone had no significant effect on bufuralol metabolism. The addition of p.Ser486Thr appeared insignificant in bufuralol metabolism either alone or in combination with the other two variants. The impact of c.1457G>C p.(Ser486Thr) may be substrate specific and is unclear at this time. Interestingly, the c.320C>T p.(Thr107Ile) variant alone reduced the affinity of codeine but not of bufuralol. It is speculated that the preferential affinity of substrates is related to the distinct substrate structure. Thus, research that fully characterizes the specific variants and the resulting substrate metabolism is warranted. This may enable determination of the true clinical implication of this variant on substrate-specific metabolism and if there is a meaningful impact for specific single amino acid substitutions.

In COS-7 cells that transiently expressed CYP2D6*17, a significant decrease in the formation of 4-hydroxy-N-desmethyltamoxifen at higher concentrations of N-desmethyltamoxifen (80 μM) was observed compared with WT (Muroi et al., 2014). Using the same transiently transfected COS-7 cell model, Muroi et al. (2014) found CYP2D6*17 had 10% the hydroxylation activity of N-desmethyltamoxifen as WT, further signifying that *CYP2D6*17* can alter enzymatic activity and influence kinetic parameters, such as intrinsic clearance.

To improve the understanding of the intrinsic properties of the *CYP2D6*17* allelic variant, Yu et al. (2002) described the Michaelis-Menten kinetic parameters for codeine, fluoxetine, and dextromethorphan. The reaction rate and estimated intrinsic clearance for dextromethorphan O-demethylation and N-demethylation were consistently significantly decreased compared with WT. Additionally, the estimated intrinsic clearance for the fluoxetine N-demethylated metabolite, norfluoxetine, decreased significantly, which was synergistic with the c.320C>T p.(Thr107Ile) substitution (Yu et al., 2002). The substitution of c.320C>T p.(Thr107Ile) reduced intrinsic clearance by sixfold following codeine administration. Overall, it was observed that CYP2D6*17 represented reduced activity and potentially impacted drug efficacy.

Due to 17%–19% prevalence of the *CYP2D6*17* variant within Ethiopian, Tanzanian, Zimbabwean, and other African populations, some clinical studies have assessed the impact of *CYP2D6*17*, thus making it possible to compare in vitro models to clinical studies. For instance, Tanzanian volunteers were genotyped, treated with four probe drugs (debrisoquine, metoprolol, codeine, and dextromethorphan), and compared with Swedish subjects (Wennerholm et al., 2002). The findings suggest that Tanzanians homozygous for *CYP2D6*17* had more than 10-fold higher parent-to-metabolite ratios of debrisoquine and dextromethorphan compared with Tanzanians with functional or WT alleles but less than four-fold higher parent-to-metabolite ratios for codeine and metoprolol. Consequently, the decreased enzymatic activity of CYP2D6*17 was less notable with two substrates, codeine and metoprolol, compared with debrisoquine and dextromethorphan, highlighting substrate specificity compared with WT. Similarly, it has been noted that the mean debrisoquine parent-to-metabolite ratio is higher in this variant compared with WT, supported by a homozygous subject expressing c.320C>T p.(Thr107Ile), c.886C>T p.(Arg296Cys), and c.1457G>C p.(Ser486Thr) variants (Masimirembwa et al., 1996). Overall, the in vitro and clinical results suggest that *CYP2D6*17* is a reduced-function variant and that among African populations, this variant may significantly impact the metabolism of CYP2D6 substrates but has a high degree of variability (7%–80% of WT; Fig. 1D), ranging from almost no metabolic activity toward the substrate to only modestly reduced activity.

CYP2D6*29. CYP2D6*29 is an AMP tier 1 variant with an activity score of 0.5, corresponding with an IM phenotype (Kane, 2021; https://files.cpicpgx.org/data/report/current/allele_function_reference/CYP2D6_allele_functionality_reference.xlsx). *CYP2D6*29* is characterized by a combination of several missense substitutions in exon 2, c.406G>A+c.408G>C p.(Val136Ile), c.886C>T p.(Arg296Cys), c.1012G>A, p.(Val338Met) c.1457G>Cp.(Ser486Thr), leading to reduced function. *CYP2D6*29* has a population-specific allele frequency of 9%–12% in Africans, <0.5% in Europeans and Asians, and 1.5% in Hispanics or Latinos (Kane, 2021; Pratt et al., 2021). However, similar to the other variants discussed, CYP2D6*29 displays substrate specificity that requires careful consideration of the substrate-dependent magnitude of effect.

For instance, in a study where CYP2D6*29 is expressed in yeast and COS-1 cells, CYP2D6*29 did not impact affinity or maximal velocity following the treatment of bufuralol compared with WT (Wennerholm et al., 2001). On the contrary, the catalytic activity of hydroxylation of the bufuralol and debrisoquine was significantly reduced in COS-1 cells. The differences in models may reflect technical variance, and the validity metrics of this model should be considered. Similarly, Gaedigk et al. (2002) found that in COS-7 cells expressing CYP2D6*29, median urinary dextromethorphan-to-dextrorphan ratio indicated significantly reduced metabolic efficiency. Additionally, the estimated intrinsic clearance of CYP2D6*29 was significantly reduced for bufuralol and dextromethorphan compared with CYP2D6*2, a normal function variant (Table 2).

Clinical studies primarily investigating the role of *CYP2D6*29* on the pharmacokinetics of CYP2D6-mediated substrates are limited. In the Wennerholm et al. (2002) study, which assessed four probe drugs in Tanzanian volunteers homozygous for *CYP2D6*17* compared with Swedish subjects, CYP2D6*29 was investigated in five of the Tanzanian subjects homozygous for **29*. The Tanzanian subjects homozygous for *CYP2D6*29* displayed reduced metabolism for dextromethorphan and metoprolol but not to codeine and debrisoquine, contrary to the reduced-function variant CYP2D6*17. Particularly, debrisoquine hydroxylase activity was significantly higher in subjects expressing CYP2D6*29 than in subjects expressing CYP2D6*17, which was not identified with the other drugs tested.

Additionally, a report of patients receiving oxycodone, hydrocodone, and other opioids has suggested that the reduced function of CYP2D6*29 may lead to accumulation of toxic metabolites resulting in adverse events (Foster et al., 2007). Thus, upon further investigation, a pilot study identified a single patient with the *CYP2D6*29* variant who exhibited altered fentanyl kinetics based on increased serum fentanyl concentrations and reduced clearance (Grimsrud et al., 2019).

Overall, CYP2D6*29 is a reduced-function variant that has a range from 26% to 64% the metabolic activity of WT across different in vitro models (Fig. 1E). The magnitude of effect may differ due to model species and technique as well as substrate-specific effects. This range in the magnitude of effect makes it likely that CYP2D6*29 has a clinically significant impact on the metabolism of some drugs, but not others, and highlights the need for additional translational studies to further characterize the impact of the variant and predict the likelihood of clinically significant effects for new CYP2D6 substrates.

CYP2D6*49. CYP2D6*49 is an AMP tier 2 variant with an activity score of 0.5, corresponding with reduced activity and an IM phenotype (Kane, 2021; Pratt et al., 2021). *CYP2D6*49* results from missense substitutions, such as c.100C>T p.(Pro34Ser), c.358T>A p.(Phe120Ile), or c.1457G>C p.(Ser486Thr) (Kane, 2021). This variant is predominantly identified in East Asian individuals, where it impacts about 1% of the population. It is currently unknown what the population-specific frequency is for European and African populations.

To understand the impact of *CYP2D6*49* on the functional activity of CYP2D6, site-directed mutagenesis was conducted to produce *Escherichia coli* expressing CYP2D6*49 and other variants and treated with dextromethorphan (Matsunaga et al., 2009). In this study, the O-demethylation and Km value was significantly increased compared with WT. Notably, the parent-to-metabolite ratios were 0.5% of the WT for bufuralol 1'-hydroxylation and 9.9% for debrisoquine 4-hydroxylation. Similarly, an in vitro model using COS-7 cells transiently expressing CYP2D6 variants found that CYP2D6*49 had a ∼30% reduction in protein expression levels and <50% reduction in bufuralol 1'-hydroxylation and dextromethorphan O-demethylation compared with WT CYP2D6 (Sakuyama et al., 2008).

Overall, data on *CYP2D6*49* is limited, and the range of effect (0.5%–50%) in vitro is limited and variable (Fig. 1F). However, it remains clear that CYP2D6*49 exhibits an IM phenotype and is associated with reduced metabolic capacity, but whether due to catalytic incompetence or a reduction in functional protein remains to be determined.

Conclusion

CYP2D6 is responsible for the metabolism of a large number of drugs, leading to concerns for safety and therapeutic response in patients with altered enzyme activity. More than 50 FDA-approved drugs have PGx information to address the impact of variable activity on substrates. For example, several FDA-approved drugs (eliglustat, pimozide, tetrabenazine, and dextromethorphan/quinidine) have genotype testing recommended as part of the FDA prescribing information to inform dose selection based on CYP2D6 genotype-inferred phenotype to reduce the risk of ineffective dosing and adverse events. Additionally, over 70 additional medications include informative statements on the impact of CYP2D6 on drug exposure within the FDA labeling. Six published CPIC guidelines have been created for CYP2D6-mediated drugs, such as tamoxifen, ondansetron and tropisetron, tricyclic antidepressants, selective serotonin reuptake inhibitors, codeine, and atomoxetine (Crews et al., 2012, 2021; Hicks et al., 2015, 2017; Goetz et al., 2018; Brown et al., 2019; Bousman et al., 2023). Although many important gene-drug interactions have been discovered, there are many barriers and challenges to implementing pharmacogenomic testing into clinical practice.

Although genotype-inferred phenotype is often used to categorize a patient's metabolic status, it is important to consider the impact of individual variants. Although some variants are relatively common and have a well characterized impact on drug metabolism, other variants are less common and are not well characterized and thus may not be routinely tested. Moreover, rare variants present a unique challenge due to low prevalence, population-specific frequency of individuals compounded by under-representation of minority groups in clinical trials, and substrate specificity. Understanding the functional impact of each variant is critical to determining whether clinical effects observed in more common alleles can be extrapolated to less common (unstudied) alleles for IM phenotypes. Specifically, rare variants with low population prevalence are nearly impossible to characterize within clinical trials. Commonly discussed as a major challenge, demographics of patients enrolled in clinical trials remain poorly representative of the American public as the majority of trials have an over-representation individuals of European ancestry (National Academies of Sciences, Engineering, and Medicine, 2022). Thus, leveraging cell-based in vitro systems may provide an insightful tool to uncover the clinical implications of rare *CYP2D6* variants and bridge the gap in knowledge that cannot be captured in clinical trials currently.

In vitro systems evaluating the substrate specificity using enzyme kinetic parameters are beneficial for many of the rare and/or understudied variants in AMP tiers 1 and 2. Particularly, studies comparing in vitro models' reproducibility, reliability, and validity may be warranted for each rare variant. As observed with investigations into *CYP2D6*29*, contrary results were observed in yeast models compared with COS-1 cell models and could lead to misinterpretation of the data as a global phenomenon and not a model-dependent result. However, our assessment of the available metabolic activity of variants across models showed that directionality was generally consistent. Although the magnitude of effect varied across different models and substrates, the similar trends in effect demonstrate the utility of in vitro models to characterize functional effects of genomic variants in drug-metabolizing enzymes.

As indicated in Fig. 1, *CYP2D6* variants may have a wide array of impacts on activity, determined by intrinsic clearance of a substrate. Although the figure is a crude representation of a variety of substrates and models, it shows consistent trends in IM phenotype across the variants. Of note, CYP2D6*10 had the lowest intrinsic clearance compared with the other variants, consistent with the reduced activity score (0.25). Variation in activity may be attributable to model species and technical differences as well as substrate specificity. Overall, in vitro models compared with clinical studies evaluating substrate exposure in genotyped patients demonstrated consistency (Fig. 2). The utilization of these models may provide a starting point for extrapolating in vitro substrate-dependent metabolism results to predict clinical effects. However, direct comparisons of multiple models, controlling for different timepoints, substrate, species, and technical assay, are needed as well as direct comparison with clinical study results to assess each model's ability to predict clinically significant gene-drug interactions.

In vitro models present a unique strategy to overcome the lack of feasibility to characterize rare variants within clinical trials, additional novel methods, and technologies such as physiologically based pharmacokinetic modeling (PBPK), quantitative structure-activity relationship modeling, and artificial intelligence, or machine learning may be used in combination to build the level of knowledge surrounding the gene-drug interactions. For instance, variants that lead to functional changes in CYP2D6 activity can be modeled using computational approaches such as quantitative structure-activity relationship and molecular dynamics (de Waal et al., 2014; Gonzalez et al., 2021). This enables the response to different substrates to be evaluated. The reaction rate and kinetic parameters of a substrate can be assessed using clinical data that collects information on drug response, organ involvement, genetic polymorphisms, and drug-drug interactions. Combining modeling, comprehensive steady-state analyses from pharmacogenomic models, and the dynamics of traditional PK models can create individualized forms of PBPK modeling. To further use a PBPK model in this way, genome-scale information and in vitro model comparison must be obtained to gain further insight into the effect of other less represented genetic variants of CYP2D6 and whether results from in vitro systems can be extrapolated to clinical practice. Similarly, mechanistic modeling, such as quantitative systems pharmacology modeling, offers a potential platform to bridge the gap between sparse clinical data and incorporates the current understanding of the genotype-phenotype relationship in a quantitative manner (https://www.fda.gov/media/101469/download). These platforms require specific technical details on the planning, conduct, and assessment of the model with biologic parameters and assumptions documented.

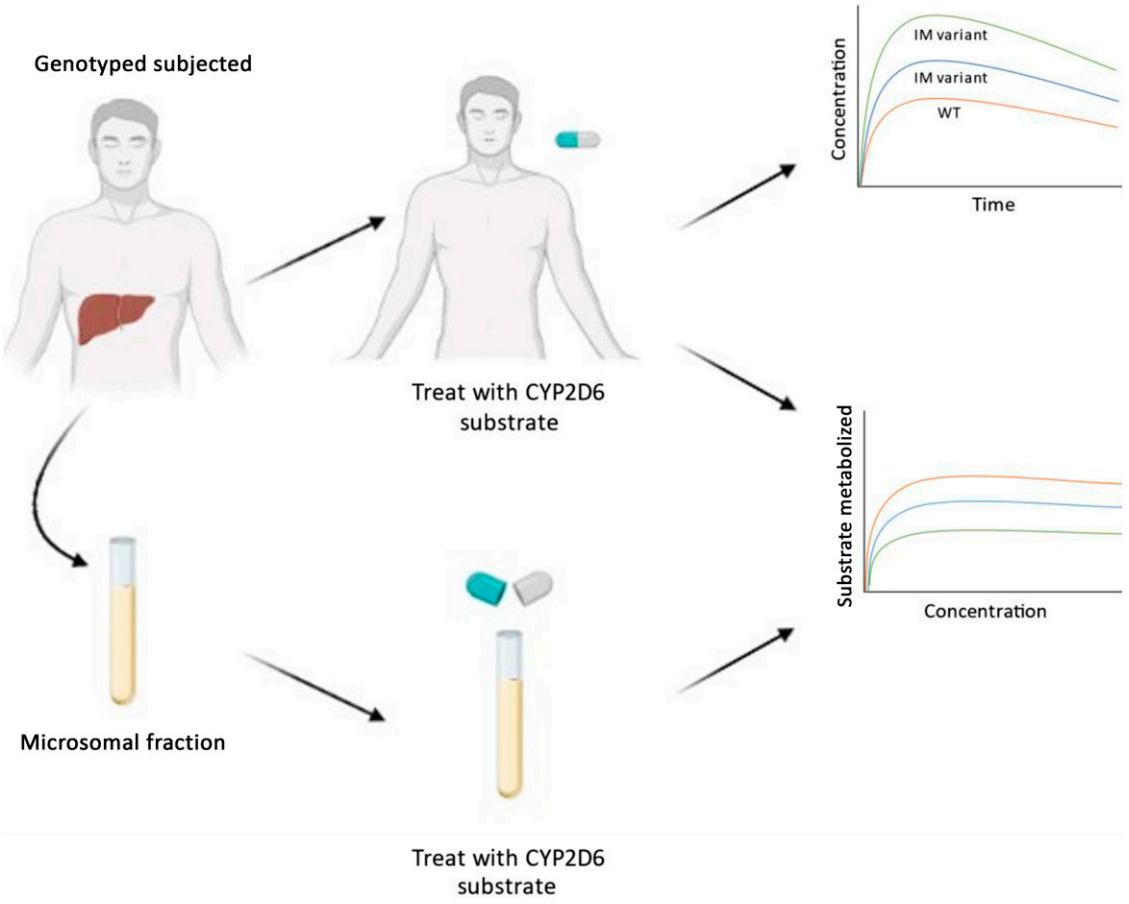

Fig. 2. Schematic of clinical study results from treated genotyped subjects or treated microsomes purified from subjects to determine variant impact on PK profile of a substrate or metabolic activity. Created with BioRender.com.

Additionally, targeting enrollment into clinical trials of minority populations that are more likely to have alleles of interest would enable the collection of clinical data to assess the impact of genomic variation more thoroughly on the drug's PK, efficacy, and safety. Lack of racial and ethnic diversity in clinical trials leads to the lack of genetic heterogeneity, ultimately skewing the results and reducing the generalizability to the American public (Clark et al., 2019). This also hinders the ability to characterize variants that are prevalent in individuals of underrepresented populations, such as CYP2D6*10, which is common in Asian populations. The FDA has outlined recommendations to improve diversity within clinical trials, such as requirements for collection and analysis of racial and ethnic data, broadening eligibility criteria to improve clinical trial participations, stakeholder engagement to develop a plan to enhance diverse recruitment and enrollment, financial reimbursements, providing language access for participants with limited English proficiency, and partnering with community-based organizations to support the study (https://www.fda.gov/media/157635/download).

In conclusion, this review highlights the use of cell-based in vitro models to evaluate the impact of rare variants of *CYP2D6* and their metabolic impact on CYP2D6 substrates. Although many individual variants are rare in the general population, these rare variants may have significant effects on drug metabolism, efficacy, and safety for some individuals and are often found in disproportionate frequencies in minority populations. Since many FDA-approved drugs have prescribing recommendations based on CYP2D6 genotype-inferred phenotype, it is important to understand the potential clinical implications of these variants. Therefore, there is an unmet need to develop and validate in vitro models and other novel methodologies, such as modeling and machine learning approaches, that can comprehensively assess rare variants and/or extrapolate findings from more common variants to understand *CYP2D6* genotype-phenotype relationships, particularly for reduced-function variants that often result in an IM phenotype. Leveraging in vitro models holds significant promise to characterize rare variants with varying IM phenotypes. However, research is still warranted to directly compare in vitro models and their ability to predict clinically significant effects for individual drugs and translate the in vitro findings into clinically actionable CYP2D6 information.

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Wrote or contributed to the writing of the manuscript: Stern, Hyland, Pacanowski, Schuck.

References

Bagheri A, Kamalidehghan B, Haghshenas M, Azadfar P, Akbari L, Sangtarash MH, Vejdandoust F, Ahmadipour F, Meng GY, and Houshmand M (2015) Prevalence of the CYP2D6*10 (C100T), *4 (G1846A), and *14 (G1758A) alleles among Iranians of different ethnicities. *Drug Des Devel Ther* **9**:2627–2634.

Bousman CA, Stevenson JM, Ramsey LB, Sangkuhl K, Hicks JK, Strawn JR, Singh AB, Ruaño G, Mueller DJ, Tsermpini EE, et al. (2023) Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2D6, CYP2C19, CYP2B6, SLC6A4, and HTR2A Genotypes and Serotonin Reuptake Inhibitor Antidepressants. *Clin Pharmacol Ther* **114**:51–68.

Brown JT, Bishop JR, Sangkuhl K, Nurmi EL, Mueller DJ, Dinh JC, Gaedigk A, Klein TE, Caudle KE, McCracken JT, et al. (2019) Clinical Pharmacogenetics Implementation Consortium Guideline for Cytochrome P450 (CYP)2D6 Genotype and Atomoxetine Therapy. *Clin Pharmacol Ther* **106**:94–102.

Cai WM, Chen B, and Zhang WX (2007) Frequency of CYP2D6*10 and *14 alleles and their influence on the metabolic activity of CYP2D6 in a healthy Chinese population. *Clin Pharmacol Ther* **81**:95–98.

Caudle KE, Sangkuhl K, Whirl-Carrillo M, Swen JJ, Haidar CE, Klein TE, Gammal RS, Relling MV, Scott SA, Hertz DL, et al. (2020) Standardizing CYP2D6 Genotype to Phenotype Translation: Consensus Recommendations from the Clinical Pharmacogenetics Implementation Consortium and Dutch Pharmacogenetics Working Group. *Clin Transl Sci* **13**:116–124.

Chen C, Gonzalez FJ, and Idle JR (2007) LC-MS-based metabolomics in drug metabolism. *Drug Metab Rev* **39**:581–597.

Clark LT, Watkins L, Piña IL, Elmer M, Akinboboye O, Gorham M, Jamerson B, McCullough C, Pierre C, Polis AB, et al. (2019) Increasing Diversity in Clinical Trials: Overcoming Critical Barriers. *Curr Probl Cardiol* **44**:148–172.

Crews KR, Gaedigk A, Dunnenberger HM, Klein TE, Shen DD, Callaghan JT, Kharasch ED, and Skaar TC; Clinical Pharmacogenetics Implementation Consortium (2012) Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for codeine therapy in the context of cytochrome P450 2D6 (CYP2D6) genotype. *Clin Pharmacol Ther* **91**:321–326.

Crews KR, Monte AA, Huddart R, Caudle KE, Kharasch ED, Gaedigk A, Dunnenberger HM, Leeder JS, Callaghan JT, Samer CF, et al. (2021) Clinical Pharmacogenetics Implementation Consortium Guideline for CYP2D6, OPRM1, and COMT Genotypes and Select Opioid Therapy. *Clin Pharmacol Ther* **110**:888–896.

Daly AK, Brockmöller J, Broly F, Eichelbaum M, Evans WE, Gonzalez FJ, Huang JD, Idle JR, Ingelman-Sundberg M, Ishizaki T, et al. (1996) Nomenclature for human CYP2D6 alleles. *Pharmacogenetics* **6**:193–201.

de Waal PW, Sunden KF, and Furge LL (2014) Molecular dynamics of CYP2D6 polymorphisms in the absence and presence of a mechanism-based inactivator reveals changes in local flexibility and dominant substrate access channels. *PLoS One* **9**:e108607.

Donato MT, Jiménez N, Castell JV, and Gómez-Lechón MJ (2004) Fluorescence-based assays for screening nine cytochrome P450 (P450) activities in intact cells expressing individual human P450 enzymes. *Drug Metab Dispos* **32**:699–706.

Fedorinov DS, Mirzaev KB, Mustafina VR, Sychev DA, Maximova NR, Chertovskikh JV, Popova NV, Tarabukina SM, and Rudykh ZA (2018) Pharmacogenetic testing by polymorphic markers G1846A (CYP2D6*4) and C100T (CYP2D6*10) of the CYP2D6 gene in coronary heart disease patients taking $\beta\beta$-blockers in the Republic of Sakha (YAKUTIA). *Drug Metab Pers Ther* **33**:195–200.

Foster A, Mobley E, and Wang Z (2007) Complicated pain management in a CYP450 2D6 poor metabolizer. *Pain Pract* **7**:352–356.

Gaedigk A, Bradford LD, Marcucci KA, and Leeder JS (2002) Unique CYP2D6 activity distribution and genotype-phenotype discordance in black Americans. *Clin Pharmacol Ther* **72**:76–89.

Gaedigk A, Casey ST, Whirl-Carrillo M, Miller NA, and Klein TE (2021) Pharmacogene Variation Consortium: A Global Resource and Repository for Pharmacogene Variation. *Clin Pharmacol Ther* **110**:542–545.

Gaedigk A, Ingelman-Sundberg M, Miller NA, Leeder JS, Whirl-Carrillo M, and Klein TE; PharmVar Steering Committee (2018) The Pharmacogene Variation (PharmVar) Consortium: Incorporation of the Human Cytochrome P450 (CYP) Allele Nomenclature Database. *Clin Pharmacol Ther* **103**:399–401.

Goetz MP, Sangkuhl K, Guchelaar HJ, Schwab M, Province M, Whirl-Carrillo M, Symmans WF, McLeod HL, Ratain MJ, Zembutsu H, et al. (2018) Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2D6 and Tamoxifen Therapy. *Clin Pharmacol Ther* **103**:770–777.

Gonzalez E, Jain S, Shah P, Torimoto-Katori N, Zakharov A, Nguyễn ĐT, Sakamuru S, Huang R, Xia M, Obach RS, et al. (2021) Development of Robust Quantitative Structure-Activity Relationship Models for CYP2C9, CYP2D6, and CYP3A4 Catalysis and Inhibition. *Drug Metab Dispos* **49**:822–832.

Grimsrud KN, Ivanova X, Sherwin CM, Palmieri TL, and Tran NK (2019) Identification of Cytochrome P450 Polymorphisms in Burn Patients and Impact on Fentanyl Pharmacokinetics: A Pilot Study. *J Burn Care Res* **40**:91–96.

Hicks JK, Bishop JR, Sangkuhl K, Müller DJ, Ji Y, Leckband SG, Leeder JS, Graham RL, Chiulli DL, LLerena A, et al.; Clinical Pharmacogenetics Implementation Consortium (2015) Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2D6 and CYP2C19 Genotypes and Dosing of Selective Serotonin Reuptake Inhibitors. *Clin Pharmacol Ther* **98**:127–134.

Hicks JK, Sangkuhl K, Swen JJ, Ellingrod VL, Müller DJ, Shimoda K, Bishop JR, Kharasch ED, Skaar TC, Gaedigk A, et al. (2017) Clinical pharmacogenetics implementation consortium guideline (CPIC) for CYP2D6 and CYP2C19 genotypes and dosing of tricyclic antidepressants: 2016 update. *Clin Pharmacol Ther* **102**:37–44.

Hicks JK, Swen JJ, and Gaedigk A (2014) Challenges in CYP2D6 phenotype assignment from genotype data: a critical assessment and call for standardization. *Curr Drug Metab* **15**:218–232.

ICH MIDD Discussion Group (2022) Considerations with respect to future MIDD related guidelines output from ICH model-informed drug development (MIDD) discussion group (DG) 2021.

Shen H, He MM, Liu H, Wrighton SA, Wang L, Guo B, and Li C (2007) Comparative metabolic capabilities and inhibitory profiles of CYP2D6.1, CYP2D6.10, and CYP2D6.17. *Drug Metab Dispos* **35**:1292–1300.

Sakuyama K, Sasaki T, Ujiie S, Obata K, Mizugaki M, Ishikawa M, and Hiratsuka M (2008) Functional characterization of 17 CYP2D6 allelic variants (CYP2D6.2, 10, 14A-B, 18, 27, 36, 39, 47-51, 53-55, and 57). *Drug Metab Dispos* **36**:2460–2467.

Kane M (2021) *CYP2D6 Overview: Allele and Phenotype Frequencies*, in Medical Genetics Summaries, National Center of Biotechnology Information, Bethesda, MD.

Lim HS, Ju Lee H, Seok Lee K, Sook Lee E, Jang IJ, and Ro J (2007) Clinical implications of CYP2D6 genotypes predictive of tamoxifen pharmacokinetics in metastatic breast cancer. *J Clin Oncol* **25**:3837–3845.

Masimirembwa C, Persson I, Bertilsson L, Hasler J, and Ingelman-Sundberg M (1996) A novel mutant variant of the CYP2D6 gene (CYP2D6*17) common in a black African population: association with diminished debrisoquine hydroxylase activity. *Br J Clin Pharmacol* **42**:713–719.

Matsunaga M, Yamazaki H, Kiyotani K, Iwano S, Saruwatari J, Nakagawa K, Soyama A, Ozawa S, Sawada J, Kashiyama E, et al. (2009) Two novel CYP2D6*10 haplotypes as possible causes of a poor metabolic phenotype in Japanese. *Drug Metab Dispos* **37**:699–701.

Micaglio E, Locati ET, Monasky MM, Romani F, Heilbron F, and Pappone C (2021) Role of Pharmacogenetics in Adverse Drug Reactions: An Update towards Personalized Medicine. *Front Pharmacol* **12**:651720.

Muroi Y, Saito T, Takahashi M, Sakuyama K, Niinuma Y, Ito M, Tsukada C, Ohta K, Endo Y, Oda A, et al. (2014) Functional characterization of wild-type and 49 CYP2D6 allelic variants for N-desmethyltamoxifen 4-hydroxylation activity. *Drug Metab Pharmacokinet* **29**:360–366.

National Academies of Sciences, Engineering, and Medicine (2022) Medicine, in *Improving Representation in Clinical Trials and Research: Building Research Equity for Women and Underrepresented Groups* (Bibbins-Domingo K and Helman A eds) pp 75–107, National Academies Press, Washington, DC.

Nofziger C, Turner AJ, Sangkuhl K, Whirl-Carrillo M, Agúndez JAG, Black JL, Dunnenberger HM, Ruano G, Kennedy MA, Phillips MS, et al. (2020) PharmVar GeneFocus: CYP2D6. *Clin Pharmacol Ther* **107**:154–170.

Oscarson M, Hidestrand M, Johansson I, and Ingelman-Sundberg M (1997) A combination of mutations in the CYP2D6*17 (CYP2D6Z) allele causes alterations in enzyme function. *Mol Pharmacol* **52**:1034–1040.

Pratt VM, Cavallari LH, Del Tredici AL, Gaedigk A, Hachad H, Ji Y, Kalman LV, Ly RC, Moyer AM, Scott SA, et al. (2021) Recommendations for Clinical CYP2D6 Genotyping Allele Selection: A Joint Consensus Recommendation of the Association for Molecular Pathology, College of American Pathologists, Dutch Pharmacogenetics Working Group of the Royal Dutch Pharmacists Association, and the European Society for Pharmacogenomics and Personalized Therapy. *J Mol Diagn* **23**:1047–1064.

Relling MV and Klein TE (2011) CPIC: Clinical Pharmacogenetics Implementation Consortium of the Pharmacogenomics Research Network. *Clin Pharmacol Ther* **89**:464–467.

Saghafi F, Salehifar E, Janbabai G, Zaboli E, Hedayatizadeh-Omran A, Amjadi O, and Moradi S (2018) CYP2D6*3 (A2549del), *4 (G1846A), *10 (C100T) and *17 (C1023T) genetic polymorphisms in Iranian breast cancer patients treated with adjuvant tamoxifen. *Biomed Rep* **9**:446–452.

Saito T, Gutiérrez Rico EM, Kikuchi A, Kaneko A, Kumondai M, Akai F, Saigusa D, Oda A, Hirasawa N, and Hiratsuka M (2018) Functional characterization of 50 CYP2D6 allelic variants by assessing primaquine 5-hydroxylation. *Drug Metab Pharmacokinet* **33**:250–257.

Shimada T, Tsumura F, Yamazaki H, Guengerich FP, and Inoue K (2001) Characterization of (+/-)-bufuralol hydroxylation activities in liver microsomes of Japanese and Caucasian subjects genotyped for CYP2D6. *Pharmacogenetics* **11**:143–156.

Shiraishi T, Hosokawa M, Kobayashi K, Tainaka H, Yamaura Y, Taguchi M, and Chiba K (2002) Effects of G169R and P34S substitutions produced by mutations of CYP2D6*14 on the functional properties of CYP2D6 expressed in V79 cells. *Drug Metab Dispos* **30**:1201–1205.

Sistonen J, Fuselli S, Levo A, and Sajantila A (2005) CYP2D6 genotyping by a multiplex primer extension reaction. *Clin Chem* **51**:1291–1295.

Taylor C, Crosby I, Yip V, Maguire P, Pirmohamed M, and Turner RM (2020) A Review of the Important Role of *CYP2D6* in Pharmacogenomics. *Genes (Basel)* **11**:1295.

van der Lee M, Guchelaar HJ, and Swen JJ (2021) Substrate specificity of CYP2D6 genetic variants. *Pharmacogenomics* **22**:1081–1089.

van der Weide J and Steijns LS (1999) Cytochrome P450 enzyme system: genetic polymorphisms and impact on clinical pharmacology. *Ann Clin Biochem* **36**:722–729.

Wang SL, Lai MD, and Huang JD (1999) G169R mutation diminishes the metabolic activity of CYP2D6 in Chinese. *Drug Metab Dispos* **27**:385–388.

Wennerholm A, Dandara C, Sayi J, Svensson JO, Abdi YA, Ingelman-Sundberg M, Bertilsson L, Hasler J, and Gustafsson LL (2002) The African-specific CYP2D617 allele encodes an enzyme with changed substrate specificity. *Clin Pharmacol Ther* **71**:77–88.

Wennerholm A, Johansson I, Hidestrand M, Bertilsson L, Gustafsson LL, and Ingelman-Sundberg M (2001) Characterization of the CYP2D6*29 allele commonly present in a black Tanzanian population causing reduced catalytic activity. *Pharmacogenetics* **11**:417–427.

Xu Y, Sun Y, Yao L, Shi L, Wu Y, Ouyang T, Li J, Wang T, Fan Z, Fan T, et al. (2008) Association between CYP2D6 *10 genotype and survival of breast cancer patients receiving tamoxifen treatment. *Ann Oncol* **19**:1423–1429.

Yu A, Kneller BM, Rettie AE, and Haining RL (2002) Expression, purification, biochemical characterization, and comparative function of human cytochrome P450 2D6.1, 2D6.2, 2D6.10, and 2D6.17 allelic isoforms. *J Pharmacol Exp Ther* **303**:1291–1300.

Zanger UM and Schwab M (2013) Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. *Pharmacol Ther* **138**:103–141.

Zhao M, Ma J, Li M, Zhang Y, Jiang B, Zhao X, Huai C, Shen L, Zhang N, He L, et al. (2021) Cytochrome P450 Enzymes and Drug Metabolism in Humans. *Int J Mol Sci* **22**:12808.

Zineh I, Beitelshees AL, Gaedigk A, Walker JR, Pauly DF, Eberst K, Leeder JS, Phillips MS, Gelfand CA, and Johnson JA (2004) Pharmacokinetics and CYP2D6 genotypes do not predict metoprolol adverse events or efficacy in hypertension. *Clin Pharmacol Ther* **76**:536–544.

Address correspondence to: Dr. Robert Schuck, Office of Clinical Pharmacology, Office of Translational Science, Center for Drug Evaluation and Research, U.S. Food and Drug Administration, 10903 New Hampshire Ave, Silver Spring, Maryland 20993. E-mail: robert.schuck@fda.hhs.gov