Drug Metabolism and Pharmacokinetics xxx (2016) 1–6

Contents lists available at ScienceDirect

Drug Metabolism and Pharmacokinetics

journal homepage: http://www.journals.elsevier.com/drug-metabolism-and-pharmacokinetics

Regular article

Effect of CYP2D6 genetic polymorphism on the metabolism of citalopram in vitro

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ARTICLE INFO

Article history:
Received 18 September 2015
Received in revised form
19 December 2015
Accepted 14 January 2016
Available online xxx

Keywords:
Cytochrome P450 2D6
Genetic polymorphism
Fluorescence
Citalopram
Enzymatic activity
Drug metabolism

ABSTRACT

Genetic polymorphisms of CYP2D6 significantly influence the efficacy and safety of some drugs, which might cause adverse effects and therapeutic failure. We aimed at investigating the role of CYP2D6 in the metabolism of citalopram and identifying the effect of 24 CYP2D6 allelic variants we found in Chinese Han population on the metabolism of citalopram in vitro. These CYP2D6 variants expressed by insect cells system were incubated with 10–1000 μM citalopram for 30 min at 37 °C and the reaction was terminated by cooling to −80 °C immediately. Citalopram and its metabolites were analyzed by high-performance liquid chromatography (HPLC). The intrinsic clearance (V_{max}/K_m) values of the variants toward citalopram metabolites were significantly altered, 38–129% for demethylcitalopram and 13–138% for citalopram N-oxide when compared with CYP2D6*1. Most of the tested rare alleles exhibited significantly decreased values due to increased K_m and/or decreased V_{max} values. We conclude that recombinant system could be used to investigate the enzymes involved in drug metabolism and these findings suggest that more attention should be paid to subjects carrying these CYP2D6 alleles when administering citalopram in the clinic.

1. Introduction

Cytochrome P450 (CYP) enzymes are products of a supergene family, playing a vital role in the metabolism of both exogenous and endogenous compounds [1]. More than 90% of current therapeutic drugs are metabolized by the CYP450 enzyme [2,3]. CYP2D6, a member of the Cytochrome P450 mixed-function oxidase system, is one of the most important enzymes involved in the metabolism of xenobiotics in the body. It is responsible for the metabolism and elimination of approximately 25% of clinically used drugs [4,5]. There is a considerable variation in the efficiency and amount of CYP2D6 enzyme produced between individuals. More than 50 kinds of drugs, including antiarrhythmics, antihistamine, antidepressants, β-adrenoceptor antagonists, neuroleptics and opioids have been identified as substrates of CYP2D6 [5,6]. Individuals eliminate these drugs in different speed, and were classified into 4 types: poor metabolizers (PMs), intermediate metabolizers (IMs), extensive metabolizers (EMs) and ultrarapid metabolizers (UMs). Hence the dose of some drugs metabolized by CYP2D6, especially with narrow therapeutic indexes, may be paid more attention and have to be adjusted to take into account of the speed [7].

Citalopram is a selective serotonin reuptake inhibitors (SSRIs) that widely used to treat depressive disorder [8,9]. Previous study showed that about 30% of patients achieved remission after taking citalopram [10,11]. It is metabolized mainly in the liver via *N*-demethylation to its principle metabolite demethylcitalopram. Besides it also can metabolize into N-oxide metabolite by CYP2D6. Studies with human liver microsomes (HLM) and expressed enzymes have indicated that the N-oxide metabolite is formed predominantly by CYP2D6, while it was known that demethylcitalopram metabolized from citalopram is involved in CYP2D6 and CYP2C19, CYP3A4 in human [9,12,13]. CYP2D6 with highly polymorphism has been found to have 128 different alleles [14] (http://www.cypalleles.ki.se/cyp2d6.htm). To date, almost 80 variant alleles for CYP2D6 have been identified [15]. Of them, the effects of CYP2D6*3, CYP2D6*4 and CYP2D6*10 have been well studied for their reduced metabolic activities toward substrates such as bufuralol, debrisoquine and dextromethorphan, both in vitro and in vivo [15,16]. Dai et al. have analyzed the CYP2D6 polymorphisms in 2129 unrelated Chinese individuals [14]. Then 22 new nonsynonymous mutation sites have

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http://dx.doi.org/10.1016/j.dmpk.2016.01.001
1347-4367/

been discovered, and 12 of them were named as *87–*93, *94A, *94B and *95–*98.

In the present study, we assessed the catalytic activities of 24 CYP2D6 alleles (*2, *10, *87–*98, R25Q, F164L, E215K, F219S, V327M, D336N, V342M, R344Q, R440C, R497C) expressed in the insect cell toward citalopram, thus proving valuable information for further studies about CYP2D6 alleles for citalopram metabolism.

2. Materials and methods

2.1. Chemicals and materials

Citalopram and venlafaxine were obtained from Tokyo Chemical Industry Co., Ltd (Tokyo, Japan). Demethylcitalopram and citalopram *N*-oxide were purchased from Toronto Research Chemicals Inc. (TRC, Canada). The reduced nicotinamide adenine dinucleotide phosphate (NADPH) were obtained from Sigma (St. Louis, MO), P450 Cytochrome b5 microsomes and recombinant human P450s CYP2D6 expressed in the microsomes from *Spodoptera frugiperda* (*Sf*) 21 insect cells were kind gifts from Beijing Hospital. A RRHD Eclipse Plus C18 column used for high-performance liquid chromatography (HPLC) was obtained from the Agilent Technologies. Other reagents and organic solvents were obtained from Chemical Industries (Beijing, China).

2.2. Conditions for enzymatic activity analysis

The incubation mixture consisted of recombinant microsomes containing 5 pmol CYP2D6*1 or 5 pmol other CYP2D6 mutants, 5 pmol purified cytochrome b5 and 4.05 μL citalopram in 100 mmol/LPBS buffer (pH 7.4). Citalopram was initially prepared in methanol solution, and the total concentration in the incubation mixture was adjusted from 10 to 1000 μM. The total concentration of methanol was less than 0.5%. The reaction was allowed to pre-incubate for 5 min in a Fisher shaking water bath. Then an NADPH regenerating system was added to start the reaction at 37 °C in a final volume of 200 μL, and the mixture was incubated at the same temperature for 30 min. Incubations were terminated by cooled to −80 °C immediately, and then add 50 μL 0.1 M HCl to the tubes when taking out of −80 °C after 15 min approximately. Venlafaxine (20 μL of 10 μg/mL in methanol solution) as an internal standard was added to the mixture followed by the addition of 0.8 mL acetic ether. After vortexing for 2 min, the incubation mixture was centrifuged at 12 000 rpm at 4 °C for 10 min, the organic phase was transferred into a clean tube, and it was dried under a nitrogen stream. The resulting residue was dissolved in 100 μL mobile phase and used for the following measurement of citalopram and its metabolites. Incubations were performed in individual tubes for each time and triplicate, and the data are presented by the mean standard deviation (SD).

High-performance liquid chromatography (HPLC) was carried out on an Agilent RRHD Eclipse Plus C18 column (3.0*100 mm, 1.8 μm, Agilent Technologies) at 30 °C. The mobile phase consisted of acetonitrile (solvent B) and 0.05% trifluoroacetic acid in water (solvent A) at an isocratic flow rate of 0.3 mL/min with 72% solvent A + 28% solvent B for 14 min. The column eluent was monitored with a Fluorescence detector at excitation and emission wavelengths of 245 and 306 nm, respectively. Under these conditions, the retention times of venlafaxine, demethylcitalopram, citalopram and citalopram *N*-oxide were 4.510, 9.169,10.113 and 13.601 min, respectively. The standard curves for citalopram and demethylcitalopram or citalopram *N*-oxide were prepared using spiked incubation samples with seven points.

2.3. Statistical analysis

Michaelis–Menten analysis was performed by non-linear regression curve fitting using the computer program Prism v 5.0 (GraphPad Software Inc., San Diego, CA). Kinetic data for each variant are presented as the mean ± S.D. of three microsomal preparations. The one-way ANOVA was used for inter group comparison. Dunnett's test was used to analyze differences in catalytic activity between CYP2D6*1 and other mutants. Statistical analyses were performed with the SPSS package (version 19.0; SPSS Inc., Chicago, IL), with $p < 0.05$ considered to be statistically significant.

3. Results

In our study, the catalytic activities of the wild-type CYP2D6*1 and 24 allelic variants were assessed using citalopram as substrate. Michaelis–Menten plots for each of the CYP2D6 variants are shown in Figs. 1 and 2, and the corresponding kinetic parameters are summarized in Tables 1 and 2. As shown in the figures and tables, almost all of the variants exhibited changed K_m or V_{max} values as compared to that of the wild-type protein. Therefore, the intrinsic clearance (V_{max}/K_m) values for citalopram demethylation and oxynitride were altered in all of the tested allelic variants, while the clearance value of identical allelic variant for the two metabolites is mainly consistent.

3.1. Demethylcitalopram

As shown in Fig. 1 and Table 1, two variants (CYP2D6*92 and CYP2D6*96) were too week to produce demethylcitalopram, resulting no detectable enzymatic activity. Except for CYP2D6*92 and CYP2D6*96, the remaining 22 defective alleles displayed much difference in K_m, V_{max} or intrinsic clearance values. According to the intrinsic clearance value compared with wild type, we classified the remaining 22 defective alleles into three categories in regard to demethylcitalopram. One variant (V342M) exhibited a decreased V_{max}value and a much lower K_m value with wild-type, resulting in higher intrinsic clearance ($p < 0.01$); four variants (CYP2D6*94, E215K, F219S and V327M) showed no significant difference (1-fold) in enzyme activity; the remaining 17 variants displayed significantly reduced intrinsic clearance values (38–82% relative clearance). As a result, eighteen of the 22 variants (CYP2D6*2, *10, *87, *88, *89, *90, *91, *93, *95, *97, *98, R25Q, F164L, D336N, V342M, R344Q, R497C and R440C) had significant difference in intrinsic clearance (V_{max}/K_m) values of citalopram demethylation compared with the wild type ($^*p < 0.05$, $^{**}p < 0.01$).

3.2. Citalopram N-oxide

The Michaelis–Menten curves and the corresponding kinetic parameter of CYP2D6 for citalopram *N*-oxide are shown in Fig. 2 and Table 2, respectively. Similar to the result for demethylcitalopram, the kinetic parameter of citalopram *N*-oxide by the variants CYP2D6*92 and CYP2D6*96 could not be determined, because of their inactivity at all substrate concentration. Regarding to the oxynitride of citalopram (citalopram *N*-oxide), the intrinsic clearance of CYP2D6*R440 was increased to 138% compared with wild type; two variants (CYP2D6*94, E215K) showed no significant difference (1-fold) in enzyme activity compared with the wild type; The remaining 19 variants displayed significantly reduced intrinsic clearance values compared with the wild type (13–66% relative clearance). As a result, twenty of the 22 variants (CYP2D6*2, *10, *87, *88, *89, *90, *91, *93, *95, *97, *98, *R25Q, F164L, F219S, V327M, D336N, V342M, R344Q, R497C and R440C) have significant

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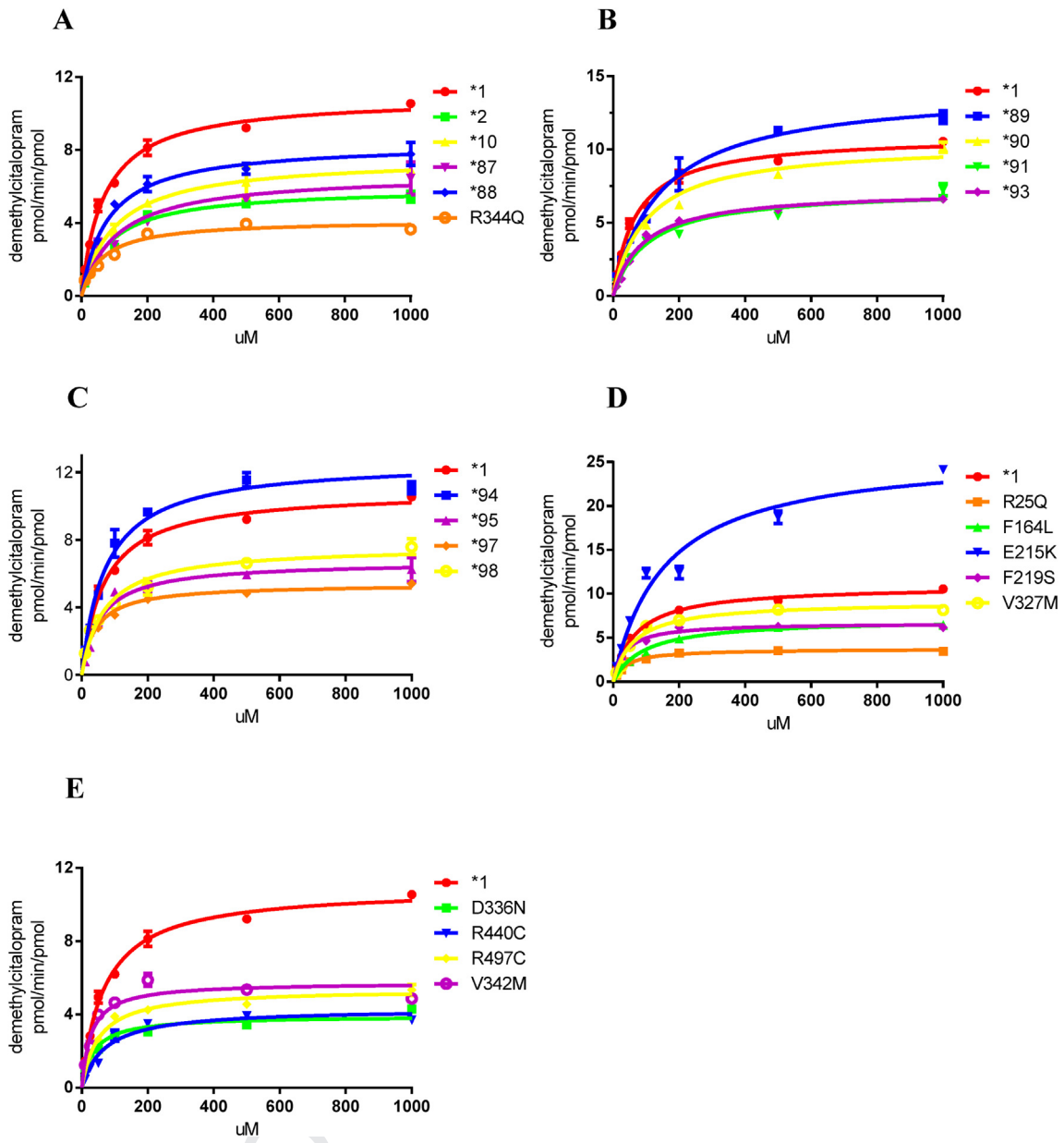

Fig. 1. Michaelis–Menten curves of the enzymatic activity of the wild-type and 24 variants toward citalopram demethylation (each point represents the mean ± S.D. of three parallel experiments). The allelic variants with designated allele names have been properly arranged into 5 groups.

difference in value of intrinsic clearance of citalopram (**$p < 0.01$) compared with the wild type.

4. Discussion

The cytochrome P450 2D6 enzyme (CYP2D6) metabolizes about 25% of prescribed drugs, and genetic polymorphisms in CYP2D6 can greatly affect its activity and lead to differences among individuals in drug efficacy and adverse drug reactions [17]. Previous studies showed that individuals lacking CYP2D6 activity have lower clearance resulting in higher steady-state plasma concentrations of substrates and lower steady-state plasma concentrations of metabolites comparing with that in normal [18,19]. Our study aims to functionally analyze the 24 variants with respect to citalopram using recombinant insect microsomes. In our study, some of these variants showed decreased enzyme activity as previous studies reported, but some caused increased or no change in enzyme activity, which is not in accordance with the results of previous research. It indicates that CYP2D6 polymorphisms have a great impact on the metabolism of citalopram, and it is necessary for this study.

Using insect cell expression system, Cai et al. (2015) found that the typical defective allele, CYP2D6*2 and CYP2D6*10, exhibited lower intrinsic clearance value for bufuralol, about 40.41% and 1.34% of wild type, respectively [20]. The study of N-desmethyltamoxifen 4-hydroxylation by Yuka Muroi et al. also reported that CYP2D6*2 exhibited a decrease of V_{max}, resulting a decreased relative clearance [21]. In our study, CYP2D6*2 caused a decrement in the V_{max} (54.49% and 50.15%)and an obvious increase in K_m values (178.76% and 185.18%) for catalysis of the demethylation and oxynitride of citalopram, which caused a decrease in the clearance rate.

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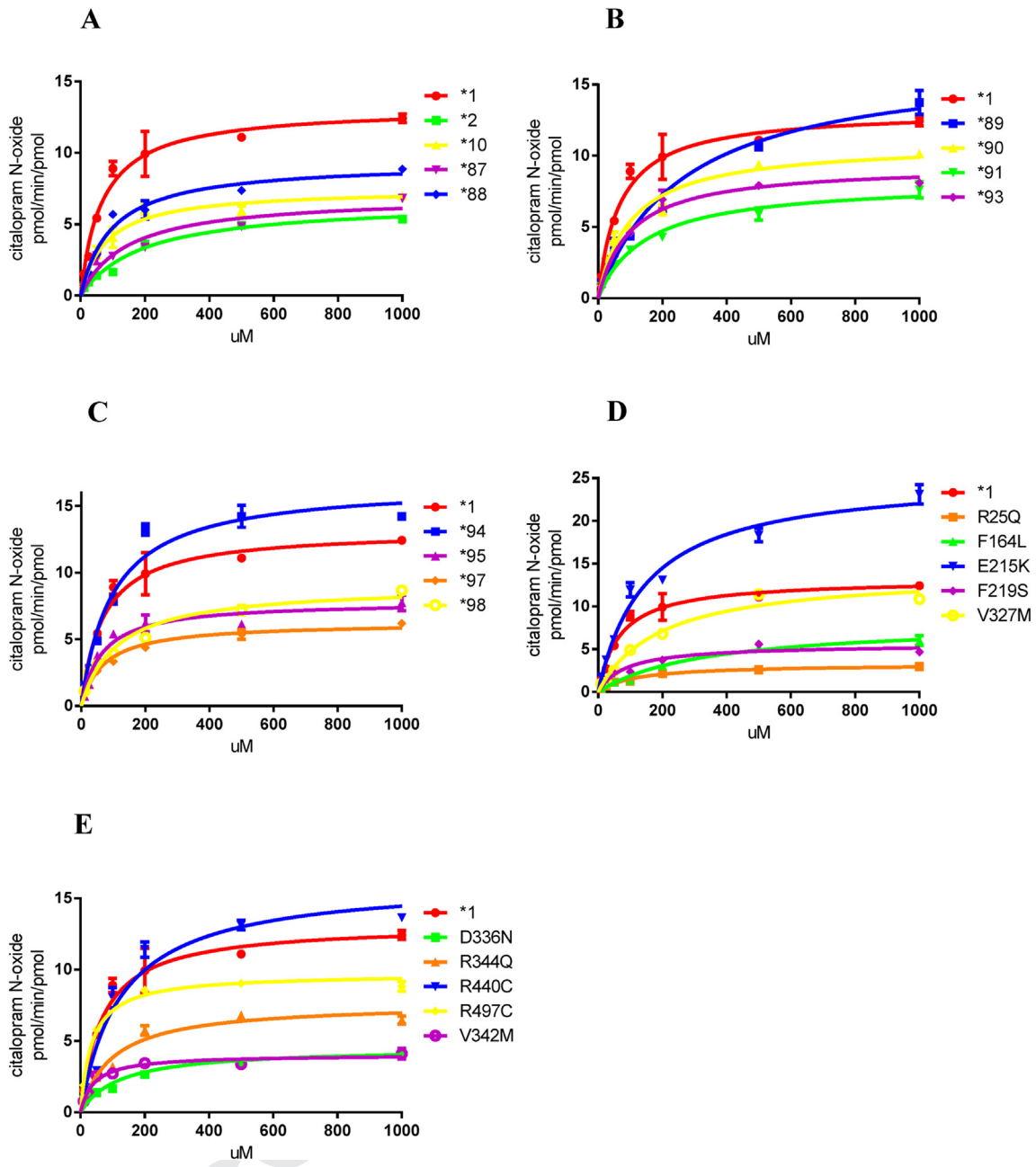

Fig. 2. Michaelis–Menten curves of the enzymatic activity of the wild-type and 24 variants toward citalopram N-oxidation (each point represents the mean ± S.D. of three parallel experiments). The allelic variants with designated allele names have been properly arranged into 5 groups.

The most widely studied allele, CYP2D6*10, is present approximately 50% in some Asian countries [22], while 42.86% in the Chinese Han population [14]. This variant contains the substitution of S486T and P34S, leading to a very unstable enzyme with reduced substrate affinity [23]. As reported by Shen et al., CYP2D6*10 yielded 1.32–27.9% of the efficiency of CYP2D6*1 toward nortriptyline (1.32%), bufuralol (3.65%), dextromethorphan (5.31%), tramadol (6.90%), (*S*)-fluoxetine (7.54%), atomoxetine (8.58%), debrisoquine (11.8%), and codeine (27.9%) in vitro [24]. While in our study of citalopram, with a decreased V_{max} and an increased K_m, the relative clearance of CYP2D6*10 is separately 49% and 47% of the two metabolites. CYP2D6*10, as a typical defective enzyme, could greatly impact on patients' plasma concentrations of drugs and should be paid more attention in clinical [19,25,26].

When we assessed the effects of these CYP2D6 variant alleles on demethylcitalopram, we found that 14 variants (CYP2D6*88–*90, *94, *95, *97, *98, R25Q, E215K, F219S, V327M, D336N, V342M and R497C) showed a higher relative clearance compared with CYP2D6*10, suggesting higher activity than it (Table 1). Most of these results accord with previous studies, but some caused change in enzyme activity. Variant E215K contained an amino acid switch from Glu to Lys at position 215. In previous study of venlafaxine and dextromethorphan, it exhibited significantly decreased clearance [20,27]. However, in our study, E215K displayed no difference in intrinsic clearance compared with CYP2D6*1, because both of its V_{max} and K_m values had a 2-fold increase approximately.

CYP2D6*89 contains one T to C substitution in site 1678 of the DNA sequence (425T > C in the cDNA) resulting in the

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Table 1
Kinetic parameters from demethylation by wild-type and 24 mutant CYP2D6 alleles on citalopram.

Variants	V_{max} (pmol/min/pmol P450)	K_m (μM)	CLint(V_{max}/K_m) (μL/min/nmol P450)	Relative clearance (% of wild type)
CYP2D6*1	10.92 ± 0.13	69.99 ± 11.67	0.16 ± 0.02	100.00
CYP2D6*2	5.95 ± 0.31**	86.98 ± 4.29	0.07 ± 0.00**	43.22
CYP2D6*10	7.67 ± 1.14**	106.74 ± 46.05	0.08 ± 0.03**	47.44
CYP2D6*87	6.78 ± 1.14**	110.73 ± 23.47	0.06 ± 0.01**	39.38
CYP2D6*88	8.45 ± 0.48**	83.50 ± 20.16	0.10 ± 0.02**	65.96
CYP2D6*89	14.30 ± 0.68**	150.43 ± 41.43**	0.10 ± 0.02**	62.99
CYP2D6*90	10.43 ± 0.61	100.99 ± 12.04	0.10 ± 0.01**	65.34
CYP2D6*91	7.29 ± 0.50**	105.90 ± 12.99	0.07 ± 0.00**	43.77
CYP2D6*92	N.D.	N.D.	N.D.	N.D.
CYP2D6*93	7.21 ± 0.22**	91.46 ± 5.75	0.08 ± 0.00**	49.93
CYP2D6*94	12.69 ± 0.30**	73.83 ± 9.87	0.17 ± 0.02	109.76
CYP2D6*95	6.77 ± 0.58**	58.78 ± 15.33	0.12 ± 0.02*	75.21
CYP2D6*96	N.D.	N.D.	N.D.	N.D.
CYP2D6*97	5.38 ± 0.09**	41.39 ± 2.10	0.13 ± 0.00*	82.21
CYP2D6*98	7.62 ± 0.75**	68.00 ± 14.06	0.11 ± 0.01**	70.82
R25Q	3.74 ± 0.22**	39.50 ± 6.77	0.10 ± 0.01**	60.63
F164L	7.13 ± 1.07**	94.37 ± 32.77	0.08 ± 0.02**	47.92
E215K	26.32 ± 0.54**	159.67 ± 30.35**	0.17 ± 0.03	106.45
F219S	6.69 ± 0.20**	35.18 ± 3.38	0.19 ± 0.02	121.05
V327M	9.10 ± 0.16**	63.08 ± 1.55	0.14 ± 0.00	91.13
D336N	3.91 ± 0.13**	36.07 ± 5.99	0.11 ± 0.01**	68.45
V342M	5.74 ± 0.27**	28.00 ± 2.40	0.21 ± 0.01**	129.33
R344Q	4.16 ± 0.09**	62.89 ± 3.90	0.07 ± 0.00**	41.88
R440C	4.33 ± 0.36**	71.53 ± 10.82	0.06 ± 0.01**	38.46
R497C	5.39 ± 0.23**	51.27 ± 3.63	0.11 ± 0.00**	66.69

*Significantly different from wild-type CYP2D6, *p < 0.05, **p < 0.01.

Table 2
Kinetic parameters from oxidational activities of wild-type and 24 mutant CYP2D6 alleles towards citalopram.

NO-variants	V_{max} (pmol/min/pmol P450)	K_m (μM)	CLint(V_{max}/K_m) (μL/min/nmol P450)	Relative clearance (% of wild type)
CYP2D6*1	13.217.85 ± 0.55	68.95 ± 8.09	0.19 ± 0.04**	100.00
CYP2D6*2	6.63 ± 0.26**	196.63 ± 22.97**	0.03 ± 0.00**	17.44
CYP2D6*10	7.63 ± 0.46**	88.54 ± 39.69	0.10 ± 0.04**	48.64
CYP2D6*87	7.01 ± 0.20**	146.13 ± 13.81	0.05 ± 0.00**	24.71
CYP2D6*88	9.37 ± 0.29**	97.53 ± 9.22	0.10 ± 0.01**	49.76
CYP2D6*89	17.22 ± 3.45**	279.00 ± 128.15**	0.07 ± 0.02**	32.88
CYP2D6*90	10.92 ± 0.40	103.73 ± 20.27	0.11 ± 0.02**	54.85
CYP2D6*91	8.22 ± 1.44**	147.43 ± 34.85	0.06 ± 0.00**	28.63
CYP2D6*92	N.D.	N.D.	N.D.	N.D.
CYP2D6*93	9.36 ± 0.24**	100.30 ± 5.60	0.09 ± 0.01**	48.22
CYP2D6*94	16.73 ± 0.42**	98.02 ± 6.57	0.17 ± 0.02	88.20
CYP2D6*95	7.85 ± 0.55**	64.69 ± 7.91	0.12 ± 0.01**	63.33
CYP2D6*96	N.D.	N.D.	N.D.	N.D.
CYP2D6*97	6.27 ± 0.18**	71.24 ± 10.10	0.09 ± 0.01**	45.77
CYP2D6*98	9.05 ± 0.27**	111.65 ± 10.57	0.08 ± 0.01**	41.81
R25Q	3.30 ± 0.34**	120.23 ± 36.71	0.03 ± 0.01**	14.70
F164L	8.33 ± 2.04**	344.87 ± 130.67**	0.02 ± 0.00**	12.61
E215K	25.35 ± 1.77**	150.20 ± 23.25	0.17 ± 0.01	87.29
F219S	5.56 ± 0.68**	85.07 ± 19.83	0.07 ± 0.01**	33.18
V327M	14.02 ± 0.97	193.93 ± 23.16*	0.07 ± 0.00**	37.28
D336N	4.54 ± 0.29**	123.80 ± 28.90	0.04 ± 0.01**	19.10
V342M	4.05 ± 0.16**	42.38 ± 7.42	0.10 ± 0.01**	39.40
R344Q	7.66 ± 0.46**	100.03 ± 6.59	0.08 ± 0.00**	66.24
R440C	16.31 ± 0.30**	127.93 ± 13.44	0.13 ± 0.02**	137.51
R497C	9.71 ± 0.13**	36.32 ± 1.97	0.27 ± 0.01**	50.06

*Significantly different from wild-type CYP2D6, *p < 0.05, **p < 0.01.

Leu142 → Ser change, and CYP2D6*93 contains a 745A > C nucleotide substitution that result in a Thr249 → Pro change in amino-acid. Using 293FT cell expression system, Dai et al. found that CYP2D6*89 and *93 exhibited >90% decreases in catalytic activity compared with CYP2D6*1 [28]. In the study of Cai et al., CYP2D6*93 also exhibited markedly reduced metabolic activity of bufuralol in vitro, while CYP2D6*89 caused moderately decrease of relative clearance [20]. In our study, CYP2D6*89, with little change in V_{max} and a significant increase in K_m values, also caused a moderate decrease in clearance, in accordance with the study of bufuralol performed by Cai et al. CYP2D6*93 produced lower intrinsic clearance values of citalopram than did wild-type CYP2D6*1 resulting from greater K_m and smaller V_{max}, and the intrinsic clearance values for CYP2D6*93 decreased about 50% compared with wild type.

CYP2D6*92 and CYP2D6*96 exhibit an undeterminable activity in citalopram metabolism, similar to previous reports about bufuralol and dextromethorphan. It may owe to that CYP2D6*92 has one nucleotide deletion at site 1995 and causes a 218 frameshift effect, leading to premature termination of protein synthesis;

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Similarly, CYP2D6*96 contains a single-nucleotide mutation C > T at position 3895 in the DNA and causes a stop codon (424STOP) [14].

In summary, this study provided another way to analyze drug metabolism in vitro and functionally assessed the demethylation and oxynitride activity of the wild-type and 24 CYP2D6 variants. To date, this is the first report of these 24 variants for citalopram metabolism. Although the allelic frequencies of these rare alleles in the Chinese population are less than 1%, functional study of these alleles is still necessary and valuable in clinical practice in view of Chinese large populations. Our result suggests that the 24 allelic variants significantly altered the intrinsic clearance value of citalopram in vitro. Furthermore, the data can complement the database of enzymatic activity of CYP2D6 variants and offer a better understanding of pharmacogenomics. It also might contribute to variability in drug dose–response relationship and tailoring drug therapy to patient in a safe and effective manner.

Declaration of interest

This work was supported by a grant from the National Health and Family Planning Commission of the People's Republic of China (No. 201302008) and a grant from the National Natural Science Foundation of China (No. 31371280).

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Acknowledgments

The authors thank the members of the Beijing Institute of Geriatrics of the Ministry of Health for their advice and assistance.

References

[1] Tharanga TD, et al. Genetic variants in the cytochrome P450 2D6 gene in the Sri Lankan population. Indian J Hum Genet 2013;19(4):392–6.
[2] Wang B, et al. Genetic polymorphism of the human cytochrome P450 2C9 gene and its clinical significance. Curr Drug Metab 2009;10(7):781–834.
[3] Zhou SF, Liu JP, Chowbay B. Polymorphism of human cytochrome P450 enzymes and its clinical impact. Drug Metab Rev 2009;41(2):89–295.
[4] Zhou SF. Polymorphism of human cytochrome P450 2D6 and its clinical significance: part I. Clin Pharmacokinet 2009;48(11):689–723.
[5] Wang HH, et al. Novel DNA sequence variations of cytochrome P450 genes in the Han Chinese population. Pharmacogenomics 2009;10(3):359–74.
[6] Griese EU, et al. Allele and genotype frequencies of polymorphic cytochromes P4502D6, 2C19 and 2E1 in aborigines from western Australia. Pharmacogenetics 2001;11(1):69–76.
[7] Walko CM, McLeod H. Use of CYP2D6 genotyping in practice: tamoxifen dose adjustment. Pharmacogenomics 2012;13(6):691–7.
[8] Brosen K, Naranjo CA. Review of pharmacokinetic and pharmacodynamic interaction studies with citalopram. Eur Neuropsychopharmacol 2001;11(4):275–83.
[9] Talarico G, et al. Serotonin toxicity: a short review of the literature and two case reports involving citalopram. Neurol Sci 2011;32(3):507–9.
[10] Bezchlibnyk-Butler K, Aleksic I, Kennedy SH. Citalopram – a review of pharmacological and clinical effects. J Psychiatry Neurosci 2000;25(3):241–54.
[11] Shiroma PR, et al. Emotion recognition processing as early predictor of response to 8-week citalopram treatment in late-life depression. Int J Geriatric Psychiatry 2014;29(11):1132–9.
[12] Chang M, et al. Impact of cytochrome P450 2C19 polymorphisms on citalopram/escitalopram exposure: a systematic review and meta-analysis. Clin Pharmacokinet 2014;53(9):801–11.
[13] von Moltke LL, et al. Escitalopram (S-citalopram) and its metabolites in vitro: cytochromes mediating biotransformation, inhibitory effects, and comparison to R-citalopram. Drug Metab Dispos 2001;29(8):1102–9.
[14] Qian JC, et al. Genetic variations of human CYP2D6 in the Chinese Han population. Pharmacogenomics 2013;14(14):1731–43.
[15] Hiratsuka M. In vitro assessment of the allelic variants of cytochrome P450. Drug Metab Pharmacokinet 2012;27(1):68–84.
[16] Daly AK, et al. Nomenclature for human CYP2D6 alleles. Pharmacogenetics 1996;6(3):193–201.
[17] Jin TB, et al. Polymorphisms and phenotypic analysis of cytochrome P450 2D6 in the Tibetan population. Gene 2013;527(1):360–5.
[18] Zanger UM, Raimundo S, Eichelbaum M. Cytochrome P450 2D6: overview and update on pharmacology, genetics, biochemistry. Naunyn Schmiedeb Arch Pharmacol 2004;369(1):23–37.
[19] Rolla R, et al. An observational study of Venlafaxine and CYP2D6 in clinical practice. Clin Lab 2014;60(2):225–31.
[20] Cai J, et al. Effects of 22 novel CYP2D6 variants found in the Chinese population on the bufuralol and dextromethorphan metabolisms in vitro. Basic Clin Pharmacol Toxicol 2015. Q2
[21] Muroi Y, et al. Functional characterization of wild-type and 49 CYP2D6 allelic variants for N-desmethyltamoxifen 4-hydroxylation activity. Drug Metab Pharmacokinet 2014;29(5):360–6.
[22] Byeon JY, et al. Effects of the CYP2D6*10 allele on the pharmacokinetics of atomoxetine and its metabolites. Arch Pharm Res 2015.
[23] Ingelman-Sundberg M. Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. Pharmacogenomics J 2005;5(1):6–13.
[24] Shen H, et al. Comparative metabolic capabilities and inhibitory profiles of CYP2D6.1, CYP2D6.10, and CYP2D6.17. Drug Metab Dispos 2007;35(8):1292–300.
[25] Wu X, et al. The impact of CYP2D6 polymorphisms on the pharmacokinetics of codeine and its metabolites in Mongolian Chinese subjects. Eur J Clin Pharmacol 2014;70(1):57–63.
[26] Zhong Y, et al. Effect of CYP2D6*10 and APOE polymorphisms on the efficacy of donepezil in patients with Alzheimer's disease. Am J Med Sci 2013;345(3):222–6.
[27] Zhan YY, et al. Effect of CYP2D6 variants on venlafaxine metabolism in vitro. Xenobiotica 2015:1–6.
[28] Dai DP, et al. In vitro functional assessment of 22 newly identified CYP2D6 allelic variants in the chinese population. Basic Clin Pharmacol Toxicol 2015;117(1):39–43. Q3

Please cite this article in press as: Hu X-x, et al., Effect of CYP2D6 genetic polymorphism on the metabolism of citalopram in vitro, Drug Metabolism and Pharmacokinetics (2016), http://dx.doi.org/10.1016/j.dmpk.2016.01.001