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Functional characterization of 17 *CYP2D6* allelic variants (CYP2D6.2, 10, 14A–B, 18, 27, 36, 39, 47–51, 53–55, and 57)

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Abstract

Cytochrome P450 2D6 (CYP2D6) is an enzyme of potential importance for the metabolism of clinically used drugs, and it exhibits genetic polymorphism with interindividual differences in metabolic activity. To date, 21 *CYP2D6* allelic variants have been identified in the Japanese population. The aim of this study was to investigate the functional characterization of CYP2D6 variants identified in Japanese subjects. Wild-type CYP2D6 and its variants, namely, CYP2D6.2, CYP2D6.10, CYP2D6.14A, CYP2D6.14B, CYP2D6.18, CYP2D6.27, CYP2D6.36, CYP2D6.39, CYP2D6.47, CYP2D6.48, CYP2D6.49, CYP2D6.50, CYP2D6.51, CYP2D6.53, CYP2D6.54, CYP2D6.55 and CYP2D6.57 were transiently expressed in COS-7 cells, and enzymatic activities of the CYP2D6 variant proteins were characterized using bufuralol and dextromethorphan. Functional characterization of 17 CYP2D6 variants revealed an absence of enzyme activity in 4 (CYP2D6.14A, CYP2D6.36, CYP2D6.47, and CYP2D6.57), low activity in 8 (CYP2D6.10, CYP2D6.14B, CYP2D6.18, CYP2D6.49, CYP2D6.50, CYP2D6.51, CYP2D6.54, and CYP2D6.55) and high activity in 1 (CYP2D6.53) as compared with the wild-type. Analysis of CYP2D6 variant proteins can be useful for predicting CYP2D6 phenotypes and could be applied to personalized drug therapy.

Introduction

Cytochrome P450 2D6 (CYP2D6) is an important drug-metabolizing enzyme involved in the metabolism of many therapeutic agents, including antidepressants, β-adrenergic antagonists, antiarrhythmics, and opioids (Zanger et al., 2004). The genetic polymorphisms of *CYP2D6* are defined in 67 allelic variants, many of which are associated with increased, decreased, or abolished enzyme functions (Human CYP Allele Nomenclature website: http://www.cypalleles.ki.se/cyp2d6.htm). These polymorphisms result in differences of up to 30- to 40-fold in substrate drug clearance, leading to drug concentrations out of the therapeutic range in treated patients. Consequently, such differences in CYP2D6 activity would not only lead to severe adverse effects in clinical therapy (Kirchheiner et al., 2004) but also to nonresponse to medications, e.g., no observable analgesic effects of prodrugs such as codeine in poor metabolizers (PM) (Gasche et al., 2004).

CYP2D6 activity widely differs among ultrarapid metabolizer (UM), extensive metabolizer (EM), intermediate metabolizer (IM), and PM phenotypes. The PM phenotype is due to 2 non-functional (null) alleles, whereas the EM phenotype is due to 1 or 2 alleles with normal function, such as *CYP2D6*1* and *CYP2D6*2*. An IM phenotype is usually observed in populations harboring a combination of 1 *CYP2D6* null allele and another allele with impaired expression and/or function, such as *CYP2D6*10* (Zanger et al., 2004). The frequency of PM is 5–10% in the Caucasian population and less than 1% in the Asian population (Sohn et al., 1991; Dahl et al., 1992; Tateishi et al., 1999). Among the variant alleles reported thus far, *CYP2D6*3*, *CYP2D6*4*, *CYP2D6*5*, and *CYP2D6*6* account for approximately 97% of the PM alleles in the Caucasian population (Sachse et al., 1997). On the other hand, studies conducted by us and other groups have identified 21 variant alleles of the *CYP2D6* gene in the Japanese population (Fig. 1) (Yokoi et al., 1996; Chida et al., 1999;

Nishida et al., 2000; Yamazaki et al., 2003; Soyama et al., 2004; Ebisawa et al., 2005; Soyama et al., 2006). A number of these variant alleles have also been described in other Asian populations (Table 1). Among them, CYP2D6**4, CYP2D6*5, CYP2D6*14A, CYP2D6*18, CYP2D6*21, CYP2D6*36,* and *CYP2D6*44* are associated with the PM phenotype (Kagimoto et al., 1990; Gaedigk et al., 1991; Yokoi et al., 1996; Chida et al., 1999; Wang et al., 1999; Yamazaki et al., 2003; Gaedigk et al., 2006). The catalytic properties of *CYP2D6*10* and *CYP2D6*2* have been extensively studied *in vivo* and *in vitro* using recombinant expression systems and reaction phenotyping. The frequency of *CYP2D6*10* is relatively high in Asians, and individuals with an IM phenotype and with *CYP2D6*10/*10* or *CYP2D6*10/*null* genotypes, respectively, exhibit lower catalytic activities toward typical CYP2D6 substrates such as dextromethorphan (Tateishi et al., 1999), metoprolol (Huang et al., 1999), and nortriptyline (Yue et al., 1998) than wild-type homozygous individuals. Subjects harboring *CYP2D6*2/*2* exhibit metabolic activity similar to that of the wild-type for various substrates (Johansson et al., 1993; Dahl et al., 1995). Functional alterations conveyed by sequence variations in *CYP2D6*14B, CYP2D6*27, CYP2D6*39, CYP2D6*47, CYP2D6*48, CYP2D6*49, CYP2D6*50, CYP2D6*51, CYP2D6*53, CYP2D6*54, CYP2D6*55,* and *CYP2D6*57,* which have been identified in the Japanese population, remain unknown.

The purpose of this study was to investigate the functional characterization of the CYP2D6 variants identified in Japanese subjects. We generated 17 expression constructs (*CYP2D6*2, CYP2D6*10, CYP2D6*14A, CYP2D6*14B, CYP2D6*18, CYP2D6*27, CYP2D6*36, CYP2D6*39, CYP2D6*47, CYP2D6*48, CYP2D6*49, CYP2D6*50, CYP2D6*51, CYP2D6*53, CYP2D6*54, CYP2D6*55,* and *CYP2D6*57*), which were transfected into COS-7 cells. The enzymatic properties of CYP2D6 variant proteins were characterized using the specific substrates bufuralol and dextromethorphan.

Materials and Methods

Construction of expression plasmids

CYP2D6 cDNA fragments were amplified from the human liver cDNA library (TaKaRa, Otsu, Japan) using PCR with the forward primer 5′-CACCATGGGGCTAGAAGCAC-3′ and the reverse primer 5′-CTAGCGGGGCACAGCACAAAG-3′ in the GeneAmp High Fidelity PCR system (Applied Biosystems, Foster City, CA). The amplified fragments were subcloned into the pENTR/D-TOPO vector (Invitrogen, Carlsbad, CA). The underlined sequence was introduced for directional TOPO cloning. Plasmids carrying the *CYP2D6*2* cDNA were used as the template to generate 17 *CYP2D6* constructs (*CYP2D6*1, CYP2D6*10, CYP2D6*14A, CYP2D6*14B, CYP2D6*18, CYP2D6*27, CYP2D6*36, CYP2D6*39, CYP2D6*47, CYP2D6*48, CYP2D6*49, CYP2D6*50, CYP2D6*51, CYP2D6*53, CYP2D6*54, CYP2D6*55*, and *CYP2D6*57*) using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA) according to the manufacturer's instructions. All constructs were sequenced to confirm successful mutagenesis. The wild-type and variant *CYP2D6* cDNAs were subsequently subcloned into the pcDNA-DEST40 mammalian expression vector (Invitrogen).

Expression of variant CYP2D6 proteins in COS-7 cells

COS-7 cells were cultured in Dulbecco's modified Eagle's medium (Invitrogen) containing 10% fetal bovine serum under 5% CO_2 at 37°C. The cells were plated at 1.8×10^6 cells in 100-mm dishes 24 h before transfection. Subsequently, the OPTI-MEM medium (Invitrogen) was added to the culture medium, and the cells were transfected with 5 μg CYP2D6 plasmids by using the TransFectin lipid reagent (Bio-Rad, Hercules, CA), according to the manufacturer's instructions. Cells were incubated for 24 h at 37°C and then scraped off the dish after washing the cells once with cold phosphate-buffered saline. Cells were then

resuspended in a homogenization buffer containing 10 mM Tris-HCl (pH 7.4), 0.25 M sucrose, and 1 mM EDTA. Microsomal fractions were prepared by differential centrifugation at 9,000 *g* for 20 min followed by centrifugation of the resultant supernatant at 105,000 *g* for 60 min. The microsomal pellet was resuspended in 10 mM Tris-HCl (pH 7.4), 10% glycerol, and 1 mM EDTA and stored at –80°C. The protein concentration was measured using the BCA protein assay kit (Pierce, Rockford, IL).

Determination of protein expression levels by immunoblotting

Immunoblotting was performed according to standard procedures with 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and 5 μg microsomal protein per lane. Recombinant CYP2D6 Baculosomes reagent (Invitrogen) was co-analyzed on each gel as the standard (range, 0.125–0.5 pmol) and for the quantification of the CYP2D6 apoprotein content. CYP2D6 protein was detected using rabbit anti-human CYP2D6 antibody (Daiichi Pure Chemicals, Tokyo, Japan) and horseradish peroxidase-conjugated goat anti-rabbit IgG (Santa Cruz, CA). Immunoblots were developed using SuperSignal West Pico chemiluminescence substrate (Pierce). Chemiluminescence quantified using the Lumino Imaging Analyzer FAS-1000 (Toyobo, Osaka, Japan) and the Gel-Pro Analyzer (Media Cybernetics, Silver Spring, MD).

Enzymatic properties of wild-type and CYP2D6 variants

Bufuralol 1′-hydroxylation

The activity of bufuralol 1′-hydroxylation was measured by the method reported by Marcucci et al. (2002) with minor modifications. The incubation mixture contained 1–640 μM bufuralol (Sigma-Aldrich, St. Louis, MO), microsomal fraction (25 μg) obtained from COS-7 cells, 1.3 mM NADP, 3.3 mM glucose-6-phosphate, 0.4 U/ml glucose-6-phosphate dehydrogenase, 3.3 mM $MgCl_2$, and 100 mM potassium phosphate buffer (pH 7.4) in a final

volume of 75 µl. The reactions were carried out at 37°C for 15 min and terminated by the addition of 75 µl methanol. The samples were then centrifuged at 10,000 *g* for 3 min to obtain a protein pellet prior to high-performance liquid chromatography (HPLC) analysis. The HPLC system consisted of a Waters 2695 Separations Module, Waters 2475 multi λ fluorescence detector (Waters Corp., Milford, MA), and a 4.6 mm × 150 mm Novapak Phenyl column (3.9 mm × 15 cm; particle size, 4 µm; temperature, 40°C; Waters Corp). The mobile phase was composed of 82% buffer (20 mM potassium phosphate and 20 mM hexane sulfonic acid, pH 4.0) and 18% acetonitrile for the first 12 min, which was subsequently replaced by 60% buffer and 40% acetonitrile from 12 to 17 min, and the mobile phase was finally restored to 82% buffer and 18% acetonitrile from 17 min to 27 min. The flow rate was set at 1.0 ml/min with fluorescence detection at excitation and emission wavelengths of 252 nm and 302 nm, respectively. Under these conditions, the retention times of 1′-hydroxybufuralol and bufuralol were 9 min and 17 min, respectively.

Dextromethorphan *O*-demethylation

The *O*-demethylation activity of dextromethorphan was measured by the method reported by Marcucci et al. (2002) with minor modifications. The reaction mixture containing 20 µM dextromethorphan (Wako Pure Chemical Industries, Osaka, Japan) was incubated for 10 min at 37°C. The reactions were terminated by the adding 75 µl methanol containing 1 nM levallorphan tartrate (Sigma-Aldrich), which was used as the internal standard. Other conditions for incubation and deproteinization were identical to those used in the assay of bufuralol 1′-hydroxylation activity. Dextrorphan was detected using the same HPLC system used for the determination of 1′-hydroxybufuralol. The mobile phase consisted of 75% buffer (20 mM potassium phosphate and 20 mM hexane sulfonic acid, pH 4.0) and 25% acetonitrile at a flow rate of 1.2 ml/min with fluorescence detection at excitation and emission

wavelengths of 280 nm and 310 nm, respectively. Under these conditions, the retention times of dextrorphan and dextromethorphan were 4 min and 16 min, respectively.

Data analysis

Kinetic parameters such as the K_m and V_{max} for bufuralol 1′-hydroxylation were determined using Eadie-Hofstee plots. The intrinsic clearance (CL_{int}) values were determined by the ratio V_{max}/K_m. All values were expressed as the mean ± standard deviation (S.D.) of 3 independent transfection experiments. Each assay was performed in triplicate. Statistical analyses were performed using the Student's *t* test, and a value of $P < 0.05$ was considered statistically significant.

Results

Expression of wild-type and variant CYP2D6 in COS-7 cells

The CYP2D6 variants were expressed in COS-7 cells, and the protein levels were measured by immunoblot analysis (Fig. 2). All CYP2D6 proteins expressed were recognized with an antibody against CYP2D6. Of the 17 variant proteins, the following 13 had expression levels reduced to 33–81% that of CYP2D6.1: CYP2D6.10, CYP2D6.14A, CYP2D6.27, CYP2D6.36, CYP2D6.39, CYP2D6.47, CYP2D6.49, CYP2D6.50, CYP2D6.51, CYP2D6.53, CYP2D6.54, CYP2D6.55, and CYP2D6.57. The expression level of CYP2D6.1 was 65.7 ± 18.7 pmol/mg microsomal protein.

Enzymatic properties of wild-type and variant CYP2D6

Bufuralol 1′-hydroxylation activity at substrate concentrations of 80 μM and dextromethorphan *O*-demethylation activity at substrate concentrations of 20 μM were determined on the basis of microsomal CYP2D6 of the wild-type protein and the 17 variant CYP2D6 proteins (Fig. 3). The bufuralol 1′-hydroxylation by CYP2D6.10, CYP2D6.18, CYP2D6.36, CYP2D6.47, CYP2D6.49, CYP2D6.50, CYP2D6.51, CYP2D6.54, CYP2D6.55, and CYP2D6.57 was reduced to 2–36% of that of CYP2D6.1. The dextromethorphan *O*-demethylation activity of CYP2D6.10, CYP2D6.18, CYP2D6.49, CYP2D6.50, CYP2D6.54, and CYP2D6.55 was reduced to 7–36% of that of CYP2D6.1. CYP2D6.14A had no detectable enzyme activity toward bufuralol and dextromethorphan.

Figure 4 shows the Michaelis-Menten curves and kinetic parameters of bufuralol 1′-hydroxylation by the wild-type and the 17 variants. The V_{max} and CL_{int} (V_{max}/K_m) values for bufuralol 1′-hydroxylation were normalized to those of the CYP2D6 protein levels. CYP2D6.1 had an apparent K_m value of 8.4 μM (the average value from 4 experiments). CYP2D6.2, CYP2D6.10, CYP2D6.14B, CYP2D6.18, and CYP2D6.55 had significantly

higher apparent K_m values that varied from 11.4 to 72.8 μM. In contrast, CYP2D6.49, CYP2D6.50, and CYP2D6.53 had apparent K_m values lower than that of CYP2D6.1, ranging from 2.5 to 3.5 μM. The V_{max} values of CYP2D6.10, CYP2D6.49, and CYP2D6.54 were 20%, 17%, and 6% that of CYP2D6.1, respectively. The CL_{int} values of CYP2D6.10, CYP2D6.14B, CYP2D6.18, CYP2D6.49, CYP2D6.54, and CYP2D6.55 were lower than that of CYP2D6.1 (7–35% that of CYP2D6.1). On the other hand, CYP2D6.53 had an apparent CL_{int} value 4 times higher than that of CYP2D6.1. CYP2D6.36, CYP2D6.47, CYP2D6.51, and CYP2D6.57 showed extremely low activity, and the kinetic parameters could not be determined. CYP2D6.14A activity was not detected at the substrate concentrations used.

Discussion

CYP2D6 is a clinically important enzyme that metabolizes numerous therapeutic drugs (Zanger et al., 2004). The present study provides comprehensive data regarding functional alterations of CYP2D6 variant proteins in the Japanese population. To assess the effects of 17 *CYP2D6* variant alleles on the protein expression levels and enzymatic activity, the wild-type and 17 variant CYP2D6 proteins were transiently expressed in COS-7 cells.

Among the 17 variant *CYP2D6* alleles, *CYP2D6*14A*, *CYP2D6*18*, and *CYP2D6*36* are associated with PM phenotype. There was a subject carrying *CYP2D6*5/*14A* which shown to be the PM phenotype (Wang et al., 1999). Shiraishi et al. (2001) have reported that a combination of G169R and P34S decreased the bufuralol 1′-hydroxylation and dextromethorphan *O*-demethylation activities. In this study, CYP2D6.14A harboring the P34S, G169R, R296C, and S486T substitutions showed no detectable activity. On the other hand, the activity of CYP2D6.14B harboring G169R, R296C, and S486T substitutions was higher than that of CYP2D6.14A. These results suggest that the relatively high activity of CYP2D6.14B may be mainly attributed to the absence of the P34S substitution. Yokoi et al. (1996) have reported the kinetic parameters for CYP2D6.18-mediated bufuralol 1′-hydroxylation activity. The K_m of CYP2D6.18 showed a 236-fold increase from the wild-type K_m (990 μM vs. 4.2 μM), a finding which is in agreement to the results we obtained in the COS-7 expression system. The COS-7 cells that expressed CYP2D6.36 exhibited decreased protein levels and bufuralol 1′-hydroxylation activities than those that expressed the wild-type; these results were consistent with those of a previous report (Johansson et al., 1994). The *CYP2D6*36* alleles have been described to be in a tandem arrangement with *CYP2D6*10* as *CYP2D6*36–*10* alleles. Recently, the duplicate-type **36*×2 in the Japanese and the single-type **36* in African Americans and Asians were found in subjects displaying

the PM phenotype (Chida et al., 2002; Gaedigk et al., 2006; Soyama et al., 2006). Since CYP2D6.36 shows extremely low activities toward bufuralol and dextromethorphan, the combined activities of CYP2D6.36 and CYP2D6.10 could be comparable to the activity of CYP2D6.10 alone.

Of the 12 functionally unknown variant proteins (Fig. 1), CYP2D6.47, CYP2D6.51, and CYP2D6.57 exhibited a drastic decrease in the enzymatic activity, retaining <5% of the wild-type activity, and these variants may cause as the PM phenotype. CYP2D6.47 has R25W, P34S, and S486T substitutions. R25 is located within the transmembrane domain and is considered to act as a halt transfer signal, which prevents the translocation of the protein into the lumen of the endoplasmic reticulum (Sakaguchi et al., 1987). CYP2D6.51 has R296C, E334A, and S486T substitutions. Based on the crystal structure of human CYP2D6 (Rowland et al., 2006), E334 is located on the J helix and conserved within P450s, further suggesting that this amino acid is critical for maintaining P450 function. The enzymatic activity of CYP2D6.57 (P34S, R62W, and 7 amino acid changes in exon 9) was approximately 4% that of the wild-type, and this decreased activity level was similar to that of CYP2D6.36. Hence, loss of CYP2D6.57 activity is probably attributable to the presence of the P34S substitution and the amino acids introduced by the exon 9 conversion. *CYP2D6*57* alleles have also been described to exist in a tandem arrangement with *CYP2D6*10* as *CYP2D6*57–*10*. The combined activities of CYP2D6.57 and CYP2D6.10 could be similar to the CYP2D6.10 activity and to the sum of the CYP2D6.36 and CYP2D6.10 activities. *CYP2D6*47*, *CYP2D6*51*, and *CYP2D6*57* have recently been detected using single nucleotide polymorphisms (SNP) analysis, but *in vivo* data have not yet been obtained.

CYP2D6.53 exhibited a 73% decrease in the K_m values, resulting in a 4-fold increase in its CL_{int} value as compared to that of CYP2D6.1. CYP2D6.53 harboring F120I and A122S

substitutions located in substrate recognition site 1. Flanagan et al. (2004) recently suggested that the F120 residue within the B-C loop of P450s would play an important role in substrate binding and orientation. Furthermore, Keizer et al. (2004) demonstrated that the mutation of F120 to alanine results in a 50% decrease in the K_m value as compared to the K_m value of CYP2D6.1. Similarly, our results revealed that CYP2D6.53 activity exhibited a lower K_m value than CYP2D6.1, suggesting that the increased affinity of CYP2D6.53 might be attributed to a natural variant of F120I. Since an increase in the CL_{int} of CYP2D6.53 is considered as the main alteration affecting substrate binding, individuals with *CYP2D6*53* might exhibit the UM phenotype.

CYP2D6.14B, CYP2D6.49, CYP2D6.50, CYP2D6.54, and CYP2D6.55 exhibited intermediate activity similar to CYP2D6.10 and could be associated with the IM phenotype. Although CYP2D6.27 (E410K), CYP2D6.39 (S486T), and CYP2D6.48 (A90V) exhibited slightly higher CL_{int} values for bufuralol, the differences were not significant. E410K, S486T, and A90V are located between the K′ and K″ helices, B helix, and β-sheet 3, respectively (Rowland et al., 2006), and these amino acids are not conserved among human P450s; therefore, they may not be critical with regard to CYP2D6 activity.

In Asians, the most abundant variant allele is *CYP2D6*10*. Several studies have reported CYP2D6.10 exhibited decreased protein levels and an increased K_m value with respect to bufuralol (Fukuda et al., 2000; Hanioka et al., 2006; Shen et al., 2007). CYP2D6.10 harbored the P34S and S486T substitutions; P34S is part of a proline-rich region that is highly conserved among microsomal P450s and may function as a hinge between the hydrophobic membrane anchor and the heme-binding portion of the enzyme (Yamazaki et al., 1993). It has been shown that subjects homozygous for *CYP2D6*10/*10* require lower drug dosages of metoprolol and nortriptyline than do *CYP2D6*1/*1* subjects in order to achieve the same

therapeutic effect. Thus, the former may be at greater risk for developing dose-dependent adverse effects (Yue et al., 1998; Huang 1999). An important example of cancer therapy is the adjuvant treatment of breast cancer with tamoxifen. Several recent retrospective and prospective studies have demonstrated the significant impact of the *CYP2D6* genotype on the plasma concentrations of the active tamoxifen metabolite endoxifen (Goetz et al., 2007). Patients with the *CYP2D6*10/*10* genotypes have extremely low levels of the endoxifen and thus benefit to a lesser extent from the treatment (Lim et al., 2007).

In order to characterize and assess the functional effect of variant alleles on CYP2D6 activity, several studies have been performed using various heterologous expression systems, including bacteria, insect cells and mammalian cells. By using these systems, CYP2D6.10 have been shown to exhibit a decreased catalytic efficiency towards bufuralol than that exhibited by CYP2D6.1; but the kinetic parameters (K_m, V_{max}, and CL_{int}) reported by the different authors were highly variable (Fukuda et al., 2000; Hanioka et al., 2006; Shen et al., 2007). The reason for the discrepancy among the laboratories might be partly due to the use of different expression systems. Therefore, a comparison of the kinetic parameters with previous reports would be difficult. In this study, the catalytic properties of a large number of *CYP2D6* allelic variants that were expressed under the same conditions were comprehensively compared with the properties of the wild-type. CYP2D6 proteins expressed in the COS-7 cells would undergo both posttranslational modification and protein degradation: the mammalian expression system would be an appropriate system for the functional characterization of the P450 enzymes.

In this study, the rate of enzymatic activity alteration between bufuralol and dextromethorphan were similar (Fig. 3). However, several researchers (Bogni et al., 2005; Cai et al., 2006; Shen et al., 2007) have reported that some allelic variants (e.g., *CYP2D6*17*) are

associated with substrate-dependent decreases in catalytic properties. In addition, some CYP2D6 variant proteins may decrease heme incorporation into the CYP2D6 apoprotein, but this possibility could not be assessed in the present study. The levels of CYP2D6 protein expressed in COS-7 cells were too low to be determined by the difference in the CO-reduced spectra. Further, *in vivo* and *in vitro* studies by using other substrates as well as the measurement of the holoprotein level are required to confirm these results.

Most of the 17 variants that were expressed in the COS-7 cells showed a significant decreased in catalytic activity. In a routine genotyping, it is important to determine all these alleles, even if the allele frequencies are low. A *CYP2D6* genotyping assay such as the DNA microarray can easily test hundreds of SNPs in a single run. Recently, the Food and Drug Administration (FDA) in the US has approved the first pharmacogenetic test—the AmpliChip CYP450— that uses a DNA microarray (Roche Molecular Diagnostics, Alameda, California, USA), to determine the genotypes of *CYP2D6* and *CYP2C19*.

In conclusion, we expressed 17 CYP2D6 variants that have previously been found in the Japanese population. Of these, 13 variants functionally affected the CYP2D6 activity *in vitro*. These data will help understand the genotype-phenotype relationships of *CYP2D6* and provide a foundation for future clinical studies regarding individual variations in drug efficacy and toxicity in Asians.

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Footnotes

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Legends for figures

Fig. 1. Structures of the *CYP2D6* alleles detected in Japanese individuals.

The 9 exons are indicated by numbered boxes. Shaded boxes represent exons that encode open reading frame sequences. DNA polymorphisms are indicated on top (ins, insertion). Predicted amino acid changes and translation termination (ter) codons are indicated below the boxes. Silent mutations and some promoter and intronic polymorphisms are not shown.

Fig. 2. Detection of CYP2D6 proteins by immunoblot analysis and the average levels of the immunoreactive proteins expressed in COS-7 cells.

Numbers correspond to each allele number. Each bar represents the mean ± S.D. of 3 separate experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$ compared to CYP2D6.1.

Fig. 3. Bufuralol 1′-hydroxylation activity (A) and dextromethorphan *O*-demethylation activity (B) of the CYP2D6 proteins expressed in COS-7 cells.

The bufuralol and dextromethorphan concentrations were 80 μM and 20 μM, respectively. Each number corresponds to an allele number. Each value is expressed as a percentage of CYP2D6.1 activity. Results represent the mean ± S.D. of 3 independent transfection experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$ compared to CYP2D6.1. N.D. denotes not done because of inadequate enzyme activity.

Fig. 4. The Michaelis-Menten curves and kinetic parameters of wild-type and CYP2D6 variants.

(A) CYP2D6.1, CYP2D6.2, CYP2D6.39, CYP2D6.51, CYP2D6.55. (B) CYP2D6.1,

CYP2D6.10, CYP2D6.36, CYP2D6.54, CYP2D6.57. (C) CYP2D6.1, CYP2D6.14A, CYP2D6.14B, CYP2D6.47, CYP2D6.49, CYP2D6.53. (D) CYP2D6.1, CYP2D6.18, CYP2D6.27, CYP2D6.48, CYP2D6.50. The variant CYP2D6 proteins were divided into 4 separate groups for analysis. Results represent the mean ± S.D. of 3 independently performed transfection experiments.*$P < 0.05$, **$P < 0.01$, ***$P < 0.005$ compared to CYP2D6.1. N.D. represents not done because of inadequate enzyme activity.

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Table 1 Allele frequency of *CYP2D6* variants for Asians

Allele	Japanese[a]	Chinese[b]	Korean[c]
Number of alleles	196–572	446	800
	(%)	(%)	(%)
*CYP2D6*1*	39.8–43.0	37.9	33.3
*CYP2D6*2*	9.2–12.3	0.0	10.1
*CYP2D6*4*	0.0–0.3	0.2	0.3
*CYP2D6*5*	4.1–7.2	7.2	6.1
*CYP2D6*10*	34.6–40.8	51.3	45.0
*CYP2D6*14*	0.0–2.2[d]	2.0[e]	0.5[d]
*CYP2D6*18*	0.0–0.5		0
*CYP2D6*21*	0.3–0.7		0.3
*CYP2D6*27*	0.2		0.4
*CYP2D6*36*[f]	0.2–0.9		0.0
*CYP2D6*39*	0.3		0.6
*CYP2D6*44*	0.0–0.2		0.0
*CYP2D6*47*	0.0–0.2		0.1
*CYP2D6*48*	0.0–0.2		0.0
*CYP2D6*49*	0.3–0.5		0.0
*CYP2D6*50*	0.2		0.0
*CYP2D6*51*	0.0–0.2		0.0
*CYP2D6*53*	0.2		0.0
*CYP2D6*54*	0.2		0.0
*CYP2D6*55*	0.2		0.0
*CYP2D6*57*[g]	0.0–0.2		0.0
*CYP2D6*1×n*	0.3–0.5	1.1[h]	0.1
*CYP2D6*2×n*	0.3–0.7		0.5
*CYP2D6*10×n*	0.6–1.6	0.2	0.5

[a]Yokoi et al., 1996; Chida et al., 1999; Nishida et al., 2000; Yamazaki et al., 2003; Soyama et al., 2004; Ebisawa et al., 2005; Soyama et al., 2006

[b]Ji et al., 2002

[c]Lee et al., 2006

[d]*CYP2D6*14A* and *CYP2D6*14B*

[e]*CYP2D6*14B*

[f]Tandem-type and single-type

[g]Tandem-type

[h]*CYP2D6*1×n* and *CYP2D6*2×n*

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Figure 1

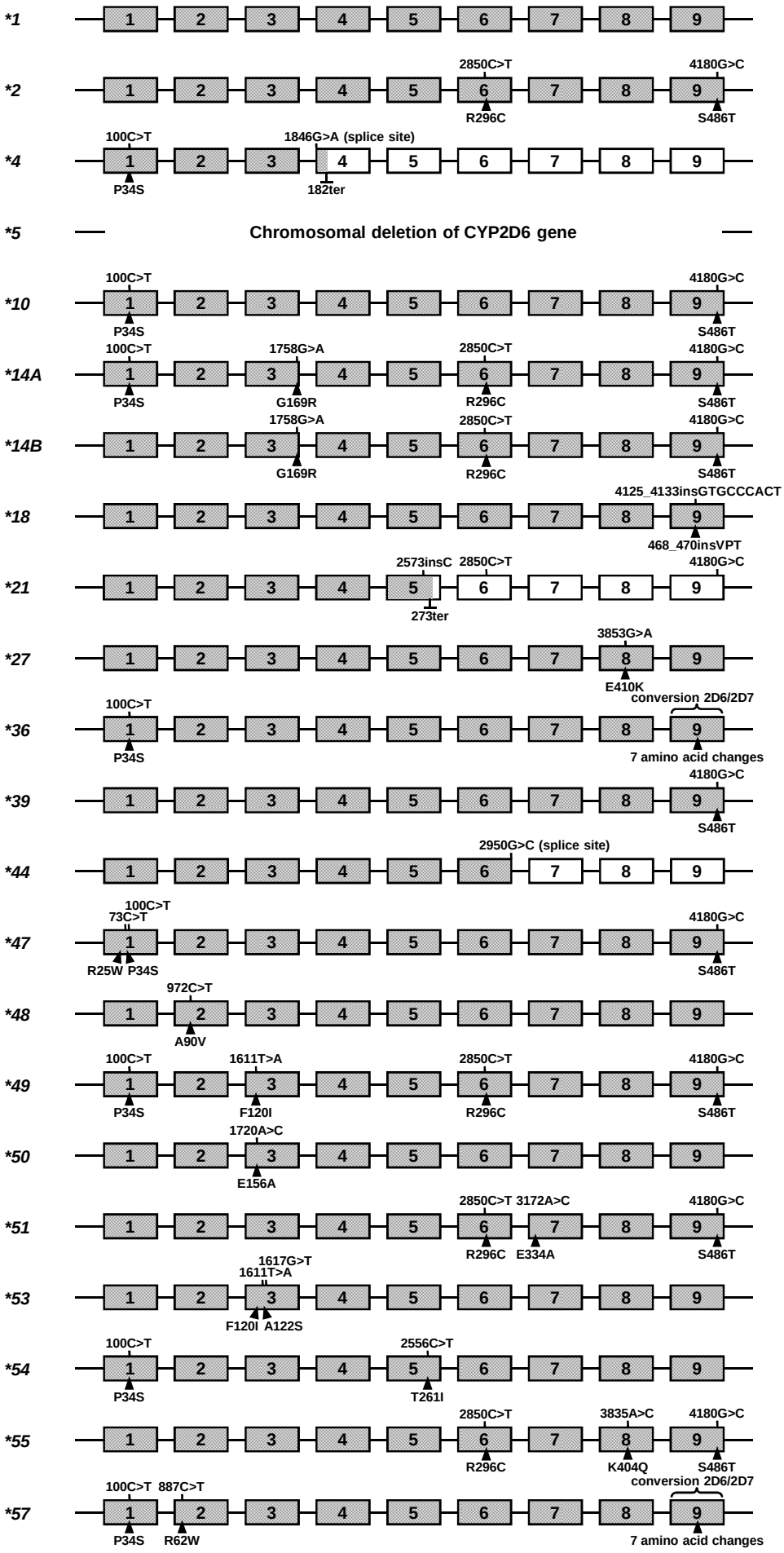

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Figure 2

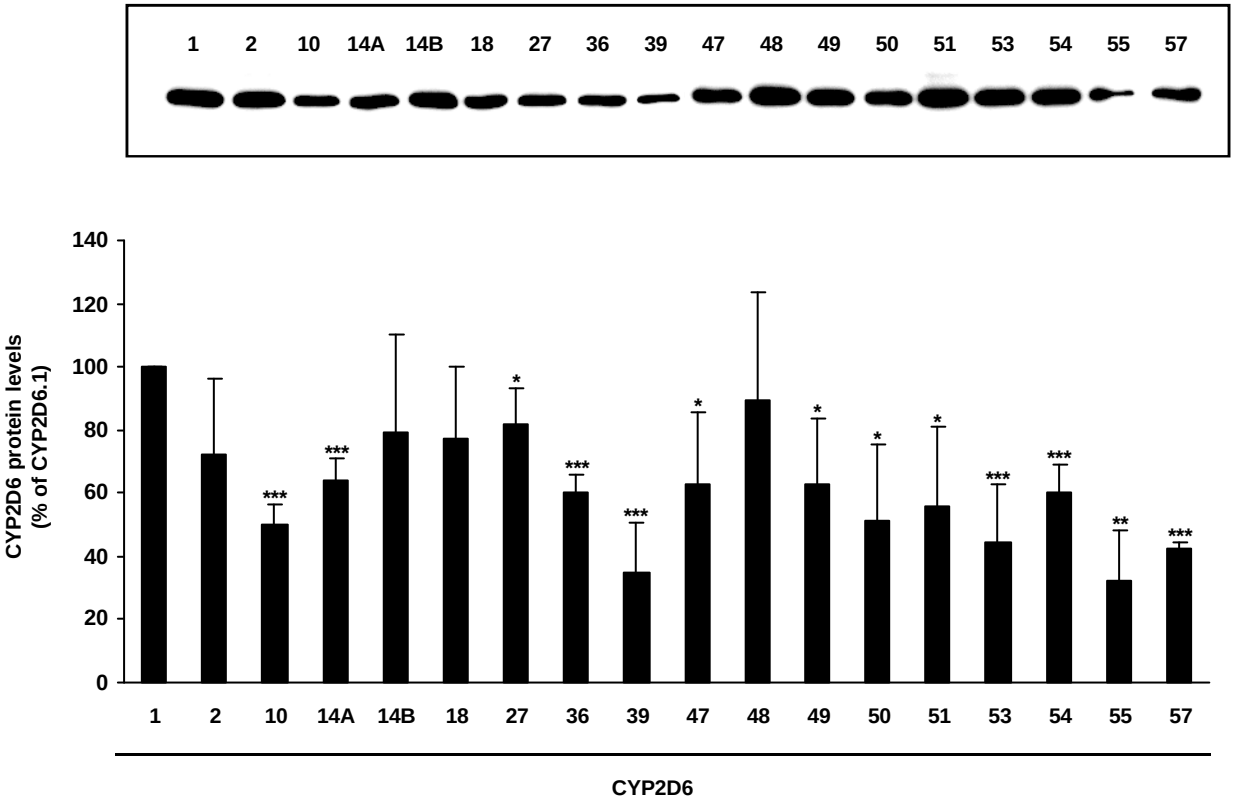

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Figure 3A,B

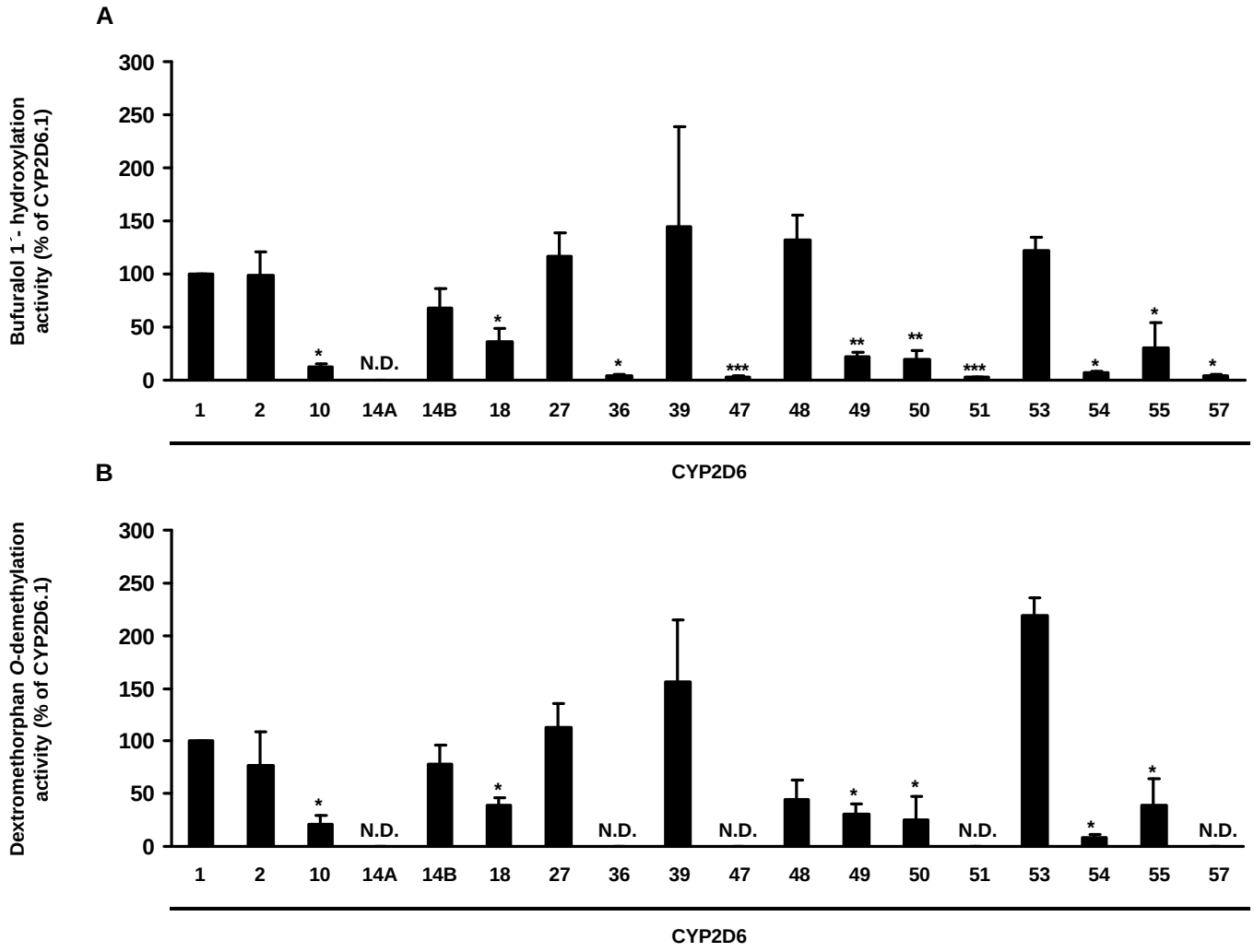

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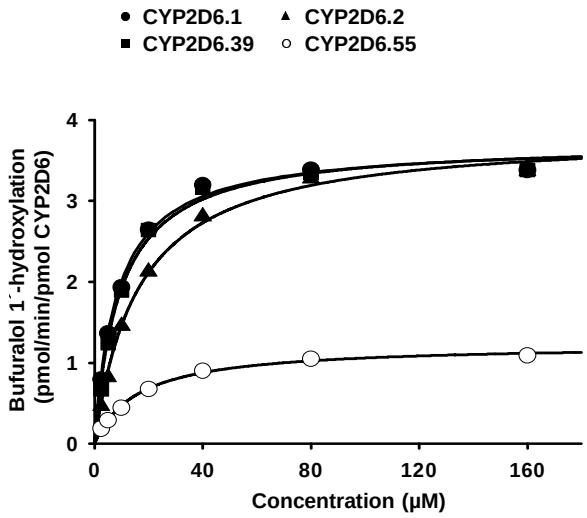
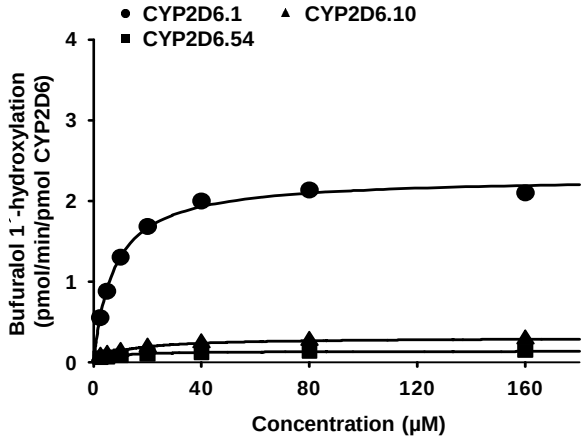
Figure 4A,B

A



Protein	K_m (μM)	V_{max} (pmol/min/pmol CYP2D6)	V_{max}/K_m (μL/min/pmol CYP2D6)
CYP2D6.1	8.71±1.51	3.73±1.54	0.42±0.11
CYP2D6.2	17.8±1.93 ***	3.97±1.82	0.22±0.07
CYP2D6.39	11.0±1.48	5.13±2.98	0.45±0.23
CYP2D6.51	N.D.	N.D.	N.D.
CYP2D6.55	14.6±0.72 ***	1.19±1.15	0.08±0.08 *

B



Protein	K_m (μM)	V_{max} (pmol/min/pmol CYP2D6)	V_{max}/K_m (μL/min/pmol CYP2D6)
CYP2D6.1	7.84±0.98	2.32±0.88	0.29±0.09
CYP2D6.10	11.4±1.48 *	0.30±0.12 *	0.03±0.01 **
CYP2D6.36	N.D.	N.D.	N.D.
CYP2D6.54	6.30±0.74	0.14±0.02 *	0.02±0.01 **
CYP2D6.57	N.D.	N.D.	N.D.

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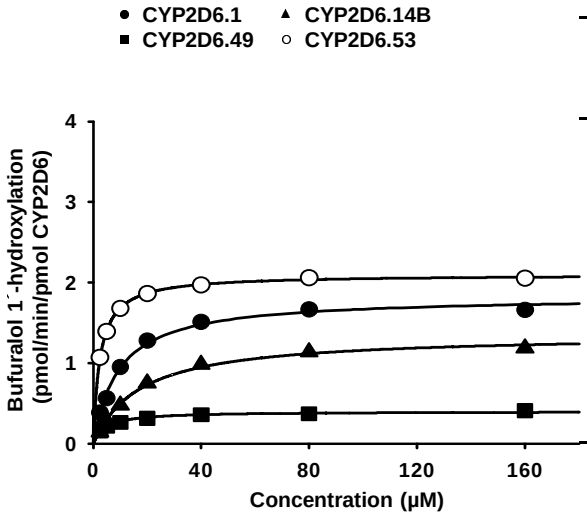
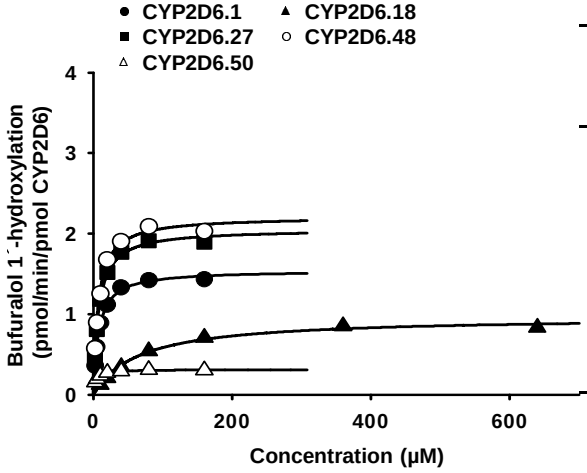
Figure 4C,D

C



Protein	K_m (μM)	V_{max} (pmol/min/pmol CYP2D6)	V_{max}/K_m (μL/min/pmol CYP2D6)
CYP2D6.1	9.20±1.22	1.84±0.47	0.20±0.02
CYP2D6.14A	N.D.	N.D.	N.D.
CYP2D6.14B	18.9±1.43 ***	1.39±0.49	0.07±0.03 **
CYP2D6.47	N.D.	N.D.	N.D.
CYP2D6.49	3.73±0.62 ***	0.36±0.20 **	0.10±0.01 *
CYP2D6.53	2.46±0.14 ***	2.10±0.69	0.86±0.32 *

D



Protein	K_m (μM)	V_{max} (pmol/min/pmol CYP2D6)	V_{max}/K_m (μL/min/pmol CYP2D6)
CYP2D6.1	7.82±0.83	1.56±0.82	0.19±0.09
CYP2D6.18	72.8±2.04 ***	0.98±0.17	0.01±0.01 *
CYP2D6.27	7.85±1.17	2.09±0.79	0.26±0.08
CYP2D6.48	7.14±0.65	2.52±0.80	0.35±0.08
CYP2D6.50	2.63±0.26 ***	0.31±0.08	0.12±0.04