Towards solving the missing heritability in pharmacogenomics

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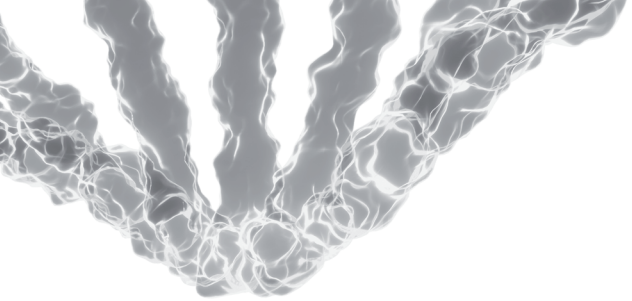

CHAPTER 6

Substrate specificity of cytochrome P450 2D6 genetic variants

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Abstract

Genetic variation in the gene encoding CYP2D6 is used to guide drug prescribing in clinical practice. However, genetic variants in *CYP2D6* show substrate specific effects which are currently not accounted for. With a systematic literature we retrieved 22 original studies describing in vitro experiments focusing on *CYP2D6* alleles (*CYP2D6**1, *2, *10 and *17) and substrates. Allele activity (clearance of the allele of interest divided by the clearance of the wildtype) was extracted.

The results support the hypothesis of the existence of substrate specificity of the *CYP2D6**17-allele (higher debrisoquine clearance), a subtle effect of the *CYP2D6**10-allele (lower dextromethorphan clearance) but no substrate specific effect of the *CYP2D6**2-allele.

While our results support substrate specificity, for most substrates data are too sparse and require further studies.

Introduction

The cytochrome P450 2D6 (CYP2D6) enzyme is involved in the metabolism of 20–30% of commonly prescribed drugs, making it one of the most important enzymes in drug metabolism [1]. The gene encoding the CYP2D6 enzyme, *CYP2D6*, is highly polymorphic and harbors single nucleotide variants as well as structural variants. These variants lead to either increased or decreased enzyme activity [2,3]. To allow for clinical interpretation, variants within the *CYP2D6* gene are translated into haplotypes using the star(*)-nomenclature [3]. Currently, more than 140 *CYP2D6* haplotypes have been described and are catalogued by the Pharmacogene Variation Consortium (PharmVar). Each allele is assigned an allele activity score, with a score of 0 for non-functional alleles, 0.25 or 0.5 for decreased activity alleles, 1.0 for fully active alleles and 2.0 for duplications or increased activity alleles. The scores of the paternal and maternal alleles are combined into a gene activity score (GAS) for the subject, which in turn is translated into predicted drug metabolizer phenotypes based on the guidelines from CPIC (Clinical Pharmacogenetics Implementation Consortium) or the DPWG (Dutch Pharmacogenetics Working Group) [4-6].

The activity scores and the genotype predicted drug metabolizer phenotypes are based on in vitro and in vivo data. Results from in vitro experiments are used to infer activity scores for *CYP2D6* alleles for which data from clinical studies is lacking [3,7,8]. Gene-drug interactions are often supported by high levels of evidence before they are included in the guidelines [9,10]. However, with estimations showing that more than 95% of the variants in pharmacogenes are rare variants [11,12], it is impossible to obtain such high-level evidence for the effect of every single haplotype in the *CYP2D6* gene. Therefore, in vitro experiments can be used to assess the activity of (novel) haplotypes and compare this to haplotypes of which the effect is known [7,13]. Even though the Pharmacogenomics Knowledge base (PharmGKB) classifies in vitro experiments amongst the lowest level of evidence, comparable to case reports [13], in vitro data can be valuable in gaining insight into the effect of (novel) variants and haplotypes. These results can be used in the greater scope of gene-drug interactions.

Generally, the genotype to phenotype translations from a particular substrate is extrapolated to all CYP2D6 substrates, assuming generalizability for allele activity and for the clinical impact of the phenotype categories [4,5]. For example, if a patient carries a *CYP2D6* *1/*4 genotype, by convention the predicted CYP2D6 phenotype is an intermediate metabolizer regardless of the CYP2D6 substrate [4,5].

However, the extent to which the metabolism is affected by a specific genetic variant may differ per substrate. This inter substrate variability is also known as substrate specificity [14,15]. Indeed, pharmacokinetic data show that for tricyclic antidepressants

the *CYP2D6**1/*4 diplotype leads to significantly decreased metabolism whereas the metabolism of the CYP2D6 substrate codeine is much less affected [9,16].

In vitro data could help to better characterize and understand the impact of specific variants in CYP2D6 substrate specificity. However, it is well recognized that the use of data from individual in vitro studies to predict in vivo variant contributions and substrate specific effects is limited due to the substantial impact of experimental conditions such as the applied vector and incubation concentrations on the results of the in vitro experiments [17-19]. This restricts the value of individual in-vitro studies regarding substrate specificity as it cannot be determined if the observed effect is the results of substrate specificity or the experimental design. In this study, we collect data from multiple in vitro kinetic studies to assess the impact of *CYP2D6* variants on different substrates with the aim to estimate the role of substrate specificity. Comparisons will be made within one study and experimental system, to correct for study-specific effects.

Methods

Literature search

Studies were selected through a systematic literature search focusing on the CYP2D6 substrates included in the Flockhart table [20]. PubMed was searched to identify and extract relevant papers until December 2020. Search terms consisted of "CYP2D6", "functional characterization" and the substrate of interest. Reference lists from reviews were manually checked to identify relevant cross-references. The full search string is provided in Supplementary File S6.1. Only papers with full text available in the English language were included. Furthermore, the studies had to include kinetic parameters including the Km (Michaelis constant) and Vmax (maximum conversion speed).

The study was limited to *CYP2D6* alleles which are included in the *-nomenclature, meaning that no individual (rare) variants were included.

Data extraction

From the selected studies the following data were extracted: kinetic parameters, incubation specifics and the transfection vector used. Four kinetic parameters were extracted or calculated based on available data: Km, Vmax, intrinsic clearance (Clint expressed as Vmax/Km), calculated enzyme activity. The calculated activity was defined as the Clint of the allele of interest normalized to the Clint of the wildtype *1 allele in the same experiment. Individual kinetic parameters (Km, Vmax and Clint) were highly variable

between studies due to study specific effects, resulting in an inability to compare these parameters between studies. To assess the substrate specific effects per allele, the calculated activity of all substrates was compared. For substrates which were studied in more than 2 studies, a comparison of multiple drugs within one study was made to assess the relative activity of an allele for the different drugs. This cut-off was made to ensure that the same substrate comparisons could be made in different studies. Moreover, this comparison negated some of the study-specific effects, as these were assumed to be identical within one study. Experiment specific parameters were extracted to determine if these factors result in differences in observed enzyme activity.

All analyses were performed with R, v3.6.3.

Results

The literature search identified 286 papers. After analysis of the full text, 22 studies were found to meet all inclusion criteria (Supplementary Figure S6.1, Supplementary Table S6.1). Manual checking of references identified bufuralol as a commonly used CYP2D6 substrate for in vitro experiments and this was added to the substrate selection. The alleles selected were *1, *2, *10 and *17, the impact of these variants on the substrate-binding site is summarized in Supplementary Figure S6.2, based on findings by Dong et al. [21]. Substrates occurring in 3 or more studies were bufuralol (7 studies), dextromethorphan (8 studies), debrisoquine (5 studies) and venlafaxine (3 studies), hereafter called the common substrates. For another 13 substrates, less than 3 studies were identified (Supplementary Figure S6.1 and Supplementary Table S6.2).

Study-specific parameters were collected and assessed on their influence on the observed activity. Investigated parameters were: vector (e.g. baculovirus, yeast cells, microsomes), incubation time and the minimum and maximum substrate concentrations. The majority of these factors did not seem to influence the outcomes in a specific direction (Supplementary Figure S6.3). The only exception was the use of yeast cells which seems to result in a slightly higher activity of *CYP2D6**10 and *CYP2D6**17 compared to the other vectors, however, for *CYP2D6**2 this effect was not observed.

*CYP2D6**2

The mean in vitro activity of *CYP2D6**2 was 0.65±0.34 (range 0.17–1.18), Supplementary Table S6.2. While this is higher than the activity of *10 and *17, there is also a suggestion of a decrease of activity compared to the wildtype allele. In clinical practice, it is currently assumed that the *2 allele has the same activity as the wildtype allele.

Of the four studies comparing bufuralol and dextromethorphan, two reported a comparable activity of *2 for both substrates [22,23] while one reported a decreased activity of the *2 allele for dextromethorphan [24] (Figure 6.2 and Supplementary Table S6.1). Of the two studies focusing on debrisoquine, Marcucci et al. reported a decreased activity compared to bufuralol (1.18 compared to 1.01) [23], while Bapiro et al. reported a slight increase (0.80 compared to 0.74) [24].

For the other substrates, a similar amount of variability is observed, with activities ranging from clear decreased activity for amitriptyline to normal activity for metoprolol (Figure 6.1). Moreover, for many of the substrates (7/15 substrates), *CYP2D6**2 was a decreased activity allele with an activity around 0.30–0.70, while the activity was comparable to *1 (0.80–1.2) for only 4/15 substrates.

*CYP2D6**10

The mean in vitro activity of *CYP2D6**10 was 0.21±0.27 (range: 0.01–0.96), which is in line with the current gene activity score of 0.25. However, a larger amount of variability remains with some studies reporting almost no activity and others normal activity (Supplementary Table S6.1 and S6.2).

For the common substrates, the results are suggestive of a lower *CYP2D6**10 activity in the metabolism of dextromethorphan compared to the other substrates (Table 6.1, Figure 6.1). Nonetheless, by comparing the metabolic activities of the different substrates within studies, this effect is no longer evident. Three studies comparing dextromethorphan to bufuralol report conflicting results (Figure 6.2, Supplementary Table S6.1). Nakamura

Table 6.1: Relative activity of alleles for common substrates
Activity is compared to *1 by dividing the intrinsic clearance of the allele of interest by the intrinsic clearance of the *1 allele within the same experiment. Data is obtained from kinetic parameters of selected in vitro studies focusing on dextromethorphan, bufuralol, debrisoquine and venlafaxine.

		Dextromethorphan	Bufuralol	Debrisoquine	Venlafaxine
*2	Observations (n)	5	5	3	2
	Mean (sd)	0.57 (0.34)	0.77 (0.28)	0.98 (0.17)	0.67 (0.57)
	Median (range)	0.44 (0.19–1.1)	0.74 (0.40–1.18)	1.01 (0.8–1.13)	0.67 (0.26–1.07)
*10	Observations (n)	6	5	2	3
	Mean (sd)	0.037 (0.02)	0.32 (0.38)	0.26 (0.20)	0.24 (0.22)
	Median (range)	0.04 (0.01–0.06)	0.24 (0.01–0.96)	0.26 (0.12–0.4)	0.22 (0.03–0.47)
*17	Observations (n)	5	4	3	1
	Mean (sd)	0.17 (0.058)	0.27 (0.07)	0.52 (0.26)	0.097 (NA)
	Median (range)	0.17 (0.10–0.25)	0.24 (0.21–0.38)	0.64 (0.22–0.7)	0.097 (NA)

et al. report a lower metabolic activity of *CYP2D6**10 for dextromethorphan compared to bufuralol (0.06 for dextromethorphan and 0.33 for bufuralol) [25]. By contrast, Cai et al. and Shen et al. report a small increase in metabolic activity for dextromethorphan compared to bufuralol (0.05 and 0.05 for dextromethorphan compared to 0.01 and 0.04 for bufuralol) [22,26].

For the other substrates, a wide range of *CYP2D6**10 activity was observed. The substrates amitriptyline, codeine, fluoxetine, metoprolol, nortriptyline, propranolol and tramadol all showed an activity below 0.13 In contrast, *CYP2D6**10 activity in regards to mexiletine metabolism (0.78 and 0.93) reflected almost normal function.

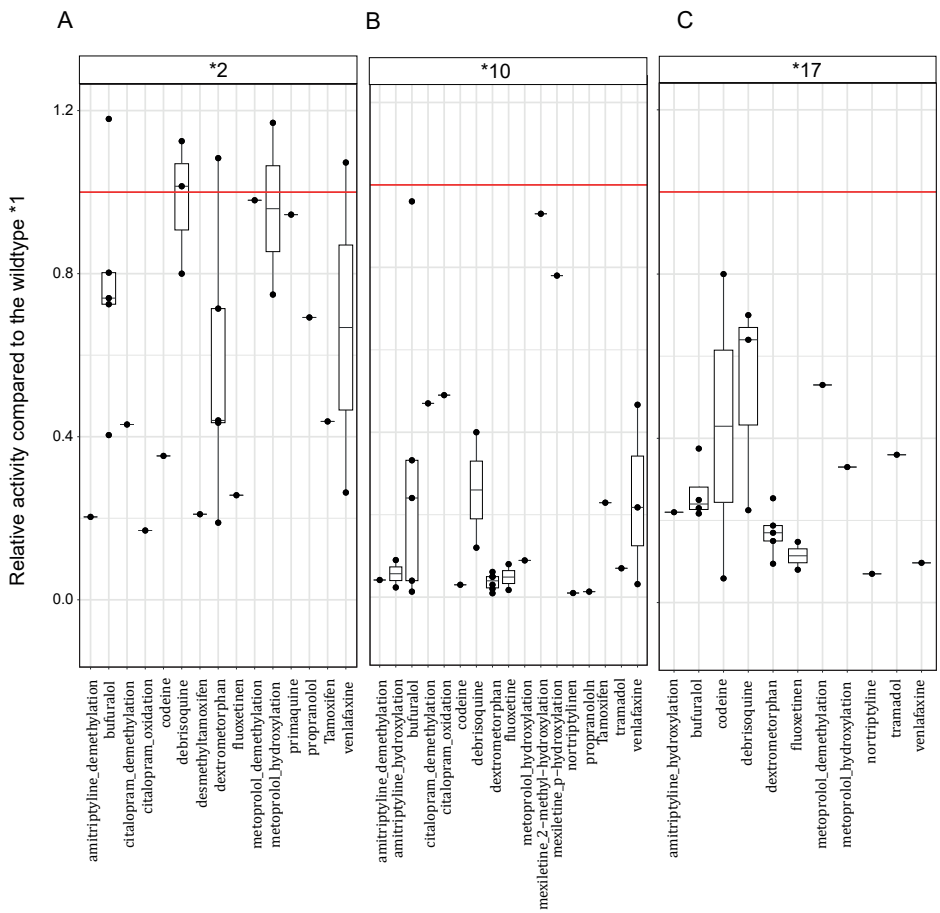

Figure 6.1: In vitro activity of *2, *17 and *2
Activity is calculated as the clearance of the allele of interest divided by the clearance of the wildtype. The red line represents the wildtype activity of 1.0. **A.** the *2 activity for 13 substrates and 15 conversions. **B.** the activity of *10 for 14 substrates and 17 metabolic steps. **C.** the activity of the *17 allele for 11 substrates and 12 metabolic conversions.

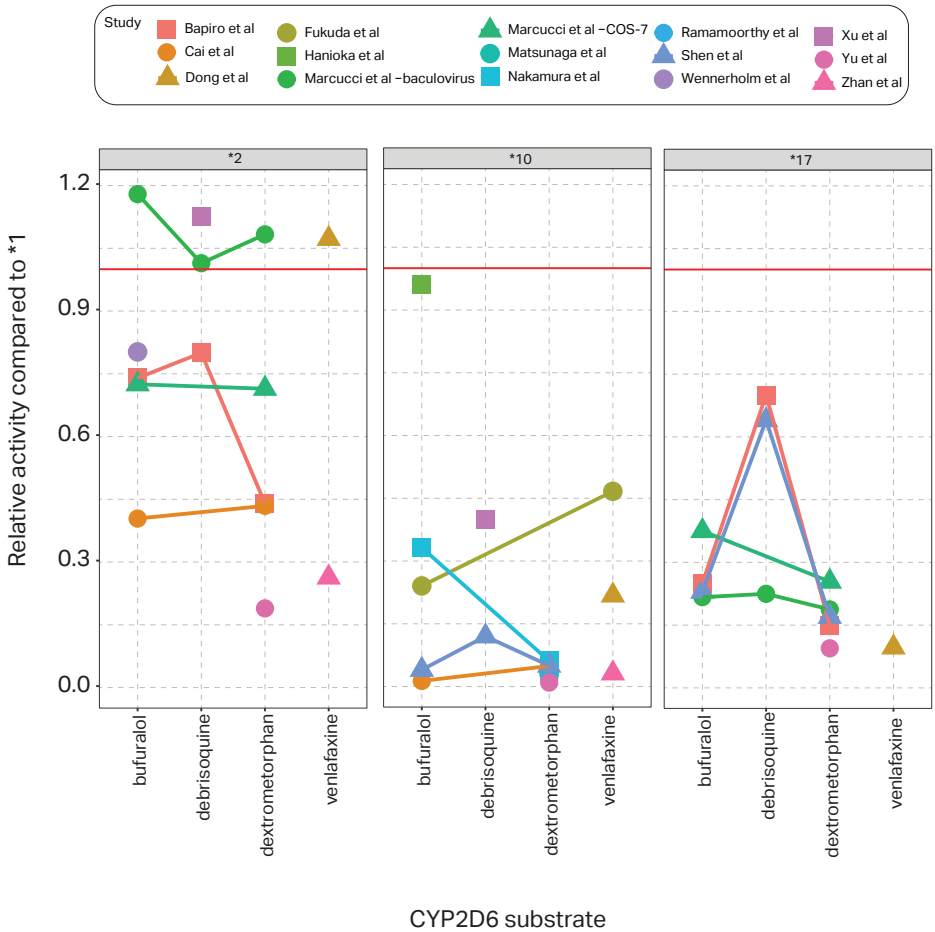

Figure 6.2: CYP2D6 substrate specificity
Within study comparisons for the commonly studies substrates; bufuralol, debrisoquine, dextromethorphan and venlafaxine. Each line connects different observations within the same study, thereby correcting for study specific effects. The majority of the studies show substrate specific activities but are not in agreement with other studies. For *CYP2D6**17 a clear substrate specific effect for debrisoquine is observed.

*CYP2D6**17

On average the in vitro *CYP2D6**17 activity was 0.28±0.21 (range 0.06–0.80), which is comparable to the *CYP2D6**10 activity and lower than the currently assigned gene activity score of 0.5 (Supplementary Table S6.2).

For the common substrates, the metabolic activity of *CYP2D6**17 for debrisoquine is almost twice as high compared to the other substrates (Table 6.1, Figure 6.1). When comparing findings within studies, 2 out of 3 studies report a similar trend (0.64 and 0.70 for debrisoquine and 0.04 and 0.25 for bufuralol) (Figure 6.2 and Supplementary Table S6.1)

[24,26]. By contrast, Marcucci et al. report an activity of 0.23 for debrisoquine compared to 0.22 for bufuralol and 0.19 for dextromethorphan when using baculovirus, indicating comparable activity for all three substrates [23]. This suggests a potential substrate specific effect of *CYP2D6**17 resulting in a higher activity for debrisoquine.

Results for the other substrate were equally diverse. Most interestingly, the two studies investigating codeine O-demethylation reported both the highest activity of *CYP2D6**17 (0.8) [26] and the lowest activity (0.06) [27]. Both of these studies used the same type of vector, namely a baculovirus. Moreover, only four observations reported an activity above 0.5, indicating a clear decreased activity function of *17 for all substrates.

Discussion

In this paper we summarize available in vitro data regarding CYP2D6 substrate specificity. Our study supports the existence of substrate specific effects of the *17 allele, a more subtle effect of the *10 allele and no substrate specificity of the *2 allele, in the metabolism of dextromethorphan, bufuralol, debrisoquine and venlafaxine. The activity of the *17 allele was higher for debrisoquine compared to bufuralol and dextromethorphan in 2 out of 3 studies, in the third study the activity was comparable. Nonetheless, the variability of reported activities for the same substrate was large between studies, making it difficult to distinguish substrate specific effects from study-specific effects.

The variants included in the characterization of *17 are 2851C>T (R296C), 4181C>G (S486T) and 1022C>T (T107I). The first two variants are also the characteristic variants of the *2 allele. Therefore, a comparison between *2 and *17 focuses on the contribution of the 1022C>T variant. The *17 allele was the first allele for which a substrate-dependent affinity was reported. As Oscarson et al. indicated, the 1022C>T mutation affected the metabolism of codeine but did not have a significant effect on the metabolism of bufuralol. To alter the metabolism of bufuralol the combination of the 1022C>T and the 2851C>T was needed [28]. It has been suggested that the *17 allele is affecting protein binding by decreasing the substrate access channel as well as a decrease in active sites of 21%. The effect of this decrease depends on the 3D structure and size of the substrate and thus results in substrate specific effects [21]. The relatively high activity of *CYP2D6**17 in the metabolism of debrisoquine can potentially be explained by the size of the drugs. Debrisoquine is substantially smaller than the other drugs at 175 g/mol for debrisoquine compared to 261 and 271 g/mol for bufuralol and dextromethorphan respectively [29]. This might allow debrisoquine to still fit through the smaller substrate-access channel caused by the *CYP2D6**17 variant, while the other drugs do not fit as easily. Additionally,

the 1022C>T mutation results in a change from the hydrophilic Thr to a hydrophobic Ile, right at the entrance of the substrate-access channel causing alteration in hydrogen bonds and potentially in the attraction of substrates [21].

Interestingly, in the metabolism of debrisoquine the activity in each study was highest for *2. This might indicate the importance of the 2851C>T (R296C) and 4181C>G (S486T) variants in the increased activity of debrisoquine metabolism. Similar to the *17 allele, the *2 allele also results in a decrease in substrate access channel albeit it is a smaller decrease compared to *17. It has been suggested that this smaller decrease results in a tighter binding pocket and therefore a better fit for some substrates [21].

The *CYP2D6**10 haplotype is characterized by 4181C>G (S486T) and 100C>T (P34S). the predicted activities for *10 differed greatly, with an activity of <0.1 for nine substrates and a group of six substrates with activities higher than 0.25. The low in vitro activity observed is in line with a previous review investigating *CYP2D6**10 substrate specificity. This review reported that the *10 allele decreased the CYP2D6 activity by more than 90% for most substrates, except for mexiletine and venlafaxine. Similar to the current study, it was also concluded that the large variability between studies complicates the interpretation and quantification of substrate specific effects [30]. Several in vitro and in vivo studies have shown that the amount of functional protein expressed by *CYP2D6**10 is many-fold lower than that of a wildtype allele [31-34]. Decreased levels of protein expression would mean that all substrates are similarly affected. Nonetheless, In addition to decreased expression, *CYP2D6**10 also leads to a smaller substrate access channel, similar to *2 and *17 [21]. This change in active sites and substrate binding channel combined with the decrease in expression can lead to a substrate specific effect in addition to a general lower activity due to a decrease in expression. This could explain that while *CYP2D6**17 has the largest changes in substrate binding sites and access channel, it is *CYP2D6**10 that displays the lowest activity.

As previously mentioned, the heterogeneity between in vitro experiments is a known problem and can potentially be explained by multiple factors. A potential source of variability in results between studies could be the transfection vector used [18,19]. Indeed, studies using yeast cells reported slightly higher CYP2D6 activities compared to the other transfection vectors. Nonetheless, this effect was limited and was not observed for the other vectors. Moreover, for *CYP2D6**17 metabolism of codeine the highest and lowest activities (0.06 and 0.8) were reported by studies using the same vector, namely a baculovirus.

Another cause for the observed heterogeneity between studies might be the genetic make-up of the *1 template used for the wildtype. While some studies used human *CYP2D6**1 cDNA which was checked by sequencing [21-25,27,35], others reverse

engineered cDNA of the variant of interest to serve as the wildtype comparator [33]. This means that there are potential genetic differences in the wildtype templates used by the different studies. Variants in the wildtype alleles can influence its activity and thereby the relative activity of the other alleles.

Moreover, for in vivo experiments a suggested cause of variation in the activity of the *2 allele is the presence of an upstream enhancer [36,37]. However, no upstream variants are included in the applied in vitro assays and therefore the role of the suggested upstream enhancer cannot be assessed with in vitro experiments.

The majority of the current PGx guidelines relate to genetic variants associated with drug metabolism. As such, predicting in vivo enzyme activity based on genetic test results is instrumental for clinical PGx. Marcath et al. have shown that for *CYP2D6**10 the activity observed in vitro does not necessarily translate to the in vivo setting. More specifically, they noted that the decrease in CYP2D6 activity was approximately 90% in most in vitro studies. However, in the human PK studies this decrease was between 15.9 and 69.9% which is substantially lower [30]. One of the main hurdles in translating in vitro results to an in vivo setting is the influence of variants of CYP2D6 protein expression levels. Protein expression can vary greatly between different transfection systems [17-19]. The parameters used in this study were all adjusted for protein content (Vmax = pmol substrate/pmol CYP2D6) to cancel out the differences in regards to protein expression. However, by using this correction it is no longer possible to detect any changes in expression caused by the introduced variants.

While our study provides insight into substrate specificity of CYP2D6 there were also several limitations. First and foremost, due to the heterogeneity between in vitro experiments it was not possible to quantitatively compare findings between studies. For only a few substrates there was sufficient data to allow within-study comparisons, thereby correcting for study-specific effects. Additionally, in vitro data in itself suffers from limitations when it comes to PGx effect predictions. Small differences in set-up (e.g. vector and origin of cDNA template) can result in large differences in outcomes. Moreover, in vitro findings do not always translate to an in vivo prediction, especially not in the case of protein expression.

In conclusion, our results support that genetic variation in *CYP2D6* show substrate specific effects in vitro. Nonetheless, for many CYP2D6 substrates there is limited data regarding in vitro enzyme activity which complicates the study of substrate specificity. Moreover, in vitro data is limited in assigning accurate quantitative activity scores due to the high heterogeneity between studies.

Future perspectives

- Pharmacogenomics informed drug prescribing is becoming standard clinical practice. However, current clinical guidelines assume that the effect of a particular genetic variant on metabolism is independent of the drug substrate.
- However, as our study has shown, there are clear substrate specific effects that can be explained by specific variant-substrate interactions. To further improve our ability to predict drug response and thereby improve therapy outcomes, substrate specific effects should be accounted for in the clinical pharmacogenomics guidelines.

Executive summary

- Data for 4 *-haplotypes (*1, *2, *10, *17) was collected and compared. Four substrates (dextromethorphan, debrisoquine, bufuralol and venlafaxine) were studied in more than 2 studies, allowing for a more detailed analysis of the substrate specific effects. Another 15 substrates were included in only 1 or 2 studies.
- There is significant variability in kinetics effects of the *CYP2D6* alleles dependent on the substrates, with differences in kinetic effects ranging 1.02–5.4 fold for the same allele. Most significantly, the *17 allele showed a 4.7-fold higher activity for debrisoquine compared to the other drugs.
- On average, *CYP2D6**10 was the least active, followed by *CYP2D6**17 and *CYP2D6**2. For *CYP2D6**10 large differences in activity were observed with an activity of <0.1 for nine substrates and a group of six substrates with activities higher than 0.25. *CYP2D6**17 was more active in regards to debrisoquine metabolism compared to the other substrates. Finally, *CYP2D6**2 displayed the largest variation in activity ranging from loss of function at 0.17 to gain of function at 1.18.
- We observed high, unexplained, variability between studies resulting in an inability to unambiguously determine the impact of variants on absolute CYP2D6 activity and to quantify the effect of substrate specificity.
- Changes in protein binding channels are potentially the cause of the substrate specific effects. These changes result in an altered affinity of the substrate for the binding site which can differ per substrate depending on substrate characteristics. This indicates that substrate specific effects originate at the variant level. However, current clinical guidelines only differentiate at the phenotype level.
- Our study confirms the existence of clear substrate specific effects of the *CYP2D6**17 allele, a subtle effect of the *CYP2D6**10 allele and no substrate specificity of the *CYP2D6**2 allele, in the metabolism of dextromethorphan, bufuralol, debrisoquine and venlafaxine.

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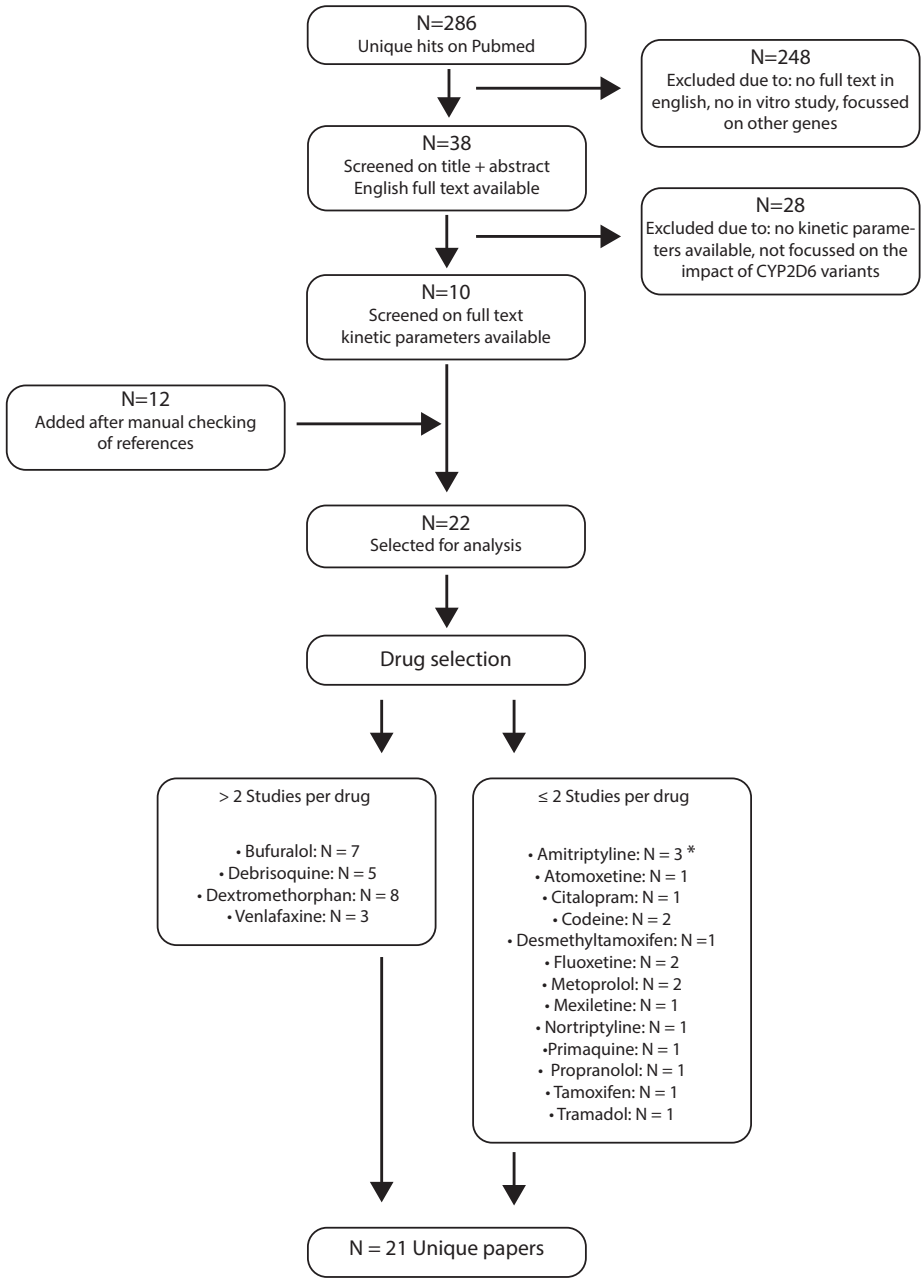
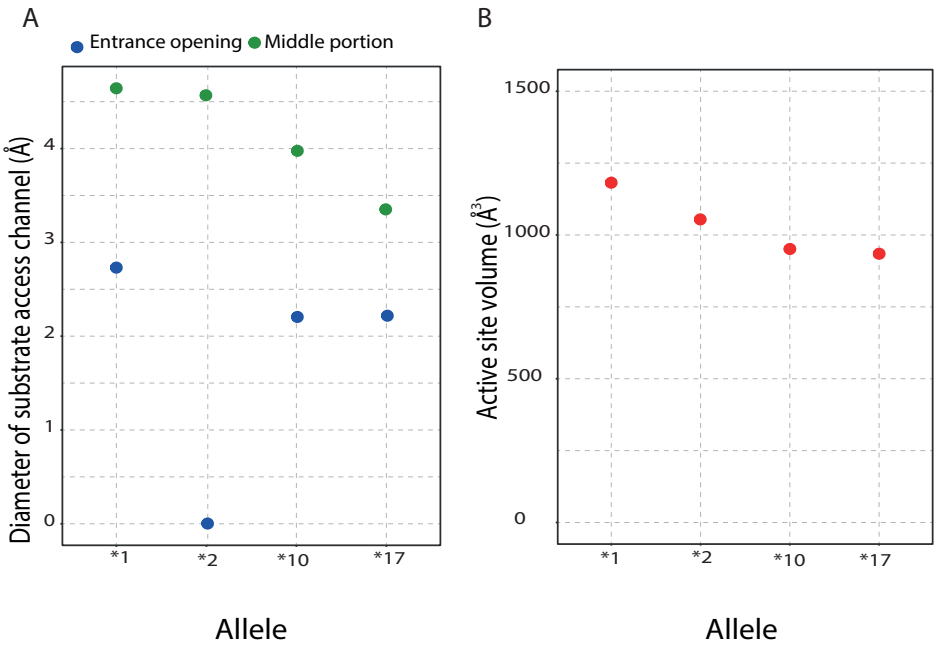
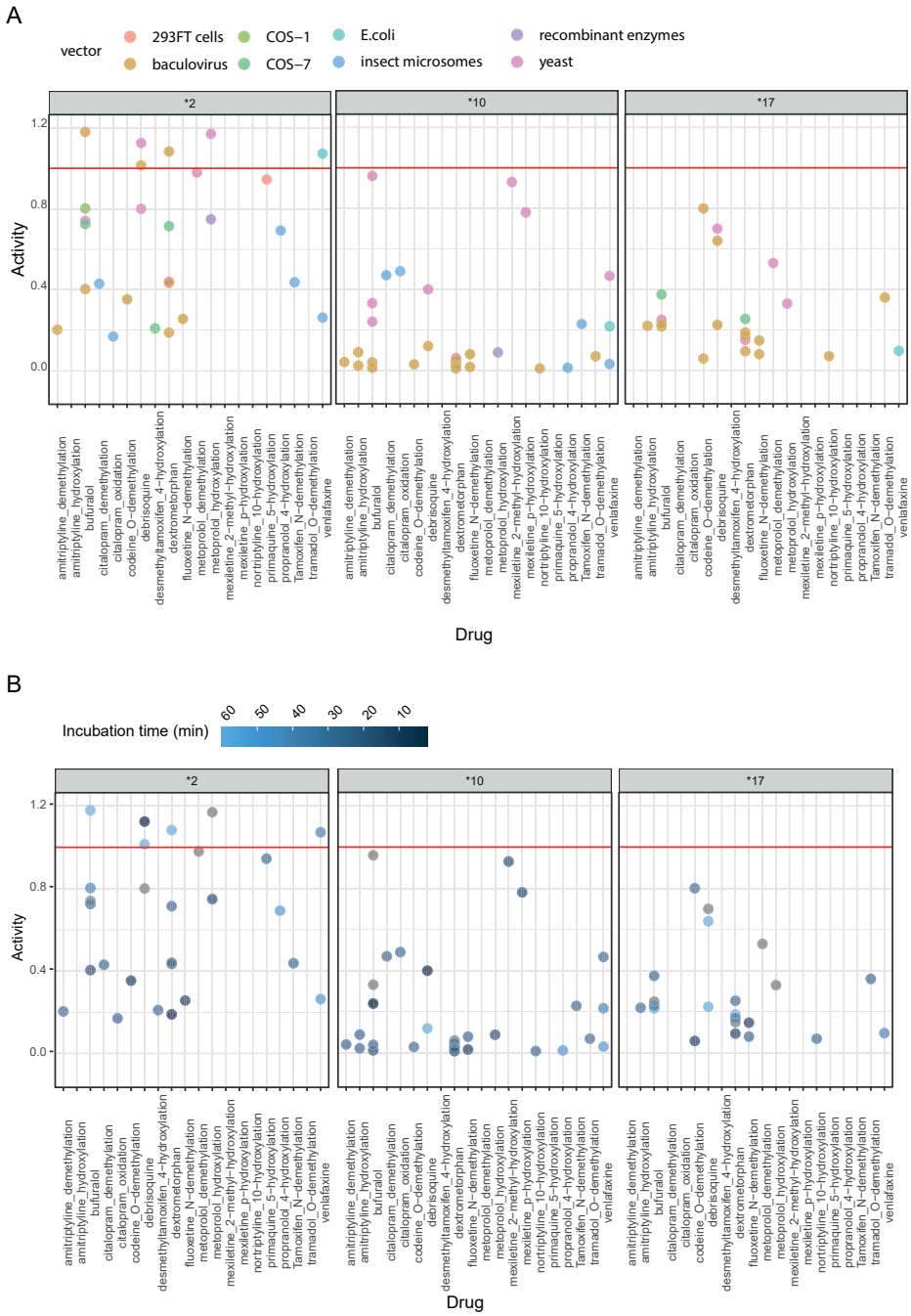
Supplementary material



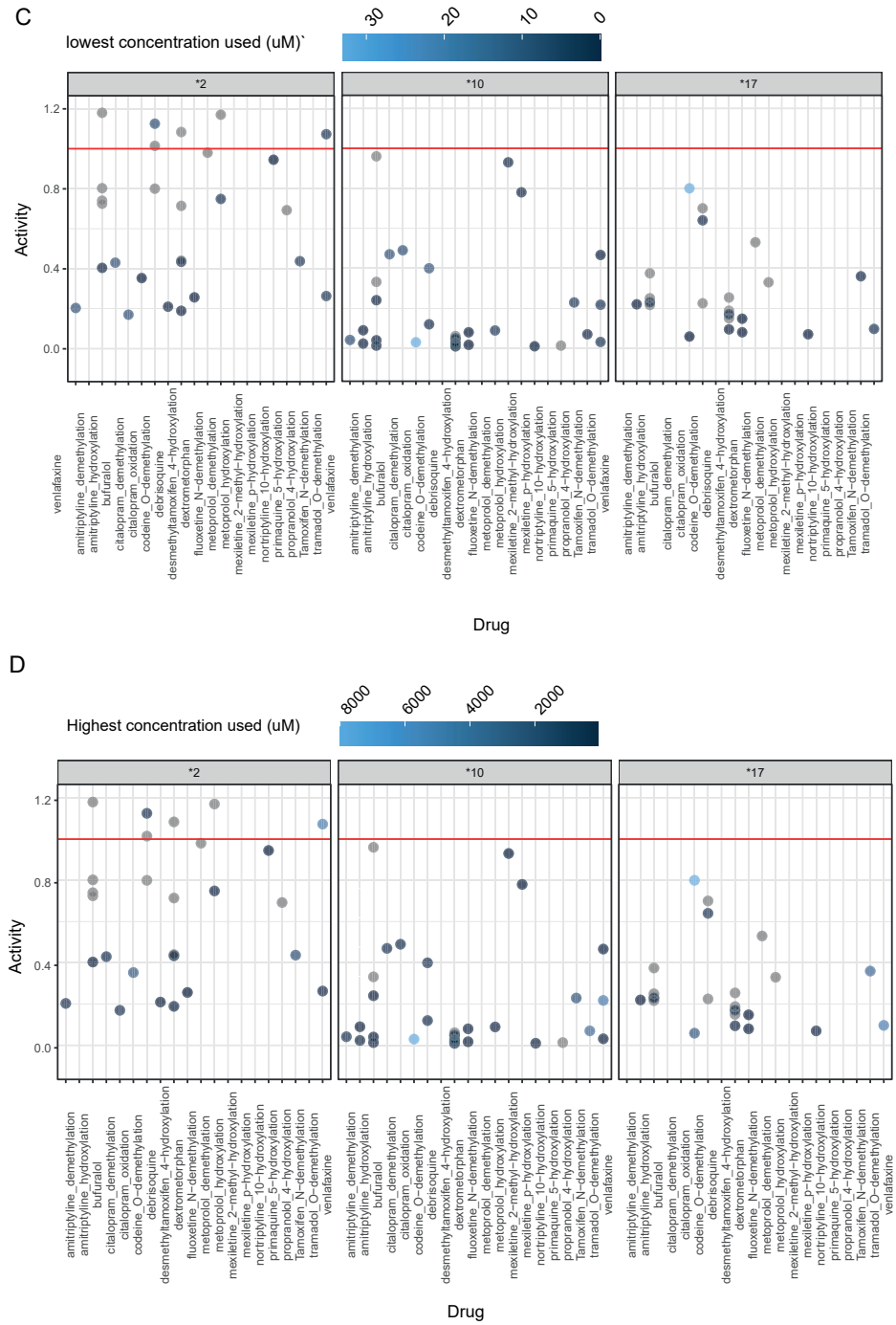
Supplementary Figure S6.1: Flowchart of paper selection
* While 3 studies investigating amitriptyline were retrieved, the studies focused on different metabolic conversion steps making them not comparable in regards to measured kinetic parameters.



Supplementary Figure S6.2: Effect of the *CYP2D62, *10 and *17 alleles on the substrate access channel (A) and the Active binding site (B)**
Data is obtained from Dong et al.



Supplementary Figure S6.3: Influence of vector (A), incubation time (B), lowest substrate concentration (C) and highest used substrate concentration (D) on in vitro enzyme activity of CYP2D6
If parameters were missing from the studies, the values are not represented in this figure.



Supplementary Figure S6.3: ***Continued***

Supplementary Table S6.1: Overview of the parameters extracted from the included studies

All parameters were normalized to reflect the same units.

drug	author	vector	allele	metabolic_step	km_mean (uM)	Vmax_mean (pmol/pmol/min)	Clin_mean (ul/min/pmol)	activity	incubation time	low_conc (uM)	high_conc (uM)
bufuralol	bapiro	yeast	*1	hydroxylation	9,65	8,640	0,890	1,000	30,0	10,0	1000,0
bufuralol	bapiro	yeast	*17	hydroxylation	21,00	4,740	0,220	0,250	30,0	10,0	1000,0
bufuralol	bapiro	yeast	*2	hydroxylation	13,00	8,480	0,660	0,740	30,0	10,0	1000,0
debrisoquine	bapiro	yeast	*1	4-hydroxylation	55,20	0,550	0,010	1,000	20,0	1,3	400,0
debrisoquine	bapiro	yeast	*17	4-hydroxylation	59,20	0,400	0,007	0,700	30,0	1,3	400,0
debrisoquine	bapiro	yeast	*2	4-hydroxylation	112,00	0,790	0,008	0,800	30,0	0,6	96,0
dextrometorphan	bapiro	yeast	*1	O-demethylation	1,30	2,210	1,770	1,000	30,0	0,6	96,0
dextrometorphan	bapiro	yeast	*17	O-demethylation	7,16	1,680	0,270	0,150	30,0	0,6	96,0
dextrometorphan	bapiro	yeast	*2	O-demethylation	2,42	1,720	0,770	0,440			
metoprolol_demethylation	bapiro	yeast	*1	O-demethylation	51,00	4,810	0,095	1,000			
metoprolol_demethylation	bapiro	yeast	*17	O-demethylation	75,00	3,730	0,050	0,530			
metoprolol_demethylation	bapiro	yeast	*2	O-demethylation	57,00	5,250	0,093	0,980	20,0	1,0	200,0
metoprolol_hydroxylation	bapiro	yeast	*1	alpha-hydroxylation	41,00	1,470	0,036	1,000	20,0	1,0	200,0
metoprolol_hydroxylation	bapiro	yeast	*17	alpha-hydroxylation	76,00	0,900	0,012	0,330	20,0	1,0	200,0
metoprolol_hydroxylation	bapiro	yeast	*2	alpha-hydroxylation	47,00	1,980	0,042	1,170	2,0	1,0	100,0
bufuralol	Cai	baculovirus	*1	hydroxylation	6,80	4,720	0,694	1,000	2,0	1,0	100,0
bufuralol	Cai	baculovirus	*10	hydroxylation	21,49	0,200	0,009	0,013			
bufuralol	Cai	baculovirus	*2	hydroxylation	10,44	2,920	0,281	0,404			
dextrometorphan	Cai	baculovirus	*1	O-demethylation	4,49	5,460	1,220	1,000	60,0		
dextrometorphan	Cai	baculovirus	*10	O-demethylation	3,94	0,220	0,060	0,049	60,0		
dextrometorphan	Cai	baculovirus	*2	O-demethylation	5,27	2,810	0,530	0,434	60,0		
venlafaxine	dong	E.coli	*1	O-demethylation	85,81	1,060	0,012	1,000	30,0		
venlafaxine	dong	E.coli	*10	O-demethylation	273,90	0,740	0,003	0,218	30,0		
venlafaxine	dong	E.coli	*17	O-demethylation	377,80	0,460	0,001	0,097	30,0		
venlafaxine	dong	E.coli	*2	O-demethylation	63,25	0,840	0,013	1,073			
bufuralol	fukuda	yeast	*1	hydroxylation	1,70	13,400	7,900	1,000			
bufuralol	fukuda	yeast	*10	hydroxylation	8,50	16,500	1,941	0,241	30,0	0,6	80,0
venlafaxine	fukuda	yeast	*1	O-demethylation	9,00	1,350	0,015	1,000	30,0	0,6	80,0
venlafaxine	fukuda	yeast	*10	O-demethylation	51,90	3,690	0,007	0,467	30,0	0,6	80,0
bufuralol	Hanioka	yeast	*10	hydroxylation	6,54	147,000	22,600	0,960	40,0		
bufuralol	Hanioka	yeast	*1	hydroxylation	2,34	57,000	23,600	1,000	40,0		
mexiletine_ 2-methyl-hydroxylation	Hanioka	yeast	*1	2-methyl-hydroxylation	13,00	7,980	0,600	1,000	30,0	10,0	1000,0
mexiletine_ 2-methyl-hydroxylation	Hanioka	yeast	*10	2-methyl-hydroxylation	18,40	9,620	0,560	0,930	30,0	10,0	1000,0
mexiletine_p-hydroxylation	Hanioka	yeast	*1	p-hydroxylation	13,50	4,360	0,320	1,000	30,0	10,0	1000,0
mexiletine_p-hydroxylation	Hanioka	yeast	*10	p-hydroxylation	23,10	5,720	0,250	0,780	30,0	10,0	1000,0
tamoxifen	Hu_2016_1	insect microsomes	*1	N-demethylation	77,07	39,960	0,480	1,000	30,0	10,0	1000,0
tamoxifen	Hu_2016_1	insect microsomes	*10	N-demethylation	132,97	14,430	0,110	0,229	30,0	10,0	1000,0
tamoxifen	Hu_2016_1	insect microsomes	*2	N-demethylation	122,10	25,030	0,210	0,438	30,0	33,0	8000,0
citalopram_demethylation	Hu_2016_2	insect microsomes	*1	demethylation	69,99	10,920	0,160	1,000	30,0	33,0	8000,0
citalopram_demethylation	Hu_2016_2	insect microsomes	*10	demethylation	106,74	7,670	0,080	0,470	30,0	33,0	8000,0
citalopram_demethylation	Hu_2016_2	insect microsomes	*2	demethylation	86,98	5,950	0,070	0,430	15,0	0,0	3000,0
citalopram_oxidation	Hu_2016_2	insect microsomes	*1	oxidation	68,95	13,210	0,190	1,000	15,0	0,0	3000,0

citalopram_oxidation	Hu_2016_2	insect microsomes	*10	oxidation	88,54	7,630	0,100	0,490	15,0	0,0	3000,0
citalopram_oxidation	Hu_2016_2	insect microsomes	*2	oxidation	196,63	6,630	0,030	0,170	15,0	0,0	3000,0
propranolol	Liang	insect microsomes	*1	4-hydroxylation	3,08	1,610	0,523	1,000			
propranolol	Liang	insect microsomes	*10	4-hydroxylation	23,43	0,178	0,008	0,013			
propranolol	Liang	insect microsomes	*2	4-hydroxylation	22,73	0,798	0,036	0,693			
bufuralol	marcucci-b	baculovirus	*1	hydroxylation	8,10	10,200	1,259	1,000	60,0		
bufuralol	marcucci-b	baculovirus	*17	hydroxylation	16,50	4,500	0,270	0,217	60,0		
bufuralol	marcucci-b	baculovirus	*2	hydroxylation	13,00	19,300	1,480	1,180	60,0		
debrisoquine	marcucci-b	baculovirus	*1	4-hydroxylation	13,40	2,800	0,209	1,000	60,0	2,7	1000,0
debrisoquine	marcucci-b	baculovirus	*17	4-hydroxylation	46,30	2,200	0,470	0,225	60,0	2,7	1000,0
debrisoquine	marcucci-b	baculovirus	*2	4-hydroxylation	21,20	4,400	0,210	1,014	60,0	2,7	1000,0
dextrometorphan	marcucci-b	baculovirus	*1	O-demethylation	1,00	2,400	2,400	1,000	5,0	10,0	1000,0
dextrometorphan	marcucci-b	baculovirus	*17	O-demethylation	6,10	2,700	0,450	0,188	5,0	10,0	1000,0
dextrometorphan	marcucci-b	baculovirus	*2	O-demethylation	1,40	3,700	2,600	1,083	5,0	10,0	1000,0
bufuralol	marcucci-c	COS-7	*1	hydroxylation	25,00	1,000	0,040	1,000	30,0	1,0	240,0
bufuralol	marcucci-c	COS-7	*17	hydroxylation	33,00	0,510	0,015	0,375	30,0	1,0	240,0
bufuralol	marcucci-c	COS-7	*2	hydroxylation	32,00	0,940	0,029	0,725	30,0	1,0	240,0
dextrometorphan	marcucci-c	COS-7	*1	O-demethylation	5,40	0,680	0,126	1,000			
dextrometorphan	marcucci-c	COS-7	*17	O-demethylation	9,90	0,320	0,032	0,254			
dextrometorphan	marcucci-c	COS-7	*2	O-demethylation	5,40	0,490	0,090	0,714			
dextrometorphan	matsunaga	E.coli	*1	O-demethylation	10,00	6,300	0,360	1,000	25,0	1,3	100,0
dextrometorphan	matsunaga	E.coli	*10	O-demethylation	63,00	1,200	0,019	0,030	25,0	1,3	100,0
desmethyltamoxifen	Muroi	COS-7	*1	4-hydroxylation	56,47	0,450	0,008	1,000	25,0	1,3	100,0
desmethyltamoxifen	Muroi	COS-7	*2	4-hydroxylation	97,17	0,160	0,002	0,210	60,0		
desmethyltamoxifen	Muroi	COS-7	*9	4-hydroxylation	42,16	0,130	0,003	0,370	60,0		
bufuralol	Nakamura	yeast	*1	hydroxylation	7,10	1,000	0,146	1,000	60,0		
bufuralol	Nakamura	yeast	*10	hydroxylation	12,40	0,600	0,049	0,332	30,0		
dextrometorphan	Nakamura	yeast	*1	O-demethylation	1,20	0,107	0,090	1,000	30,0		
dextrometorphan	Nakamura	yeast	*10	O-demethylation	3,60	0,020	0,006	0,061	30,0		
amitriptyline	ramamoorthy	baculovirus	*1	hydroxylation	1,80	1,600	0,890	1,000	15,0	3,5	2000,0
amitriptyline	ramamoorthy	baculovirus	*10	hydroxylation	5,80	0,120	0,021	0,024	15,0	3,5	2000,0
dextrometorphan	ramamoorthy	baculovirus	*1	NA	0,80	2,100	2,600	1,000			
dextrometorphan	ramamoorthy	baculovirus	*10	NA	5,00	0,260	0,052	0,020			
primaquine	Saito	293FT cells	*1	5-hydroxylation	15,70	1,390	0,076	1,000	15,0	0,5	312,5
primaquine	Saito	293FT cells	*2	5-hydroxylation	32,40	2,320	0,072	0,945	30,0	0,5	312,5
primaquine	Saito	293FT cells	*9	5-hydroxylation	20,20	1,060	0,052	0,687	30,0	0,6	320,0
amitriptyline	shen	baculovirus	*1	4-hydroxylation	2,73	19,100	6,990	1,000	30,0	0,6	320,0
amitriptyline	shen	baculovirus	*10	4-hydroxylation	3,45	2,070	0,600	0,090	30,0	0,6	320,0
amitriptyline	shen	baculovirus	*17	4-hydroxylation	8,33	12,800	1,530	0,220	5,0	0,0	500,0
bufuralol	shen	baculovirus	*1	hydroxylation	10,20	6,290	0,617	1,000	5,0	0,0	500,0
bufuralol	shen	baculovirus	*10	hydroxylation	12,96	0,290	0,023	0,040	15,0	0,0	500,0
bufuralol	shen	baculovirus	*17	hydroxylation	9,47	1,330	0,140	0,230	15,0	0,0	500,0
codeine	shen	baculovirus	*1	O-demethylation	1079,00	3,680	0,003	1,000	30,0	0,1	200,0
codeine	shen	baculovirus	*10	O-demethylation	1206,00	1,140	0,001	0,030	30,0	0,1	200,0
codeine	shen	baculovirus	*17	O-demethylation	1470,00	4,030	0,003	0,800	30,0	0,1	200,0
debrisoquine	shen	baculovirus	*1	4-hydroxylation	73,70	1,320	0,018	1,000	15,0	0,0	100,0
debrisoquine	shen	baculovirus	*10	4-hydroxylation	94,50	0,200	0,002	0,120	15,0	0,0	100,0

Supplementary Table S6.1 continues on next page.

Supplementary Table S6.1: ***Continued***

debrisoquine	shen	baculovirus	*17	4-hydroxylation		108,00	1,240	0,016	0,640	15,0	0,0	100,0
dextrometorphan	shen	baculovirus	*1	O-demethylation		2,97	3,490	1,180	1,000	15,0	0,0	100,0
dextrometorphan	shen	baculovirus	*10	O-demethylation		9,14	0,570	0,062	0,050			
dextrometorphan	shen	baculovirus	*17	O-demethylation		17,30	3,410	0,197	0,170			
fluoxetine	shen	baculovirus	*1	N-demethylation		0,83	0,414	0,496	1,000			
fluoxetine	shen	baculovirus	*10	N-demethylation		0,46	0,017	0,037	0,080			
fluoxetine	shen	baculovirus	*17	N-demethylation		2,57	0,104	0,041	0,080			
nortriptyline	shen	baculovirus	*1	10-hydroxylation		4,07	0,960	0,236	1,000			
nortriptyline	shen	baculovirus	*10	10-hydroxylation		48,10	0,150	0,003	0,010	200,0	5,0	500,0
nortriptyline	shen	baculovirus	*17	10-hydroxylation		18,50	0,320	0,017	0,070	20,0	5,0	500,0
tramadol	shen	baculovirus	*1	O-demethylation		286,00	12,900	0,045	1,000	20,0	5,0	500,0
tramadol	shen	baculovirus	*10	O-demethylation		331,00	1,030	0,003	0,070	20,0	2,0	200,0
tramadol	shen	baculovirus	*17	O-demethylation		209,00	3,360	0,016	0,360	20,0	2,0	200,0
amitriptyline	Weng	baculovirus	*1	N-demethylation		20,37	2,540	0,125	1,000	20,0	2,0	200,0
amitriptyline	Weng	baculovirus	*10	N-demethylation		229,14	1,210	0,052	0,042	20,0	2,0	200,0
amitriptyline	Weng	baculovirus	*2	N-demethylation		128,23	3,250	0,025	0,203	30,0	0,6	320,0
bufuralol	wennerholm	COS-1	*2	hydroxylation		23,60	23,300	0,987	0,802	30,0	0,6	320,0
bufuralol	wennerholm	COS-1	*1	hydroxylation		21,00	25,900	1,230	1,000	30,0	0,6	320,0
debrisoquine	Xu	yeast	*10	4-hydroxylation		18,18	2,820	0,160	0,400	30,0	0,5	250,0
debrisoquine	Xu	yeast	*1	4-hydroxylation		10,04	4,030	0,400	1,000	30,0	0,5	250,0
debrisoquine	Xu	yeast	*2	4-hydroxylation		9,00	4,080	0,450	1,125	30,0	0,5	250,0
metoprolol_hydroxylation	Yang	recombinant enzymes	*1	alpha-hydroxylation		11,31	85,060	7,520	1,000	50,0		
metoprolol_hydroxylation	Yang	recombinant enzymes	*10	alpha-hydroxylation		31,46	21,080	0,670	0,089	50,0		
metoprolol_hydroxylation	Yang	recombinant enzymes	*2	alpha-hydroxylation		13,88	78,230	5,630	0,749	50,0		
codeine	yu	baculovirus	*1	O-demethylation		190,00	6,400	0,340	1,000	30,0	5,0	2500,0
codeine	yu	baculovirus	*10	O-demethylation	ND	ND	ND	ND		30,0	5,0	2500,0
codeine	yu	baculovirus	*17	O-demethylation		860,00	1,700	0,002	0,059	30,0	5,0	2500,0
codeine	yu	baculovirus	*2	O-demethylation		480,00	5,680	0,012	0,353	30,0	1,8	4000,0
dextrometorphan	yu	baculovirus	*1	O-demethylation		3,04	9,000	2,960	1,000	30,0	1,8	4000,0
dextrometorphan	yu	baculovirus	*2	O-demethylation		26,30	14,600	0,560	0,189	30,0	1,8	4000,0
dextrometorphan	yu	baculovirus	*17	O-demethylation		30,70	8,600	0,280	0,095	40,0	5,0	5000,0
dextrometorphan	yu	baculovirus	*10	O-demethylation		92,10	2,600	0,028	0,009	40,0	5,0	5000,0
fluoxetine	yu	baculovirus	*1	N-demethylation		2,84	0,653	0,230	1,000	40,0	5,0	5000,0
fluoxetine	yu	baculovirus	*10	N-demethylation		13,40	0,056	0,004	0,017	40,0	5,0	5000,0
fluoxetine	yu	baculovirus	*17	N-demethylation		8,31	0,281	0,003	0,148	30,0	1,0	500,0
fluoxetine	yu	baculovirus	*2	N-demethylation		4,33	0,257	0,059	0,257	30,0	1,0	500,0
venlafaxine	Zhan	insect microsomes	*1	O-demethylation		11,48	2,180	0,190	1,000	50,0	5,0	500,0
venlafaxine	Zhan	insect microsomes	*10	O-demethylation		29,83	0,170	0,006	0,032	50,0	5,0	500,0
venlafaxine	Zhan	insect microsomes	*2	O-demethylation		17,17	0,920	0,050	0,263	50,0	5,0	500,0

Supplementary File S6.1: Literature search

In PubMed the following terms were used: CYP2D6 AND functional characterization AND *DRUG*

With for *drug* the following drugs:

- amitriptyline
- amphetamine
- atomoxetine
- bufuralol (in vitro substrate)
- carvedilol
- citalopram
- codeine
- debrisoquine
- dextromethorphan
- fluoxetine
- nortriptyline
- paroxetine
- propafenone
- propranolol
- risperidone
- metoprolol
- sparteine
- tamoxifen
- tramadol
- venlafaxine

Exclusion criteria were:

- reviews
- non-English
- no full text available
- in vivo studies
- absence of values of kinetic parameters