*CYP2D6*10* polymorphism and the enantioselective O-desmethylation of S-(+)- and R-(-)-venlafaxine in Japanese psychiatric patients

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Abstract

According to previous studies, R-(-)-venlafaxine (VEN) has higher enantioselectivity than S-(+)-VEN, and the plasma concentration of R-(-)-VEN varies depending on CYP2D6 activity.

Therefore, we examined the pharmacokinetic effects of *CYP2D6*10* genotypes on the steady-state concentrations of the enantiomers of VEN. The individuals were 71 Japanese depressed patients treated with racemic VEN. The concentrations of the enantiomers of VEN and O-desmethylvenlafaxine (ODV) were measured. Polymerase chain reaction (PCR) was used to determine the *CYP2D6*10* genotypes. The plasma concentrations of S-(+)-VEN were approximately 1.9-fold higher than those of R-(-)-VEN. The plasma concentrations of S-(+)-VEN and R-(-)-VEN seemed to be higher in individualss with two mutant alleles of *CYP2D6*10*, although no significant differences were found in the plasma levels of S-(+)-VEN and R-(-)-VEN between *CYP2D6*10* genotypes. The number of mutant alleles of *CYP2D6*10* was a significant factor associated with the R-(-)-ODV/R-(-)-VEN ratio ($p=0.004$) in multiple regression analysis. This suggests that *CYP2D6*10* mutations affect the metabolism of R-(-)-VEN and S-(+)-VEN. Further studies are needed to examine how these findings affect clinical practice.

Keywords

*CYP2D6*10*, enantioselectivity, venlafaxine, depression, pharmacokinetics

Introduction

The enantiomers of enantioselective chemicals are known to have different pharmacological effects. Ignoring enantioselectivity when considering the use of drugs based on the totality of pharmacokinetics, pharmacology, pharmacodynamics, and toxicology can lead to a wide range of undesirable consequences. For example, thalidomide is synthesized as a racemic mixture of equal amounts of S-(+)-thalidomide and R-(-)-thalidomide, where R-(-)-thalidomide is hypnotic and S-(+)-thalidomide is teratogenic. [1, 2] For this reason, in recent years, the field of pharmacological research has begun to monitor the blood levels of biological samples, and much attention has been given to measuring enantiomeric concentrations to assess their toxicity and their effects on humans for treatment. Venlafaxine (VEN) is used clinically for depressed patients as a mixture of racemic S-(+)-enantiomers and R-enantiomers. Gex-Fabry et al. hypothesized that S-(+)-VEN selectively inhibits serotonin reuptake, whereas R-(-)-VEN inhibits both serotonin and noradrenaline uptake, [3] although there are no direct data on the differences in the pharmacological properties between the enantiomers of VEN.

The main metabolic pathway of VEN involves its metabolism to O-desmethylvenlafaxine (ODV) (Figure 1). [4] In addition to ODV, the metabolites of VEN include N-desmethylvenlafaxine (NDV) and N-O-desmethylvenlafaxine (DDV), which are produced by the enzymes encoded by CYP2D6 and CYP3A and by CYP2C19 and CYP2C9. [3, 5-8] ODV has similar pharmacological activity to the unchanged forms of VEN, whereas NDV and DDV are significantly less active. [9-12] Otton et al. used microsomes of human hepatocytes and yeast cells expressing human CYP2D6 cDNA to perform O-demethylation of VEN enantiomers. Using the kinetic parameters maximum velocity (Vmax) and Michaelis constant (km), the Vmax/km ratio was measured to

predict the relative hepatic metabolic clearance in vivo, and R-(-)-VEN showed 1.5- to 2-fold higher enantioselectivity than S-(+)-VEN. On the other hand, S-(+)-VEN showed faster N-demethylation by CYP3A than R-(-)-VEN, but the degree of enantioselectivity was less than 2-fold higher than that of R-(-)-VEN. [7] CYP2D6 activity ranges considerably within a population and includes ultrarapid metabolizers (UMs), extensive metabolizers (EMs), intermediate metabolizers (IMs) and poor metabolizers (PMs). Eap et al. showed that quinidine, a CYP2D6 inhibitor, decreased the oral clearance of S-(+)-VEN and R-(-)-VEN by 4-fold and 12-fold, respectively. [13] The oral clearance of R-(-)-VEN was 9-fold higher ($p<0.005$) in the CYP2D6 EM group than in the PM group, whereas the oral clearance of S-(+)-VEN was only 2-fold higher in the former group ($p<0.05$), [13] suggesting that CYP2D6 has enantioselectivity in the O-demethylation of VENs. In addition, based on these findings, the plasma concentrations of S-(+)-VEN are less affected by CYP2D6 activity, whereas those of R-(-)-VEN vary depending on CYP2D6 activity. However, there has been no information on the enantioselectivity of VEN pharmacokinetics in depressed patients receiving VEN.

The important variants of CYP2D6 are *CYP2D6*2, *3, *4, *5, *10, *17* and **41*. Among them, **10* (40.87%) is found to be most important in Japanese individuals because of the high allele frequency in Asia. [14] A previous study discussed the effects of *CYP2D6*10* gene polymorphisms on VEN metabolism in healthy Japanese subjects. [15] However, no studies have examined the pharmacokinetics of the steady-state VEN enantiomers S-(+)-VEN and R-(-)-VEN in clinical settings. Therefore, we examined the pharmacokinetic effects of the *CYP2D6*10* genotype on the steady-state concentrations of the enantiomers of VEN.

Materials and methods

The study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology policy for experimental and clinical studies. [16]

Patients

Ninety-one blood samples were collected from 78 Japanese psychiatric patients. Of the 78 subjects, however, 3 subjects whose drug concentrations were below the limit of quantification (LOQ) were excluded, and the data from the remaining 75 patients (28 men, 47 women; age median, 49.0 years, range, 20-84 years; body weight median, 56.0 kg, range, 31.02–130.0 kg) were subjected to statistical analysis. Fifty subjects were psychiatric patients at Dokkyo Medical University Hospital, 17 subjects were psychiatric patients at Hirosaki University Hospital, 6 subjects were patients at Tochigi Prefectural Okamotodai Hospital, and 2 were patients at Sakura La Mental Clinic. To confirm the effect of only *CYP2D6*10*, we analyzed the subjects (n=71) with *CYP2D6**10 and without *CYP2D6*5* with valid data (Table 1). The dosages of VEN (venlafaxine HCl; Effexor®; Pfizer Japan Inc., Tokyo, Japan) administered were 37.5–225 mg/day (median, 75.00 mg/day) or 0.2885–7.253 mg/day/kg body weight (median, 1.442 mg/day/kg body weight) (Table 1). The same daily dose was maintained for more than 1 week to achieve steady-state plasma levels. If several samples had been taken from the same patient, priority was given to the most recently collected sample. Patients receiving neuroleptics or barbiturates as subordinate prescriptions were excluded. No additional antidepressants, including tricyclic antidepressants or selective serotonin reuptake inhibitors (e.g., paroxetine, fluvoxamine), were allowed. Benzodiazepines at common doses were allowed for sleep disturbances or anxiety. Out of 71 patients, 5 patients (7.04%) were smokers, and 66 (92.96%) were nonsmokers (Table 1). Patients with a severe general

medical condition or major abnormal laboratory findings were excluded. Psychiatric diagnoses were made using the DSM-5 criteria, resulting in a diagnosis of major depressive disorder in 57 patients, bipolar affective disorder in 1 patient, panic disorder in 3 patients, adjustment disorder in 1 patient, social anxiety disorder in 3 patients, generalized anxiety disorder in 1 patient, unspecified anxiety disorder in 4 patients, and dementia in 1 patient.

This study was approved by the Ethics Committees of Dokkyo Medical University Hospital, Hirosaki University Hospital, Tochigi Prefectural Okamotodai Hospital and Sakura La Mental Clinic. Written informed consent to participate in this study was obtained from the patients or their families prior to the study.

Blood sampling

Patients were maintained on racemic venlafaxine for over 1 week. Nine to thirteen hours after the last evening dose (postdose sampling time = 10.0 ± 1.47 h), 10 mL of venous blood was collected using Venoject® tubes containing heparin-Na (Terumo Japan, Tokyo, Japan) and centrifuged at 3000 × g for 10 min. Separated plasma and cell fractions were aliquoted and stored frozen at -80 °C until assayed.

Determination of drug concentration

Steady-state plasma concentrations of S-(+)-VEN, R-(-)-VEN, S-(+)-ODV, and R-(-)-ODV were measured using stereoselective liquid chromatography for mirtazapine enantiomers described by Paus et al. with minor modifications. [17] The assay standards of S-(+)-VEN, R-(-)-VEN, S-(+)-ODV, and R-(-)-ODV were purchased from Santa Cruz, Inc. (Santa Cruz, CA, USA). The high-performance liquid chromatography (HPLC)

system consisted of an LC-10A pump system (Shimadzu Corporation, Kyoto, Japan), an AS-(+)-8020 autosample processor (Tosoh Corporation, Tokyo, Japan), and an SPD-10A ultraviolet detector (Shimadzu Corporation, Kyoto, Japan) equipped with an Astec Chirobiotic V stainless column, 250 × 4.6 mm, 5-μm particle size (Supelco, PA, USA). The HPLC system was set to a flow rate of 0.6 mL/min, and the solvent (mobile phase) was a methanol-ethanol-0.02 mol/L potassium dihydrogen phosphate solution (20:10:70, vol/vol/vol).

One hundred microliters of 8-OH-mirtazapine (1 μg/mL, internal standard), 0.5 mL of standard buffer solution at a pH of 10 (Nacalai Tesque, Kyoto, Japan), and 3.5 mL n-heptane-chloroform (70:30, vol/vol) were added to 1 mL of heparinized plasma. Extraction was performed on a rotary shaker for 10 min. After centrifugation (10 min, 3000 × g), the organic phase was transferred to a new tube and evaporated to dryness by centrifugal vacuum evaporation. The residue was dissolved in 0.3 mL of the mobile phase, and 100 μL was injected into the HPLC system.

The LOQ of each enantiomer of VEN and ODV was 2.5 ng/mL. Measured concentrations of less than the LOQ were considered to be 2.5 ng/mL. Linearity was confirmed between 5 ng/mL and 150 ng/mL for S-(+)-VEN, R-(-)-VEN, S-(+)-ODV, and R-(-)-ODV. The intraassay coefficients of variation for S-(+)-VEN, R-(-)-VEN, S-(+)-ODV, and R-(-)-ODV were 2.3–2.5%, 2.2–2.7%, 2.2–2.4%, and 2.2–2.3%, respectively. The interassay coefficients of variation for S-(+)-VEN, R-(-)-VEN, S-(+)-ODV, and R-(-)-ODV were 5.6–8.6%, 5.9–8.5%, 5.4–7.8%, and 5.2–8.1%, respectively.

CYP2D6 genotyping

DNA was isolated from the cell fraction of each blood sample using a QIAamp Blood

Kit (QIAGEN Inc., Valencia, CA). The *CYP2D6*1, CYP2D6*2* and *CYP2D6*10* alleles were identified using the mutation-specific polymerase chain reaction (PCR) amplification method described by Johansson et al. [18]. The *CYP2D6*5* allele was identified using the long-PCR analysis described by Steen et al. [19]

Statistical analysis

Three subjects whose plasma levels of S-(+)-VEN, R-(-)-VEN, S-(+)-ODV and R-(-)-ODV were below the LOQ were classified as nonadherent and were excluded from analysis, resulting in a total of 75 patients. To confirm the effect of only *CYP2D6*10*, we analyzed the subjects without *CYP2D6*5* with valid data (Table 1).

The dosage of VEN administered, the plasma concentrations of S-(+)-VEN, R-(-)-VEN, S-(+)-ODV, and R-(-)-ODV (corrected for dose and body weight), and the S-(+)-ODV/S-(+)-VEN and R-(-)-ODV/R-(-)-VEN ratios among subjects with different numbers of mutant alleles of *CYP2D6*10* were compared using the Kruskal-Wallis test. The Kolmogorov-Smirnov test was used to assess the normality of the distribution of the data. The chi-square and Kruskal-Wallis tests and one-way analysis of variance (ANOVA) were performed to compare sex, age, smoking status, and body weight among genotypes. Stepwise multiple regression analysis was performed to analyze the relationship between independent variables (sex, age, smoking status, and number of mutant alleles of *CYP2D6*10*) and subject-dependent variables (plasma concentrations of S-(+)-VEN, R-(-)-VEN, S-(+)-ODV, and R-(-)-ODV (all corrected for dose and body weight) and the S-(+)-ODV/S-(+)-VEN and R-(-)-ODV/R-(-)-VEN ratios). Dummy variables were given for sex (male=0, female=1) and smoking status (nonsmoker=0, smoker=1) as independent variables. All statistical tests were two-tailed, and p values of <0.05 were considered

189 significant. Statistical analyses were conducted using IBM SPSS statistics software
190 version 26.0 (Japan IBM, Tokyo, Japan) and GraphPad Prism® version 8.42 (GraphPad
191 Software Inc., San Diego, CA, USA).
192
193 **Results**
194 The CYP2D6 genotypes were determined for each of the 75 subjects: *CYP2D6*1/*1*
195 (n = 23), *CYP2D6*1/*2* (n = 11), *CYP2D6*2/*2* (n = 1), *CYP2D6*1/*5* (n = 2),
196 *CYP2D6*1/*10* (n = 17), *CYP2D6*2/*10* (n = 7), *CYP2D6*10/*10* (n = 12), and
197 *CYP2D6*5/*10* (n = 2).
198 Demographic characteristics, dosage of VEN administered, and plasma concentrations
199 of enantiomers of VEN and its metabolites are shown in Table 1.
200 Positive and significant relationships between the daily dose of VEN (corrected for
201 body weight) and the plasma concentrations of S-(+)-VEN ($r=0.452$, $p<0.001$), R-(-)-
202 VEN ($r=0.450$, $p<0.001$), S-(+)-ODV ($r=0.390$, $p=0.001$) and R-(-)-ODV were found
203 ($r=0.326$, $p=0.006$) (Figure 2).
204 The plasma levels of S-(+)-VEN and R-(-)-VEN seemed to be higher in subjects with
205 two mutant alleles of CYP2D6 (22.5 ng/mL/mg/kg and 10.08 ng/mL/mg/kg, respectively)
206 than in those with no mutant alleles (10.3 ng/mL/mg/kg and 3.2 ng/mL/mg/kg,
207 respectively); there was a significant difference in the S-(+)-VEN but not in the R-(-)-
208 VEN (S-(+)-VEN, $p=0.048$; R-(-)-VEN, $p=0.054$, Kruskal-Wallis test) (Table 2). The
209 plasma levels of R-(-)-ODV (corrected for dose and body weight) appeared to be lower
210 in subjects with two mutant alleles of CYP2D6 (R-(-)-ODV=12.9 ng/mL/mg/kg) than in
211 those with no mutant alleles (R-(-)-ODV=34.9 ng/mL/mg/kg); however, the difference
212 was not significant (R-(-)-ODV, $p=0.216$, Kruskal-Wallis test) (Table 2).

The S-(+)-ODV/S-(+)-VEN and R-(-)-ODV/R-(-)-VEN ratios decreased as the number of CYP2D6 mutant alleles increased (S-(+)-ODV/S-(+)-VEN, p=0.003; R-(-)-ODV/R-(-)-VEN, p=0.005, Kruskal-Wallis test) (Table 2). A post hoc test revealed that the S-(+)-ODV/S-(+)-VEN and R-(-)-ODV/R-(-)-VEN ratios in subjects with no mutant alleles of CYP2D6 were significantly higher than those in subjects with one mutant allele (p=0.006 and p=0.015, respectively; Mann-Whitney U test) (Figure 3). Additionally, the S-(+)-ODV/S-(+)-VEN and R-(-)-ODV/R-(-)-VEN ratios in subjects with no mutant alleles of CYP2D6 were significantly higher than those in subjects with two mutant alleles (p=0.008 and p=0.005, respectively; Mann-Whitney U test) (Figure 3). On the other hand, a post hoc test revealed that the S-(+)-ODV/R-(-)-ODV ratio in subjects with no mutant alleles of CYP2D6 was significantly lower than that in subjects with one mutant allele (p=0.025, Mann-Whitney U test, Figure 4). Additionally, the S-(+)-ODV/R-(-)-ODV in subjects with no mutant alleles of CYP2D6 was significantly lower than that in subjects with two mutant alleles (p=0.025, Mann-Whitney U test) (Figure 4).

The number of mutant alleles was a significant factor associated with the corrected plasma concentration of S-(+)-VEN (p=0.007) in stepwise multiple regression analysis (Table 3). Sex was a significant factor associated with the corrected plasma concentration of S-(+)-ODV (p=0.0011) (Table 3). The number of mutant alleles was a significant factor associated with the S-(+)-ODV/S-(+)-VEN ratio (p=0.008) and the R-(-)-ODV/R-(-)-VEN ratio (p=0.008) (Table 3). Sex and the number of mutant alleles were significantly correlated with the S-(+)-ODV/R-(-)-ODV ratio (p=0.043 and 0.010, respectively) (Table 3).

To confirm the effect of another CYP2D6 genotype, we analyzed subjects (n=4) with the *CYP2D6*5* allele using the same procedures. The results including subjects (n=75)

with either *CYP2D6*5* or *10 showed the same tendencies as described above.

Discussion

The results of this study showed that the R-(-)-ODV/R-(-)-VEN ratio was significantly lower in the CYP2D6 mutant allele group. In addition, multiple regression analysis showed a significant correlation between the number of *CYP2D6*10* mutant alleles and the R-(-)-ODV/R-(-)-VEN ratio. Although the result did not reach statistical significance, an increasing trend in the plasma concentration of R-(-)-ODV with the number of *CYP2D6*10* mutant alleles was observed. Therefore, these findings suggest that the main pathway of the metabolism of R-(-)-VEN to R-(-)-ODV is affected by the *CYP2D6*10* gene polymorphism in Japanese subjects. On the other hand, there were significant differences in the S-(+)-ODV/S-(+)-VEN ratio among CYP2D6 genotype groups, although there was no association between the number of *CYP2D6*10* mutant alleles and the S-(+)-ODV/S-(+)-VEN ratio. Multiple regression analysis revealed an association between the number of *CYP2D6*10* mutant alleles and the S-(+)-ODV concentration. Furthermore, an increasing trend in the plasma concentration of S-(+)-ODV with the number of *CYP2D6*10* mutant alleles was also observed. Therefore, the metabolism of S-(+)-VEN to S-(+)-ODV is, to some extent, also affected by the *CYP2D6*10* gene polymorphism, although no clear enantioselectivity of O-desmethylation of venlafaxine was found. These findings are in line with an in vitro study using yeast transformed with an expression plasmid containing human CYP2D6 cDNA showing that R-(-)-VEN and S-(+)-VEN are metabolized by CYP2D6, and that R-(-)-VEN shows 1.5- to 2-fold higher enantioselectivity than S-(+)-VEN. [7]

This is the first study to show the significant effect of *CYP2D6*10* on the O-

demethylation of the enantiomers of VEN in a clinical setting. However, because of interindividual variations in the enantiomers of VEN and ODV, factors other than *CYP2D6**10 should be investigated to predict plasma drug concentrations. Karlsson et al. investigated the significant effects of the gene polymorphisms *CYP2D6*3, *4, *5*, and **6* on the VEN metabolic ratio and the S/R ratio, showing that CYP2D6 had a significant effect on the ODV/VEN ratio (p=0.003). These results suggest that the CYP2D6 gene polymorphism affects the O-demethylation of VEN, particularly the stereoselective metabolism of CYP2D6 to R-(-)-VEN. [20] The O-demethylation rates (ODV/VEN ratio) of S-(+)-VEN and R-(-)-VEN were significantly affected by CYP2D6 gene polymorphisms, which is similar to the results of a previous study. However, the S/R ratio of VEN was affected by the CYP2D6 gene polymorphism in the previous study, while there was no significant difference in the S/R ratio of VEN but a significant effect on the S-(+)-ODV/R-(-)-ODV ratio in the present study. This may be because *CYP2D6*4, *5,* and **6* were targeted as CYP2D6 mutant alleles in previous studies, whereas this study targeted mainly *CYP2D6*10*.

Recently, a comprehensive review was published by Milosavljevic et al. Compared with the CYP2D6 EM group, significant but marginal increases in racemic VEN were observed in the intermediate (IM) plus PM group (8 studies; 716 patients), although the number of studies was not enough to determine this conclusion. However, this study did not analyze enantiomer of VEN. [21] Further pharmacokinetic studies regarding enantiomers of VEN are required to perform a meta-analysis of the association between enantiomers of VEN and CYP2D6 polymorphisms.

Gex-Fabry et al. measured stereoselective plasma concentrations of VEN in 35 depressed patients, classified them as nonresponders, transient responders, early

persistent responders, and delayed persistent responders, and compared them by VEN racemic concentration and enantiomeric ratio. The results showed that the shortening of the time to onset of therapeutic response was significantly correlated with an increase in VEN+ODV plasma concentration ($p=0.023$) or a decrease in the S-(+)-VEN/R-(-)-VEN ratio ($p<0.05$). [3] In the present study, *CYP2D6**10 mutated allele carriers tended to have a lower S-(+)-VEN/R-(-)-VEN ratio than noncarriers. This suggests that the *CYP2D6**10 mutated allele group may be more likely to achieve early therapeutic effects, while it may also affect the appearance of adverse events. Furthermore, Gex-Fabry et al. investigated interindividual variability in stereoselective plasma concentrations of VENs and noted that R-(-)-VEN was more susceptible to impairment of O-demethylation than S-(+)-VEN, suggesting that S-(+)-VEN and R-(-)-VEN exert clinically different pharmacological effects. [5] Therefore, the plasma concentrations of enantiomers of VEN may provide potential information.

Because of interindividual variations in the enantiomers of VEN and ODV in our study, factors other than CYP2D6 should be investigated to predict plasma drug concentrations. Karlsson et al. suggested that the S/R ratio of VEN was also significantly affected by not only CYP2D6 but also CYP2C19 gene polymorphisms. [20] They reported in their analysis of 94 forensic autopsy cases that CYP2C19 showed N-stereoselective metabolism of S-VEN, the S/R ratio of VEN was higher and the S/R ratio of ODV was lower in PM (CYP2C19*2 holding group). [20] This suggests that the association between CYP2C19/CYP2D6 genotypes and VENs can be quantified with sufficient precision to serve as a scientific basis for dosing recommendations based on CYP2D6/CYP2C19 genotypes. [20] Therefore, the influence of CYP2C19 gene polymorphisms on the pharmacokinetics of VEN, possibly its enantioselectivity, should

be examined in the Japanese population.

This study encompasses several limitations. First, we did not analyze all CYP2D6 gene polymorphisms, particularly the less frequent polymorphisms. Second, the results of a previous study suggested that the effect of age on VEN plasma concentrations is dependent on CYP gene polymorphisms, [22] which we did not examine in the present study. Third, this study did not analyze CYP2C19 gene polymorphisms and could not evaluate the effect of S-VEN on conversion to S-ODV. In patients without CYP2D6 activity, the other metabolic pathways may play an important role, and there are potential risks of drug interactions with inhibitors of CYP2C19 or CYP3A4. Fourth, we did not analyze metabolites other than those produced from the main metabolic pathway (e.g., DDV) or the pathway leading to glucuronic acid conjugates. Fifth, no evaluation scales, such as the Hamilton Depression Scale (HAM-D) or the Montgomery-Asberg Depression Rating Scale (MADRS), were investigated. We did not evaluate adverse events using the Udvalg for kliniske undersogelser (UKU) Side Effects Rating Scale, and clinical responses were not discussed, although enantioselective pharmacological function was suggested. Finally, the sample size might not be enough to avoid type I errors. Plasma levels of R-(-)-ODV (corrected for dose and body weight) appeared to be lower in subjects with two mutant alleles of CYP2D6 (R-(-)-ODV=12.9 ng/mL/mg/kg) than in those with no mutant alleles (R-(-)-ODV=34.9 ng/mL/mg/kg); however, the difference was not significant (R-(-)-ODV, p=0.216, Kruskal-Wallis test). Further research is needed to overcome these problems.

Conclusion

The *CYP2D6*10* gene polymorphism affects the enantioselective O-demethylation

rates of R-(-)-VEN and, to some extent, S-(+)-VEN in Japanese depressed patients, although the effect on the plasma concentrations of the enantiomers is not significant. This suggests that *CYP2D6*10* mutations affect the metabolism of R-(-)-VEN and S-(+)-VEN. Further studies are needed to examine how these findings affect clinical practice.

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Conflict of Interest Statement

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Figure legends

Figure 1: Structural formulae of the enantiomers of VEN and ODV. The metabolic pathways of VEN are also shown (modified in part from Otton et al.) (7)

Figure 2: Positive and significant relationships between the daily dose of VEN (corrected for body weight) and the plasma concentrations of S-(+)-VEN ($r=0.457$, $p<0.001$), R-(-)-VEN ($r=0.465$, $p<0.001$), S-(+)-ODV ($r=0.412$, $p<0.001$) and R-(-)-ODV were found ($r=0.325$, $p=0.004$).
Blank circles indicate subjects with the *CYP2D6*1/*1, CYP2D6*1/*2* and *CYP2D6*2/*2* genotypes, blank squares indicate subjects with the *CYP2D6*1/*10* and *CYP2D6*2/*10* genotypes, and filled circles indicate subjects with the *CYP2D6*10/*10* genotype. Dotted lines indicate regression lines.

Figure 3: Relationship between *CYP2D6*10* genotype and S-(+)-ODV/S--(+)-VEN (upper panel) and R-(-)-ODV/R-(-)-VEN (lower panel) ratios. Horizontal bars show the median value of each group. Blank circles, blank squares and filled circles indicate patients with the *CYP2D6*1/*1, CYP2D6*1/*2* and *CYP2D6*2/*2* genotypes, the *CYP2D6*1/*10* and *CYP2D6*2/*10* genotypes, and the *CYP2D6*10/*10* genotypes, respectively. After the Kruskal-Wallis test, post hoc analysis was performed using the Mann-Whitney U test.

Figure 4: Relationship between CYP2D6 genotype and S-(+)-ODV/R- (-)-ODV ratio. Horizontal bars show the median value of each group. Blank circles, blank squares and filled circles indicate patients with the *CYP2D6*1/*1, CYP2D6*1/*2* and *CYP2D6*2/*2*

453 genotypes, the *CYP2D6*1/*10* and *CYP2D6*2/*10* genotypes, and the *CYP2D6*10/*10*
454 genotypes, respectively. After the Kruskal-Wallis test, post hoc analysis was performed
455 using the Mann-Whitney U test.
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Table 1 Demograhic characteristics, dosage of venlafaxine administered, plasma concentrations of enantiomers of venlafaxine and its metabolites.

Parameter	
Sex(male/female)	27/44
Age(years)	50.1±18.3
Body weight(kg)	60.3±17.7
Daily dose of VEN(mg/day)	97.2±60.6
Daily dose of VEN corrected for body weight(ymg/day/kg body weight)	1.8±1.3
Smoking status(non-smokers/smokers)	66/5
S-(+)-VEN(ng/mL)	23.1±26.3
R-(-)-VEN(ng/mL)	12.7±15.5
S-(+)-ODV(ng/mL)	41.8±46.6
R-(-)-ODV(ng/mL)	44.2±50.1
S-(+)-ODV/S-(+)-VEN	2.7±2.4
R-(-)-ODV/R-(-)-VEN	6.5±8.4
S-(+)-VEN/R-(-)-VEN	2.2±1.6
S-(+)-ODV/R-(-)-ODV	1.2±0.5
S/R-ODV/VEN	0.7±0.6

abbreviations:VEN; venlafaxine, ODV; O-desmethylvenlafaxine

Data are expressed as mean±SD.

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Table 2 Relationship between number of *CYP2D6*10* alleles and corrected plasma levels of VEN, ODV, S-(+)-VEN, R-(-)-VEN, S-(+)-ODV, and R-(-)-ODV (all corrected for dose and body weight)(n=71).

	Number of *CYP2D6*10* alleles			
Parameter	**0**	**1**	**2**	**P-value**
Sex(male/female)[a]	13/22	8/16	7/5	0.282
Age(years)[b]	46(20-84)	46(26-77)	68.5(22-83)	0.103
Body weight(kg)[c]	56.0(31.0-100.0)	57.6(38.2-99.3)	52.8(42.0-130.0)	0.858
Daily dose of VEN(mg/day)[c]	75(37.5-225)	75(37.5-150)	56.3(37.5-225)	0.488
Daily dose of VEN corrected for body weight(mg/day/kg body weight)[c]	1.6(0.5-7.3)	1.1(0.6-3.6)	1.0(0.3-4.2)	0.631
Smoking status (non-smokers/smokers)[a]	34/1	20/4	12/0	0.073
S-(+)-VEN(ng/mL/mg/kg)[c]	10.3(0.8-63.9)	9.8(1.4-63.9)	22.5(2.7-83.7)	**0.048**
R-(-)-VEN(ng/mL/mg/kg)[c]	3.2(0.3-57.9)	4.7(1.6-22.8)	10.8(1.6-52.7)	0.054
S-(+)-ODV(ng/mL/mg/kg)[c]	24.0(1.9-75.3)	9.5(1.4-92.5)	18.7(7.1-77.6)	0.303
R-(-)-ODV(ng/mL/mg/kg)[c]	34.9(1.7-84.7)	9.8(2.5-83.0)	12.9(4.5-71.0)	0.216
S-(+)-ODV/S-(+)-VEN[c]	2.9(0.5-12.4)	1.5(0.5-4.9)	1.0(0.3-8.6)	**0.003**
R-(-)-ODV/R-(-)-VEN[c]	6.8(1.1-42.8)	1.6(0.4-16.1)	1.5(0.2-12.0)	**0.005**
S-(+)-VEN/R-(-)-VEN[c]	2.1(0.2-8.3)	1.6(0.1-7.9)	1.7(1.0-7.6)	0.569
S-(+)-ODV/R-(-)-ODV[c]	0.8(0.6-2.7)	1.2(0.1-2.1)	1.5(0.6-3.0)	**0.029**
S/R-ODV/VEN[c]	1.8(0.2-11.4)	1.2(0.6-11.9)	1.3(0.7-3.0)	0.054

abbreviations: VEN; venlafaxine corrected for dose and body weight , ODV; O-desmethylvenlafaxine corrected for dose and body weight

Data are expressed median (range).

aP-value was calculated by the Chi-square test.

bData are expressed as median (range), and P-value was calculated by analysis of variance(ANOVA).

cData are expressed as median (range), and P-value was calculated by Kruskal-Wallis test.

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Table 3: The result of standardized partial correlation coefficient in multiple regression analyses.

Independent variable	S-(+)-VEN (ng/mL/mg/kg)	R-(-)-VEN (ng/mL/mg/kg)	S-(+)-ODV (ng/mL/mg/kg)	R-(-)-ODV (ng/mL/mg/kg)	S-(+)-ODV / S-(+)-VEN	R-(-)-ODV / R-(-)-VEN	S-(+)-VEN / R-(-)-VEN	S-(+)-ODV / R-(-)-ODV	S / R-ODV / VEN
Sex	-	-	-0.301(0.011)	-	-	-	-	0.237(0.043)	-
Age (years)	-	-	-	-	-	-	-	-	-
Smoking status	-	-	-	-	-	-	-	-	-
Number of mutated alleles	0.317(0.007)	-	-	-	- 0.311(0.008)	- 0.314(0.008)	-	0.303(0.010)	-

abbreviations: VEN; venlafaxine corrected for dose and body weight , ODV; O-desmethylvenlafaxine corrected for dose and body weight

Stepwise multiple regression analysis was performed to analyze the relationship between independent variables (sex, age, smoking habit and number of mutant allele and subject-dependent variables (plasma concentrations of S-(+)-VEN, R-(-)-VEN, S-(+)-ODV and R-(-)-ODV (all corrected for dose and body weight) and the S-(+)-ODV/S-(+)-VEN, R-(-)-ODV/R-(-)-VEN, S-(+)-VEN/R-(-)-VEN and S-(+)-ODV/R-(-)-ODV ratio. Dummy variables were given for sex (male=0, female=1) and smoking habit (nonsmoker=0, smoker=1) as independent variables.

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