that differences in religion or race between Caucasians and Asians may result in less sensitivity of detection, such that a cultural difference cannot be excluded.

Similar to our finding, there was no difference between EM and IM in Japanese subjects [18]. Only three genotypes of *10/*10 and *1/*1 and *1/*10 were identified in this study, although the frequency of mutations other than CYP2D6*10 which predominantly affects CYP2D6 activity is relatively high in Japanese, e.g. CYP2D6*5. Therefore, this negative association between CYP2D6 activity and personality traits in Japanese was first demonstrated by our detailed study.

In conclusion, the present study demonstrated no significant association between CYP2D6 activity and personality trait in Japanese subjects. A significant factor affecting this outcome is the extremely rare frequency of subjects with outlying activity, leading to low interindividual variability in CYP2D6 activity in Japanese subjects.

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**Regular Article**

# Different serum concentrations of steady-state valproic acid in two sustained-release formulations

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**Abstract** Recently a new sustained-release formulation of valproic acid has been developed in Japan. The sustained-release mechanism of the new formulation was different from the conventional formulation. The aim of the present study was to compare the pharmacokinetic characteristics of valproic acid in two sustained-release formulations. Different sustained-release formulations of valproic acid (Depakene R and Selenica R) were administered in a randomized cross-over fashion in repeated doses in 24 psychiatric patients. After ≥4 weeks administration of valproic acid once daily, blood samples were taken just before (0 h) and 8, 12, 24 h after the morning dose. Blood sampling was performed in the same manner in the same patients 4 weeks after switching from one to the other formulation of valproic acid. Serum concentrations of valproic acid at 0 h (50.7 ± 19.4 vs 44.9 ± 21.8 μg/mL, $P < 0.05$) and 24 h (52.3 ± 19.54 vs 6.2 ± 22.2 μg/mL, $P < 0.05$) were significantly higher during Selenica R than during Depakene R treatment, whereas the serum concentration of valproic acid at 8 h (49.7 ± 19.2 vs 62.4 ± 25.6 μg/mL, $P < 0.01$) was significantly lower during Selenica R treatment than during Depakene R treatment. Serum concentrations of valproic acid at 12 h were not different. The present study demonstrated that steady-state serum concentrations were different because of the different dissolution profiles. When a prescription for valproic acid is switched from one drug to the other, prescribers should be aware that the therapeutic drug monitoring data are not consistent.

**Key words** dissolution, steady-state serum concentration, sustained-release formulation, valproic acid.

## INTRODUCTION

Successful long-term treatment of patients with epilepsy requires selection of an appropriate anti-epileptic regimen, optimal dosing and patient compliance.[1] Recent advances in the choice of treatment options are transforming the global management of these patients.[1] Although the achievement of seizure freedom remains the primary goal of any anti-epileptic treatment, issues associated with drug acceptability and tolerability, and with quality of life have gained increasing attention as major determinants of ultimate therapeutic success.[1,2] Sustained-release formulations of anti-epileptic drugs can be very helpful in achieving treatment objectives. Stable serum levels without marked peak-to-trough fluctuations, reduced frequency of dosing and the possibility of dosing flexibility may all improve compliance, patient satisfaction and ultimately quality of life.[3–5]

Valproic acid has been widely used in the last decade and is now considered a relatively safe and effective anticonvulsant agent.[6] Recently, several investigators have proposed its use in the treatment of anxiety, alcoholism and mood disorders.[7,8] Valproic acid is characterized by dose-limited absorption, non-linear plasma protein binding, and multiple metabolic pathways of elimination.[6,9,10] Once absorbed, valproic acid is largely bound to plasma proteins and has a relatively small volume of distribution. Its concentration in

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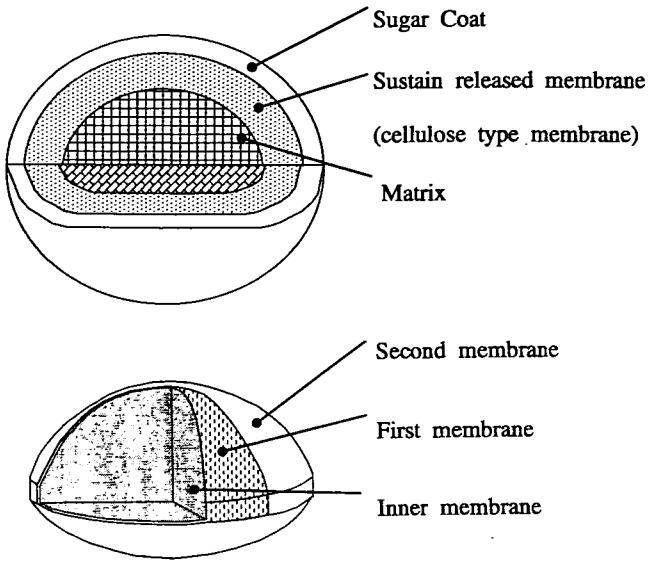


**Figure 1.** Cross-section of the slow-release Depakene R and Selenica R tablets.

cerebrospinal fluid is approximately one-tenth that in plasma and is directly correlated with the concentration found in tears.[10] At therapeutic doses, valproic acid half-life varies from 10 to 20 h in adults, while it is significantly shorter (6–9 h) in children.[6,10] Valproic acid undergoes extensive liver metabolism.[10,11] Numerous metabolites have been positively identified and there is reasonable evidence that several of them contribute to its pharmacological and toxic actions.[10,11]

Valproic acid is available in different dosage forms for parenteral and oral use. All available oral formulations are almost completely bioavailable, but they differ in dissolution characteristics and absorption rates. The sustained-release formulation (Depakene R, Kyowa Hakko Kogyo Co. Ltd, Tokyo, Japan) can therefore be given once or twice daily.[12,13] The tablet core consists of a matrix structure that is covered with the sustained-released membrane. The elution of valproic acid is controlled as the substance passes through the core of the matrix structure and further through the sustained-released membrane (Fig. 1).

Recently another sustained-release formulation containing 200 mg valproic acid (Selenica R, Nikken Chemicals Co. Ltd, Tokyo, Japan) has been developed and available in Japan.[14] Selenica R has a double-coating system that provides a mechanism that is different from the conventional sustained-release formulation (Fig. 1). We therefore compared the pharmacokinetic parameters in two formulations of valproic acid in psychiatric patients.

## METHODS

The Ethics Committee of Hirosaki University School of Medicine approved this study protocol, and written informed consent was been obtained from each participant before any examinations.

The subjects were 24 patients (15 male, nine female) receiving valproic acid, who were diagnosed as having bipolar disorders and schizophrenia. The mean (and range) of age and bodyweight were 51 years (21–68 years) and 58 kg (38–98 kg), respectively. Twelve patients received valproic acid at 08.00 hours in the conventional sustained-release formulation, Depakene R, for at least 4 weeks, and the other 12 patients received the valproic acid at 08.00 hours in the new sustained-release formulation, Selenica R for at least 4 weeks. Co-administered medications were as follows: risperidone ($n = 10$), olanzapine ($n = 8$), haloperidol ($n = 4$), zotepine ($n = 3$), levomepromadine ($n = 3$), lithium ($n = 2$), flunitrazepam ($n = 12$), brotizolam ($n = 8$), diazepam ($n = 5$), biperiden ($n = 7$), and sennoside ($n = 13$). These medications were fixed throughout the study period. After ≥4 weeks of administration, blood sampling (5 mL each) was performed just before and 8, 12 and 24 h after administration. The sustained-release formulation of valproic acid administered to the patients was switched to the other type. Four weeks after the switching, blood sampling (5 mL each) was performed in the same way. Clinical global impression (CGI) score for patient psychiatric condition was monitored at blood sampling.[15] There was no difference between administration days of Depakene R and Selenica R.

The serum samples were frozen and kept at –20°C until analysis. Serum concentrations of valproic acid were quantified with enzyme immunoassay (EIA). The detection limit was 1.0 μg/mL. The inter- and intra-assay coefficient of variation (CV) for plasma concentrations of valproic acid were <5.5% for all quality control concentrations.

Pharmacokinetic parameters were determined on a non-compartment model with WinNonLin software (Pharsight, Cary, NC, USA). Apparent volume of distribution (Vd/F), absorption constant (Ka), elimination constant (Ke), lag time (Tlag) were calculated as first parameters. Area under the drug concentration–time curve (AUC), apparent clearance (CL/F), time to peak concentration ($T_{max}$), peak concentration ($C_{max}$), peak concentration at steady state ($Css_{max}$) and minimum concentration at steady state ($Css_{min}$) were estimated as second parameters.

Statistical analysis was performed by the use of repeated measures of ANOVA in SPSS (SPSS, Chicago, IL, USA). Post-hoc analysis was done using Bonferroni

correction with paired *t*-test. All tests were two-tailed and were considered to be statistically significant for $P < 0.05$.

## RESULTS

There were no patients who had an adverse event after switching valproic acid formulation. Daily change in serum concentration of valproic acid during treatments with Depakene R and Selenica R are shown in Fig. 2. Serum concentrations of valproic acid at 0 h (44.9 ± 21.8 vs 50.7 ± 19.4 μg/mL, $P < 0.05$) and 24 h (46.2 ± 22.2 vs 52.3 ± 19.5 μg/mL, $P < 0.05$) during Depakene R treatment were significantly lower than those during Selenica R treatment (Fig. 2). Serum concentration of valproic acid at 8 h (62.4 ± 25.6 vs 49.7 ± 19.2 μg/mL, $P < 0.01$) during Depakene R treatment was significantly higher than the concentration during Selenica R treatment (Fig. 2). However, no difference was found in steady-state serum concentration of valproic acid at 12 h (62.4 ± 25.6 vs 49.7 ± 19.2 μg/mL, n.s.; Fig. 2).

The results of pharmacokinetics parameters simulated by the Pharmacokinetic Analysis program (WinNonLin) are shown in Table 1. The Tlag (1.3 ± 1.4 vs 6.8 ± 1.6 h, $P < 0.001$) and Tmax (9.4 ± 2.2 vs 15.8 ± 2.1 h, $P < 0.001$) during Selenica R treatment were significantly later than during Depakene R treatment. No difference in the estimated $C_{ss}$max and $C_{ss}$min were found between the two formulations (Table 1).

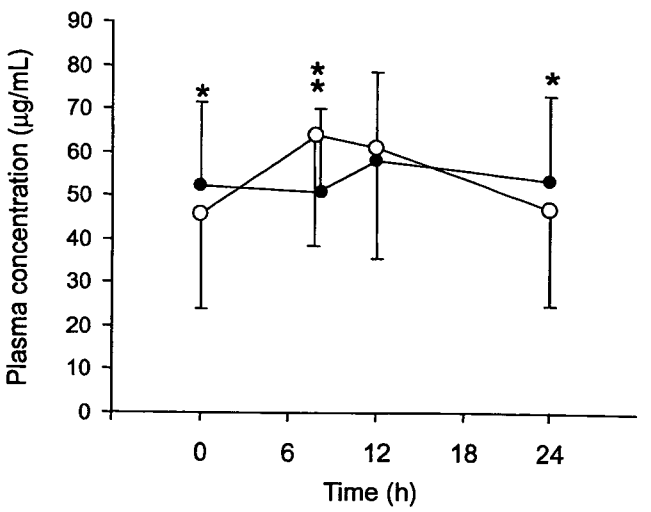


**Figure 2.** Steady-state serum concentration–time curves after repeated oral doses of Depakene R and Selenica R. Error bars indicate SD. (○), Depakene R; (●), Selenica R. *$P < 0.05$, **$P < 0.01$.

**Table 1.** Estimated pharmacokinetic parameters in patients receiving repeated doses of valproate in two sustained-release formulations ($n = 24$)

| Parameters | Depakene R (mean ± SD) | Selenica R (mean ± SD) | *P* |
|---|---|---|---|
| Vd/F (L) | 5.45 ± 0.59 | 7.22 ± 2.14 | <0.01 |
| Ka (1/h) | 0.092 ± 0.063 | 0.068 ± 0.026 | <0.05 |
| Ke (1/h) | 0.075 ± 0.022 | 0.073 ± 0.026 | NS |
| T lag (h) | 1.3 ± 1.4 | 6.8 ± 1.6 | <0.001 |
| AUC (h·μg/mL) | 1334 ± 531 | 1305 ± 467 | NS |
| CL/F (L/h) | 0.50 ± 0.32 | 0.49 ± 0.26 | NS |
| Tmax (h) | 9.4 ± 2.2 | 15.8 ± 2.1 | <0.001 |
| Cmax (μg/mL) | 43 ± 23 | 46 ± 19 | NS |
| Cssmax (μg/mL) | 65 ± 20 | 60 ± 20 | NS |
| Cssmin (μg/mL) | 35 ± 12 | 31 ± 11 | NS |

AUC (0–∞), area under the curve from 0 to infinity; Cl/F, apparent total clearance; $C_{max}$, peak concentration; $Css_{max}$, peak concentration in the steady state; $Css_{min}$, trough concentration in the steady state; Ka, absorption constant; Ke, elimination constant; Tlag, lag time; $t_{max}$, time to peak concentration in serum; Vd/F, apparent volume of distribution.

There was no difference in CGI scores between Depakene R and Selenica R (3.9 ± 1.2 vs 4.0 ± 1.3, n.s.).

## DISCUSSION

Bioequivalence is an important component of the development of anti-epileptic drugs.[16] Development of new formulations after the original testing of any drug requires demonstration that the compounds are therapeutically equivalent and additional efficacy studies may not be required. Sustained-release formulations may reduce toxicity with a lower maximum blood concentration ($C_{max}$) and improve efficacy with a higher minimum blood concentration ($C_{min}$). Obtaining an equivalent AUC while slowing gastrointestinal transit and avoiding food effects and dose dumping among a population with epilepsy with individual variability requires extensive engineering of the formulation.[16]

The results of the preliminary study using a single oral dose showed no differences in $C_{max}$ or AUC of valproic acid between the new and conventional formulations,[17] suggesting that these two formulations are bioequivalent. However, absorption speed, that is, Tlag, and hence $T_{max}$ were very different between the two formulations in the single oral dose study. These findings suggest that valproic acid dissolved from the Depakene R tablet appears earlier in the blood and brain than that from the Selenica R tablet. These

findings resulted from the different mechanisms of the sustained release between Depakene R and Selenica R, namely, the elution of valproic acid in Depakene R is controlled in such a way that the substance passes through the core of a matrix structure and further through the sustained-released membrane, while Selenica R has a mixed membrane composed of ethylcellulose and methacrylate copolymer-L (Fig. 1).[14]

The result of a repeated-dose study in psychiatric patients showed significant differences in steady-state serum concentration of valproic acid at 0, 8 or 24 h after administration. Steady-state serum concentrations at 0 and 24 h during Selenica R were higher than those during Depakene R, while the steady-state serum concentration at 8 h during Selenica R was lower than during Depakene R. The difference may be explained by the difference in Tlag, and hence $T_{max}$, observed in the single-dose study and the difference in dissolution. As a result, the fluctuation of serum valproic acid concentration in the steady state during Selenica R is shifted to 5–6 h later compared with Depakene R. Because blood sampling for therapeutic drug monitoring (TDM) of valproic acid is generally recommended just before administration, previous therapeutic drug monitoring data are not available when a sustained-release preparation is switched to another formulation. Simulation curve of serum concentration of valproic acid in the steady state including $C_{ss}$max and $C_{ss}$min from 1-point sampling data should be available for TDM.

In contrast, no changes in the mental status of psychiatric patients were observed between Selenica R treatment and Depakene R treatment in the present study. This is explained by similar exposure (AUC) in the two formulations. Thus, when a sustained-release preparation is switched to another formulation, the inconsistency of TDM data does not necessarily mean alternation in clinical response.

Many medications were concomitantly administered with valproic acid in Selenica R and Depakene R formulations. However, it is unlikely that the difference in the two formulations led to the significant difference in the results of the present study because co-administered medications were fixed throughout the study period.

Steady-state serum concentrations of valproic acid in the present study were slightly lower than the therapeutic concentration for epilepsy. If doses are escalated up to therapeutic levels, we concluded that the significant difference of TDM data does not necessarily mean alternation in clinical response, because the drug $C_{ss}$max or exposure (AUC) in the two formulations probably associated with pharmacodynamic action are not different.

In order to improve compliance, a small tablet is preferable for patients, particularly for children. The size of the current commercial once-a-day controlled-release tablet is as follows: Depakote Tablet (Abbott Laboratories, Abbott Park, IL, USA; containing 500 mg divalproex sodium), 0.9 × 1.9 cm, 1.0 g; Depakene R 200 mg Tablet, 0.66 × 1.06 cm, 0.52 g; and Selenica R 200 mg Tablet, 0.50 × 0.92 cm, 0.25 g. From the point of view of easy swallowing, Selenica R Tablet may have the advantage.

In conclusion, the present study demonstrated that steady-state serum concentrations of valproic acid were different because of the different dissolution profiles. When drug prescription for valproic acid is switched from one drug to the other, prescribers should know that TDM data are not consistent.

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# Association between major Multidrug Resistance 1 (MDR1) gene polymorphisms and plasma concentration of prolactin during risperidone treatment in schizophrenic patients

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**Abstract**

An in vitro study has suggested that risperidone is a substrate of P-glycoprotein, which is coded by *MDR1* gene. The rate of P-glycoprotein efflux transport can mediate brain penetration of lipophilic drugs. We therefore studied the effects of major polymorphisms of *MDR1* gene on plasma concentrations of prolactin. Subjects included *175* schizophrenic patients (68 males, *107* females) who were receiving 3 mg of risperidone twice daily for at least 4 weeks. Sample collections were conducted 12 h after the bedtime dosing. The plasma concentrations of prolactin in females were significantly higher than in males (54.3±27.2 versus 126.8±70.2 ng/ml, $p<0.001$). There was no difference in mean (±SD) plasma concentration of prolactin between *C3435T* genotypes [*C/C*, *C/T*, *T/T*; 62.3±33.3, 49.4±15.6, 53.2±33.2 ng/ml, ns] or *G2677T/A* genotypes [*G/G*, *G/T* or *A*, *T* or *A/T* or *A*; 58.0±27.7, 58.5±35.0, 46.1±20.7 ng/ml, ns] in males nor between *C3435T* genotypes (123.6±65.0, 127.8±79.2, 130.4±49.7 ng/ml, ns) or *G2677T/A* genotypes (123.3±67.0, 97.7±71.2, 144.9±69.9 ng/ml, ns) in females. Multiple regression analyses including plasma drug concentration and age revealed that plasma concentration of prolactin correlated with gender (standardized beta=0.540, $p<0.001$) and negatively with age (standardized beta=−0.183, $p<0.01$). No correlations were found between prolactin concentration and *MDR1* genotypes. These findings suggest that prolactin concentrations in females are much higher than in males but the major *MDR1* variants are not associated with the plasma concentration of prolactin.


*Keywords:* Genotype; *MDR1*; Prolactin; Risperidone

## 1. Introduction

Hyperprolactinemia has been assumed to be an inevitable consequence of treatment with conventional antipsychotic agents (Petty, 1999). Major effects of hyperprolactinemia in women are amenorrhea, galactorrhea, cessation of normal cyclic ovarian function, loss of libido, occasional hirsutism and long-term risk of osteoporosis (Rubin, 1987; Petty, 1999). The effects in men include impotence, loss of libido, and hypospermatogenesis (Rubin, 1987; Petty, 1999). On the other hand, the prolactin response to antipsychotic drugs has been regarded as an index of antidopaminergic activity of antipsychotic drugs (Rubin, 1987). This has been partially confirmed by a PET study indicating a close relationship between prolactin response and dopamine $D_2$ receptor occupancy (Nordström and Farde, 1998). The prolactin response to antipsychotic drugs should therefore be evaluated not only from a clinical but also a pharmacological point of view.

Risperidone has a potent serotonin $5HT_2$ and a milder dopamine $D_2$ antagonistic activity (Schotte et al., 1995). However, adverse effects associated with hyperprolactinemia (Dickson et al., 1995; Kim et al., 1999; Popli et al., 1998) have been reported. With the exception of a retrospective analysis (Kleinberg et al., 1999), it has been demonstrated that the

*Abbreviations:* MDR1, Multidrug resistance 1; LC-MS-MS, Liquid chromatography-mass spectrometry-mass spectrometry; CV, coefficient variation; ANOVA, analysis of variance.

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pronounced hyperprolactinemia (Shiwach and Carmody, 1998; Caracci and Ananthamoorthy, 1999; David et al., 2000; Lavalaye et al., 1999; Yasui-Furukori et al., 2002) is consistently induced by risperidone treatment compared with conventional antipsychotic agents. However, inter-individual variability in plasma concentration during risperidone treatments has been implied (Yasui-Furukori et al., 2002). In addition, among women, the risperidone *dose* did not correlate with adverse events, nor did the adverse events correlate with endpoint prolactin levels (Kleinberg et al., 1999).

P-glycoprotein, which is encoded by *MDR1* gene, is involved in the acquisition of multidrug resistance phenotypes not only in cancer cells but also in normal tissues such as brain, kidney, liver and intestine (Thiebaut et al., 1987). Its major physiologic role is to serve as a barrier to entry and as an efflux mechanism for xenobiotics and cellular metabolites (Cordon-Cardo et al., 1989). Not only may P-glycoprotein limit intestinal drug absorption to constrain oral drug bioavailability, but the rate of P-glycoprotein efflux transport can also mediate brain penetration of lipophilic drugs (Ambudkar et al., 1999; Benet et al., 1999).

Recently, an in vitro study has shown that quetiapine and risperidone have stronger affinity to P-glycoprotein than other atypical antipsychotic drugs, suggesting that quetiapine and risperidone are substrates of P-glycoprotein (Boulton et al., 2002). Moreover, our in vivo study showed that verapamil increased risperidone exposure, suggesting that risperidone as a substrate of P-glycoprotein is clinically relevant (Nakagami et al., 2005).

Hoffmeyer et al. (2000) suggested that a single-nucleotide polymorphism in exon 26 of the *MDR1* gene (*C3435T*) was associated with a lower level of intestinal MDR1 expression. Moreover it has been reported that another single-nucleotide polymorphism in exon 21 of the *MDR1* gene (*G2677T/A*) is also linked with a lower function of P-glycoprotein (Siegmund et al., 2002).

We hypothesized that inter-individual variability in plasma prolactin concentration can be explained by major MDR1 polymorphism to some extent. Therefore, the effect of the *MDR1* gene polymorphisms on the plasma concentration of prolactin was examined in schizophrenic patients.

## 2. Methods

### 2.1. Subjects

The subjects were *175* schizophrenic Japanese inpatients (68 males and *107* females) who fulfilled the criteria for schizophrenia according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition. Some of the patients participated in our previous studies on the relationship between steady-state plasma drug concentrations and *CYP2D6* or MDR1 genotype (Mihara et al., 2003; Yasui-Furukori et al., 2003, 2004). The mean±SD (range) of age, body weight and duration of illness were 43.2±18.9 (18–75) years, 58.6±12.6 (37–105) kg and 162±128 (4–448) months, respectively. The study was approved by the Ethics Committee of Hirosaki University Hospital, and written informed consent to participate in this study was obtained from the patients and their families.

### 2.2. Protocol

The subjects had received risperidone 3 mg twice a day (8 a.m. and 8 p.m.) for 4 to 79 weeks. The elimination half-lives of risperidone and 9-hydroxyrisperidone were reported to be 3 to 20 h and 20 to 29 h, respectively. Therefore, plasma concentrations of these compounds already reached steady state in all of the subjects before initiating the study. The drugs coadministered were flunitrazepam 1–6 mg/day in 105 cases, diazepam 2–30 mg/day in 25 cases, lorazepam 1–3 mg/day in 12 cases and alprazolam 0.8–2.4 mg/day in 25 cases, biperiden 4–6 mg/day in 69 cases, trihexyphenidyl 4–10 mg/day in 18 cases, sennoside 12–60 mg/day in 71 cases.

### 2.3. Assays

Plasma concentrations of risperidone and 9-hydroxyrisperidone were measured using liquid chromatography-mass spectrometry-mass spectrometry (LC-MS-MS) method. Extraction procedure was as follows: 200 μl of 0.1 M phosphate buffer (pH 7), 50 μl of internal standard solution (R068808: Jansen Research Foundation) and 100 μl of methanol were added to 200 μl of plasma sample. Thereafter, 400 μl of 0.1 M Borax was added. The mixture is vortexed and poured over an Extrelut NT 1 (Merck) column, which is eluted with 7 ml of ethyl acetate. The eluate was evaporated under a nitrogen stream at 65 C°, and was redissolved in 100 μl of methanol which is again evaporated under a nitrogen stream at 65 C°. The residues were redissolved in 200 μl of acetonitrile/0.01 M ammonium acetate (50/50, pH 9.0), and 5 μl were injected onto the LC-MS-MS system. The system consisted of API 3000 (Sciex) and a column (Hypersil BDS C18 100×4.6, 3 μm). The mobile phase was gradient ammonium acetate (0.01 M, pH 9.0)-acetonitrile. Among the fragment ions of the compounds, the mass-to-charge ratio (m/z) 207.0 for risperidone, m/z 191.0 for 9-hydroxyrisperidone, and m/z 201.0 for the internal standard, were selected for ion monitoring. The lower limit of detection was 0.1 ng/ml for risperidone and 9-hydroxyrisperidone, and the values of the intra-assay and inter-assay coefficient of variation were less than 5% at all the concentrations (0.1–100 ng/ml) of calibration curves for both compounds.

Plasma prolactin concentration was determined using enzyme immunoassay (IMX Prolactin Dainapack, Dainabot,

Table 1
Clinical profiles, plasma drug concentrations and plasma concentration of prolactin in C3435T genotypes

| | CC | CT | TT |
|---|---|---|---|
| | (*n*=54) | (*n*=82) | (*n*=39) |
| Age | 46.6±15.0 | 45.5±16.2 | 42.8±15.4 |
| Gender (male/female) | 19/35 | 27/55 | 22/17 |
| Plasma drug concentration (ng/ml) | | | |
| Risperidone | 6.2±7.2 | 8.4±14.9 | 12.0±20.9 |
| Nine-hydroxyrisperidone | 48.6±15.6 | 41.5±17.1 | 45.2±21.8 |
| Active moiety | 47.7±30.0 | 49.9±23.8 | 57.2±28.8 |
| Prolactin concentration (ng/ml) | | | |
| Total | 102.0±62.9 | 102.0±75.0 | 86.8±56.2 |
| Male | 62.3±33.3 | 49.4±15.6 | 53.2±33.2 |
| Female | 123.6±65.0 | 127.8±79.2 | 130.4±49.7 |

Table 2
Clinical profiles, plasma drug concentrations and plasma concentration of prolactin in G2677T/A genotypes

| | GG | GT or GA | TT, TA or AA |
|---|---|---|---|
| | ($n$=34) | ($n$=79) | ($n$=62) |
| Age | 42.5±16.2 | 50.4±15.2 | 46.0±14.4 |
| Gender (male/female) | 15/19 | 31/48 | 22/40 |
| Plasma drug concentration (ng/ml) | | | |
| Risperidone | 8.4±14.7 | 7.6±8.6 | 9.3±17.4 |
| Nine-hydroxyrisperidone | 40.5±20.5 | 42.6±20.6 | 46.6±23.4 |
| Active moiety | 48.9±28.0 | 46.3±20.3 | 55.8±33.2 |
| Prolactin concentration (ng/ml) | | | |
| Total | 97.7±63.5 | 80.5±60.6 | 109.8±74.5 |
| Male | 58.0±27.7 | 58.5±35.0 | 46.1±20.7 |
| Female | 123.3±67.0 | 97.7±71.2 | 144.9±69.9 |

Table 4
Partial correlation coefficients and multiple correlation coefficient in multiple regression analyses in total, male and female subjects between prolactin level and various factors

| Factors | Total subjects | Male | Female |
|---|---|---|---|
| | ($N$=175) | ($n$=68) | ($n$=107) |
| Age | −0.183** | −0.248 | −0.236* |
| Plasma drug concentration (ng/ml) | | | |
| Risperidone | −0.113 | 0.079 | −0.180 |
| Active moiety | −0.065 | 0.093 | 0.056 |
| *MDR1* polymorphism | | | |
| C3435T | −0.031 | −0.127 | 0.011 |
| G2677T/A | 0.084 | −0.120 | 0.122 |
| Gender | 0.540*** | | |
| Multiple correlation coefficient | 0.562*** | 0.317 | 0.297 |

*$p$<0.05, **$p$<0.01, ***$p$<0.001.

Osaka, Japan). The lowest limit of detection was 0.6 ng/ml, and inter-assay CV were 3.7, 3.5 and 3.5% at the concentrations of 8, 20 and 40 ng/ml for prolactin, respectively.

### 2.4. Analyses for MDR1 genotypes

For the determination of MDR-1 genotype, DNA was isolated from peripheral leukocytes by a guanidium isothiocyanate method. The C3435T alleles were detected by Taq-Man allelic discrimination methods (Verstuyft et al., 2003). G2677T/A alleles were identified using direct sequence methods (Horinouchi et al., 2002).

### 2.5. Data analyses and statistics

The comparison of several factors including the plasma concentration of prolactin and each MDR1 genotype was performed with use of one-way ANOVA. Multiple regression analyses were used to detect correlation between plasma concentration of prolactin and several factors including MDR1 genotypes (C3435T and G2677T/A), plasma drug concentration and age. The number of mutated alleles was used as independent variables and gender difference was analyzed using dummy variables (male=0, female=1). Because C3435T (Hoffmeyer et al., 2000) and G2677T/A (Siegmund et al., 2002) genotype proportionately expressed P-glycoprotein in the intestine, dummy variables were used for analyses of MDR1 genotype effects as follows: CC=2, CT=1 and TT=0 for C3435T, and GG=2, GA or TA=1 and TT, TA or AA=0 for G2677T/A. A $p$ value less than 0.05 was regarded as statistically significant. All analyses were performed using SPSS 12.0J for windows (SPSS Japan Inc., Tokyo, Japan).

Table 3
Plasma prolactin concentration in combination of C3435T and G2677T/A

| C3435T | G2677T/A | Male | Female |
|---|---|---|---|
| | | ($n$=68) | ($n$=107) |
| C/C | G/G | 71.0±36.3 | 104.4±34.2 |
| C/C | G/T or A | 43.6±15.1 | 128.9±90.5 |
| C/C | T or A/T or A | 46.9±12.9 | 147.7±74.9 |
| C/T | G/G | 56.2±25.0 | 136.5±82.7 |
| C/T | G/T or A | 48.6±10.3 | 75.1±45.3 |
| C/T | T or A/T or A | 54.1±18.8 | 147.9±79.3 |
| T/T | G/G | 73.6±24.5 | 112.3±33.1 |
| T/T | T or A/T or A | 53.5±17.4 | 137.9±54.7 |

## 3. Results

The patients had the following *MDR1* genotypes: *C/C* (54 cases), *C/T* (82) and *T/T* (39) for *C3435T* and *G/G* (34), *G/T* or *A* (79) and *T* or *A/T* or *A* (62) for *G2677T/A*, respectively. There was no difference in age or duration of illness between *C3435T* genotype or between *G2677T/A* genotype. However, plasma concentrations of risperidone and active moiety differed between C3435T, and plasma concentrations of 9-hydroxyrisperidone and active moiety differed between G2677T/A.

The plasma concentration of prolactin in females was significantly higher than males (137.4±81.6 ng/ml vs 56.8±30.9 ng/ml, $p$<0.001). Therefore, we analyzed prolactin concentration in males and in females separately. There were no differences in prolactin concentration in males or females between C3435T (Table 1) or between G2677T/A (Table 2). Plasma prolactin concentrations in the combination of these two genotypes (diplotypes) are shown in (Table 3). There was no difference in males ($F$=1.806, $df$=7, 60, $p$>0.05) or females ($F$=1.667, $df$=7, 98, $p$>0.05).

Multiple regression analyses including *C3435T* and *G2677T/A* allele for *MDR1*, gender difference and age showed that the plasma prolactin concentration correlated with gender (standardized beta=0.540, $p$<0.001) and negatively with age (standardized beta=−0.183, $p$<0.01), while there was no correlation between prolactin concentration and C3435T or G2677T/A (Table 4). When the multiple regression analyses without gender difference were further analyzed, the plasma prolactin concentration correlated negatively with age in females (standardized beta=−0.236, $p$<0.05), but not in males. Also, no correlations were found between prolactin concentration and MDR1 genotypes for C3435T (standardized beta=−0.031, ns) and G2677T/A (standardized beta=0.084, ns) (Table 4).

## 4. Discussion

P-glycoprotein is found in the epithelial cells lining the luminal surface of many organs often associated with an excretory or barrier function, i.e., the hepatic bile canalicular membrane, renal proximal tubule, villus-tip enterocyte in the small intestine, and the endothelial cells making up the blood–brain and blood–testes barriers (Ambudkar et al., 1999; Cordon-Cardo et al., 1989). A kinetic study showed large differences in brain concentration between the knockout animal, *mdr1a* (−/−) and *mdr1a/1b* (−/−) mice and normal animal, *mdr1a* (+/+) and *mdr1a/1b* (+/+) mice (Rao et al., 1999). Particularly, several animal studies showed that mdr1 knockout mice had extremely high brain concentrations of risperidone (Doran et al., 2005; Wang et al., 2004), suggesting that MDR1 protein plays an important role in penetration of risperidone from blood to brain. It is therefore more likely that *MDR1* variants affect risperidone concentration in brain, and hence prolactin concentration.

Contrary to our expectation, however, the plasma concentration of prolactin was not different between *MRD1* genotypes despite the fact that the active moiety concentration in the subjects with *MDR1* mutated alleles was higher than the subjects without *MDR1* mutated alleles. We have no clear explanation for this negative finding. A double-blind placebo-controlled in vivo study using healthy subjects demonstrated that prolactin response to a single oral dose of risperidone after verapamil treatment, which is a potent inhibitor of P-glycoprotein, was lower than placebo treatment (Nakagami et al., 2005). Therefore, it appears that MDR1 function in human blood–brain barrier and its role in prolactin concentration during risperidone treatment are unexpectedly complex.

Plasma concentrations of prolactin did not correlate with plasma drug concentration of risperidone or active moiety, although our previous studies showed positive correlation between plasma drug concentration and prolactin concentration during bromperidol and haloperidol treatments in males (Yasui et al., 1998; Yasui-Furukori et al., 2001). This discrepancy may be due to the relatively complex pharmacological profile of risperidone and its active metabolite, 9-hydroxyrisperidone. Risperidone has a potent serotonin $5HT_2$ and a milder dopamine $D_2$ antagonistic activity, (Schotte et al., 1995) and the $5HT_2$ antagonistic effect is associated with inhibition of prolactin secretion (Rubin, 1987).

It should be noted that the incidence of side effects related to hyperprolactinemia was not evaluated in the present study. In large clinical trials, risperidone-associated increase in serum prolactin levels did not correlate significantly with the emergence of possible prolactin-related side effects (Kleinberg et al., 1999). This may be due to the considerable number of patients who are without adverse effects despite having a high prolactin level. Nevertheless, prolactin monitoring during risperidone treatment should be conducted especially in premenopausal women who potentially suffer from potential adverse effects associated with hyperprolactinemia.

Patients receiving 6 mg/day of risperidone might have around 90% dopamine D2 receptor occupancy probably due to tight tight-binding to dopamine D2 receptor (Kapur and Seeman, 2001). According to the rapid dissociation model, tight-binding atypical agents are hypothesized to have an antipsychotic action when they cause other effects of dopamine blockade such as raised prolactin levels or extrapyramidal side effects (Kapur and Seeman, 2001). Although the MDR1 polymorphisms did not correlate with prolactin concentration, the possibility that the DRD2 variants rather than MDR1 may have predominant effect on dopamine D2 receptor occupancy of risperidone and other clinical responses cannot be excluded entirely.

An in vitro study has shown that quetiapine and risperidone have stronger affinity to P-glycoprotein than other atypical antipsychotic drugs, suggesting that quetiapine and risperidone are substrates of P-glycoprotein (Boulton et al., 2002). However, there is no in vivo data indicating that quetiapine or risperidone as a substrate of P-glycoprotein is of clinical relevance. Further information is required for antipsychotics to determine whether these *MDR1* genotypes are clinically relevant or not.

## 5. Conclusion

The present results indicate that the major *MDR1* polymorphisms are not associated with prolactin concentration during risperidone treatment in schizophrenic patients. Further studies will be valuable to determine whether or not *MDR1* genotypes are clinically relevant in the treatment with antipsychotics.

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**Short Communication**

# Effective electroconvulsive therapy in a 92-year-old dementia patient with psychotic feature

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**Abstract** A 92-year-old woman who suffered from dementia with psychotic feature was admitted to a psychiatric ward. She refused to eat or take any medications. After 0.5 mg i.v. injection haloperidol, prolongation of QTc interval occurred in the electrocardiogram. Therefore two sessions of electroconvulsive therapy (ECT) were performed carefully after informed consent was obtained by her family. Almost no psychotic symptoms were observed after the first ECT. No cognitive side-effects were observed during and after the two ECT sessions. This demonstrates that ECT can be used as an alternative treatment when elderly dementia patients with psychotic feature cannot tolerate medication.

**Key words** ECT, dementia, elderly.

## INTRODUCTION

Neuropsychiatric disturbances are a core feature of dementia and worsen many clinical outcomes.[1] Among 342 Alzheimer disease patients 75 (22%) had delusions only, nine (3%) had hallucinations only and 30 (9%) had both delusions and hallucinations.[2] Antipsychotics are important in the treatment of dementia patients with psychotic feature but they have moderate efficacy and often cause adverse events.[3,4] Recent safety warnings by the Food and Drug Administration (FDA)[5] about increased frequency of cerebrocardiovascular adverse events in elderly patients who use atypical antipsychotics mean that physicians now face a dilemma when weighing the benefits and risks of using antipsychotics in this patient group. The only empiric study in this area conducted to date indicated that antipsychotics are associated with a worse quality of life for nursing home patients.[6] Electroconvulsive therapy (ECT) is now regarded as an effective treatment for drug-resistant schizophrenia or in depressed patients who are at risk of suicide. It is therefore likely that ECT is an alternative treatment for elderly patients who cannot tolerate medication. Here we describe the case of a 92-year-old woman who suffered from dementia with psychotic feature that was dramatically improved by ECT.

## CASE REPORT

A 92-year-old woman who had no previous history of psychiatric illness and a stable family situation was referred to Hirosaki University School of Medicine with a 2-year history of cerebrovascular dementia. Computed tomography showed multiple microinfarcts and age-matched cerebral atrophy.

As a result of severe delusions such as 'Someone steals her money' or 'Someone adds poison to her food', she was excited and she refused to eat for at least 1 month in a previous hospital. She was diagnosed with dementia with psychotic feature and was admitted to Hirosaki-Aiseikai Hospital. Clinical laboratory data at admission were within normal limits except for urea nitrogen 63 mg/mL. Behave-AD scored 20 out of 57 points, where Behave-AD is the evaluation scale for behavioral and psychological symptoms with dementia.[7] She refused to take quetiapine 50 mg/day or risperidone oral solution 0.5 mg/day. Therefore we injected 0.5 mg i.v. haloperidol. Although no subjective

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symptoms were observed, prolongation of QTc interval from 415 mm at admission to 468 mm occurred in the electrocardiogram. Because an urgent treatment was required physically, we discussed whether the patient was a suitable candidate for ECT at the Department of Neuropsychiatry, Hirosaki University School of Medicine and informed consent was obtained from her family.

The ECT was carefully administered by two well-trained anesthesiologists in the psychiatric intensive care unit at Hirosaki Aiseikai Hospital after preoperative evaluation by the anesthesiologists. The patient was monitored with sphygmomanometer, electrocardiogram, SpO2 monitor and bispectral index (BIS) monitor. Propofol (40 mg), succinylcholine (20 mg) and diltiazem (5 mg) were used. Electrodes were placed in the traditional bilateral fronto-temporal manner. ECT was conducted for 5 s with 100 V sine wave apparatus (C-1, Sakai Medical, Tokyo, Japan). Motor and electroencephalogram (EEG) seizure duration of 40 s and 58 s, respectively, were recorded during the first session. After the first session her refusing attitude disappeared and score on Behave-AD dropped to 5 points. After the second ECT no psychotic symptoms were observed and Behave-AD scored 0 points. On the basis of clinical observations, no cognitive side-effects were observed after the two ECT sessions. We tried to monitor her cognitive function but Mini-Mental State Examination (MMSE)[8] was not available because of her refusal to cooperate before the ECT sessions. At interview the next day, MMSE for the first and second sessions scored 12 and 16 points, respectively.

The patient received atypical antipsychotics, perospirone 4 mg/day for 1 month after the ECT treatments, and no psychotic symptoms were observed during treatment with this medication and after its discontinuation. She was discharged from Hirosaki Aiseikai-Hospital and admitted to a nursing home after 2 months.

## DISCUSSION

This case shows the successful use of ECT for a patient suffering from dementia with psychotic feature. Recent studies have shown that antipsychotic agents have moderate effectiveness in the short- to medium-term management of these symptoms.[3,4] However, the FDA has issued an advisory stating that atypical antipsychotic medications increase mortality among elderly patients, compared with placebo,[5] probably because of cerebrocardiac adverse events. After injection of low-dose (0.5 mg/day) haloperidol we stopped this medication due to prolongation of QTc interval in the electrocardiogram.[9] Therefore ECT may be one of the alternative treatments in elderly dementia patients with psychotic feature.

The American Psychiatric Association (APA) 1990 task force advises that ECT can be used regardless of age.[10] Similarly, Kamholz and Mellow describe the use of ECT for the elderly in glowing terms: "It is increasingly advised as a first-line therapy for severely ill patients who are badly malnourished or who are at risk for suicide".[11] They also recommend it for patients who cannot tolerate antidepressants because of cardiac disease. Thus it is likely that ECT even in elderly dementia patients with psychotic feature is safer than some medications when perioperative care with the highest standard of preparation, sensitivity to clinical issues, vigilance, and meticulous attention to all details of perioperative management, is given.

In contrast, the APA report does warn that "some elderly patients may have an increased likelihood of appreciable memory deficits and confusion during the course of treatment", although there is no suggestion that ECT poses a special threat to the vulnerable brain or cardiovascular system of the elderly.[11] In a curious twist, an article by Burke *et al.*[12] is listed in the bibliography of the APA report but not cited in the actual discussions of the elderly. Burke *et al.* found a high rate (35%) of complications among the elderly.[12] They suggested that common complications in the elderly include severe confusion, falls, and cardiorespiratory problems. To avoid the cardiac events therefore we used 5 mg diltiazem just before the ECT sessions. No cardiac adverse effects including cardiac arrhythmias, angina, myocardial infarction, and persistent hypertension were observed in the present case. In addition, we performed careful monitoring of cognitive function on Glasgow Coma Scale during anesthesia and on MMSE after ECT sessions and no memory deficits and confusion were observed.

The BIS is an EEG-derived value that measures the sedative component of the anesthetic state.[13,14] Because of pharmacokinetic[15] and pharmacodynamic reasons,[16] the elderly are at particular risk of incurring unwanted side-effects from drugs commonly used in anesthesia. Accordingly, the BIS is a useful guidance for titration of anesthetic drugs in the elderly.[17] By means of BIS we achieved adequate anesthetic conditions for the present patient after only 40 mg propofol.

In conclusion, the present case demonstrates that ECT is an alternative treatment for elderly dementia patients with psychotic feature who cannot tolerate medication and who require urgent treatment physically and mentally.

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# Association Between Cytochrome P450 (CYP) 2C19 Polymorphisms and Harm Avoidance in Japanese

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**Polymorphic enzyme cytochrome P450 (CYP) 2C19 is expressed not only in the liver but also in the brain and mediates the biotransformation of 5-hydroxytriptamine (5-HT). We investigated possible association between genetic polymorphism of CYP2C19 and individual personality traits, possibly influenced by neurotransmitters. Mentally and physically healthy Japanese subjects were enrolled in this study (n = 352). Temperament and Character Inventory (TCI) and *CYP2C19* genotyping were performed in all subjects. We detected CYP2C19*2 and *3 (http://www.imm.ki.se/CYPalleles/) using Amplichip CYP450 DNA tip. The number of genotypes classified as homozygous extensive metabolizer (EM), heterozygous EM, and poor metabolizer were 113, 181, and 58, respectively. Significant difference was found in TCI score in harm avoidance (HA; $F = 3.138$, $P < 0.05$). Post hoc analysis showed that TCI score in harm avoidance in homozygous EM was significantly lower than that in heterozygous EM ($P < 0.05$) or PM ($P < 0.05$). In sub-item analyses, HA3 (shyness with strangers, $P < 0.01$) and HA1 (anticipatory worry, $P < 0.05$) of TCI scores were significantly different among *CYP2C19* genotypes. Meanwhile, there were no differences in TCI scores of novelty seeking (NS; $F = 0.350$, n.s.), reward dependence (RD; $F = 1.080$, n.s.), or persistence (P; $F = 0.786$, n.s.) among *CYP2C19* genotypes. This study demonstrated that a significant association between CYP2C19 activity and HA is present in Japanese.** © 2007 Wiley-Liss, Inc.

**KEY WORDS: CYP2C19; harm-avoidance; personality; TCI**

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## INTRODUCTION

Brain cytochrome P450s (CYPs) were originally reported to occur at only 1% of the levels found in liver [Warner et al., 1988], but the levels of CYPs in specific neurons can be as high or higher than levels in hepatocytes [Miksys et al., 2002]. Although it is unlikely that brain CYPs contribute to overall clearance of xenobiotics, they are able to metabolize a variety of compounds, including many drugs that cross the blood–brain barrier to produce their pharmacological effects within the brain. Brain CYPs are also thought to participate in the metabolism of some neurotransmitters, endogenous steroids, and neurosteroids [Miksys and Tyndale, 2002]. Therefore, this aspect of their function may be important in influencing neural development and integration of overall brain function.

5-hydroxytryptamine (5-HT) is believed to be primarily metabolized by monoamine oxidase A, which deaminates 5-HT, yielding 5-hydroxyindole acetaldehyde that is converted to 5-hydroxyindole acetic acid (5-HIAA) by an aldehyde dehydrogenase. However, a recent in vitro study demonstrated that 5-HT relaxed precontracted isolated aortic rings, with or without endothelium in the presence of CYP2B6, 2C9, and 2C19, suggesting that 5-HT is biotransformed by CYP2B6, 2C9, and 2C19 [Fradette et al., 2004].

CYP2C19 enzyme is expressed not only in the liver but also in the brain [Miksys and Tyndale, 2002]. CYP2C19 mediates the metabolism of many drugs including antidepressants, benzodiazepine, and proton pump inhibitors [Desta et al., 2002]. The CYP2C19 polymorphism results in three phenotypic groups: homozygous extensive metabolizer (EM), heterozygous EM, and poor metabolizer (PM). Although more than 18 mutated alleles affecting CYP2C19 activity have been identified (see http://www.imm.ki.se/CYPalleles/), only CYP2C19*2 and *3 among these alleles are able to explain most phenotypes of CYP2C19 activity in Japanese.

Cloninger et al. [1993] demonstrated that human personality consists of seven dimensions including three temperament dimensions and four character dimensions, and on the basis of this model, Cloninger developed the Temperament and Character Inventory (TCI), a questionnaire for assessing personality traits. The four temperament dimensions, which include novelty seeking (NS), harm avoidance (HA), reward dependence (ED), and persistence (P), have been assumed to be related to monoamine neurotransmitters: NS with dopaminergic activities [Menza et al., 1993, 1995], HA with serotonergic activities [Demitrack et al., 1992; Stein et al., 1993], and RD with noradrenergic activities [Garvey et al., 1996; Curtin et al., 1997].

Therefore, the aim of the present study was to clarify the possible relationship between the genotypes of CYP2C19 polymorphism and behavioral traits in a large sample of Japanese, as measured by the TCI.

## SUBJECTS AND METHOD

This study was carried out after obtaining approval from the Ethics Committee of Hirosaki University School of Medicine.

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The subjects enrolled consisted of all Japanese students in a medical school and medical staff (n = 352, M = 186, $F$ = 166). Their mean (±SD) age was 29.9 ± 11.3 years (range: 18–69 years). After giving the subjects a full description of the study, written informed consent to participate was obtained from each of them. The subjects were asked to complete the 240 items of the Japanese version of the TCI, whose reliability and validity had been established by Kijima et al. [1996]. Genomic DNA was extracted from 5 ml of peripheral blood using DNA purification kit (QIAGEN). Genotypings of CYP2C19 were performed using AmpliChip CYP450 Test® DNA tip (Roche Diagnostics, Tokyo, Japan). This microarray-based assay screens for *CYP2C19*2* and *CYP2C19*3*, as well as 31 known mutations in *CYP2D6*. The subjects were allocated into three groups based on the number of mutated alleles for CYP2C19: homozygous EM (**1/*1*), heterozygous EM (**1/*2* or **1/*3*), and PM (**2/*2*, **2/*3* or **3/*3*).

For statistical analysis, the SPSS for Windows ver 13.0 (SPSS Japan, Inc., Tokyo, Japan) software package was used. Genotype deviation from the Hardy–Weinberg equilibrium was evaluated by chi-squared test. The mean scores for the seven factors (novelty seeking, harm avoidance, reward dependence, persistence, self-directedness, cooperativeness, self-transcendence) of the TCI were compared among the three genotype groups using ANOVA. Post hoc analysis was performed using Scheffe test. $P < 0.05$ was regarded as significant.

## RESULTS

The frequencies of genotypes for **1/*1*, **1/*2*, **1/*3*, **2/*2*, **2/*3*, and **3/*3* were 0.32, 0.34, 0.17, 0.065, 0.082, and 0.017, respectively. The number of homozygous EM, heterozygous EM, and PM were 113, 181, and 58, respectively. The genotype distribution was not significantly different from that expected according to the Hardy–Weinberg equilibrium. There was no difference in age ($F = 1.105$, df = 2, 349, $P < 0.332$) or gender ($F = 1.125$, df = 2, 349, $P < 0.326$) among these *CYP2C19* genotypes.

Significant difference was found in TCI score in harm avoidance (HA; $F = 3.138$, df = 2, 349, $P < 0.016$) (Table I). Post hoc analysis showed that TCI score in harm avoidance in homozygous EM was significantly lower than that in heterozygous EM ($P < 0.047$) or PM ($P < 0.048$). In sub-item analyses, HA3 (shyness with strangers) ($F = 6.656$, df = 2, 349, $P < 0.001$) and HA1 (anticipatory worry) ($F = 3.940$, df = 2, 349, $P < 0.020$) had significant difference in CYP2C19 genotype, while *CYP2C19* genotypes differed neither scores of HA2 (fear of uncertain) ($F = 0.460$, df = 2, 349, $P < 0.631$) nor HA4 (fatigability) ($F = 1.530$, df = 2, 349, $P < 0.218$) (Table II). There were no differences in TCI scores of novelty seeking (NS; $F = 0.350$, df = 2, 349, $P < 0.705$), reward dependence (RD; $F = 1.080$, df = 2, 349, $P < 0.314$), or persistence (P; $F = 0.786$, df = 2, 349, $P < 0.415$) among *CYP2C19* genotypes (Table I). In addition, self-directedness ($F = 1.647$, df = 2, 349, $P < 0.208$), cooperativeness ($F = 0.547$, df = 2, 349, $P < 0.579$), self-transcendence ($F = 2.261$, df = 2, 349, $P < 0.106$) did not differ among the *CYP2C19* genotype groups (Table I).

## DISCUSSION

The results of this study showed that scores of TCI of HA were significantly different among *CYP2C19* genotypes in Japanese. TCI score of HA in homozygous EM was significantly lower than that in heterozygous EM or PM in this study. This is the first study suggesting association between CYP2C19 activity and some personality characteristics. Since CYP2C19 mediates biotransformation of 5-HT, low activity of CYP2C19 might be linked with high concentration of 5-HT in the brain. It might therefore be reasonable to correlate the activity of CYP2C19 with HA because it has been suggested that HA is associated with serotonergic activity [Demitrack et al., 1992; Stein et al., 1993].

5-TH has been believed to be primarily metabolized by monoamine oxidase A, which deaminates 5-HT, yielding 5-hydroxyindole acetaldehyde that is converted to 5-hydroxyindole acetic acid (5-HIAA) by an aldehyde dehydrogenase. However, metyrapone and ketoconazole increases 5-HT concentration in the brain [Kennett et al., 1985; Murphy, 1997; Leret et al., 1998; Healy et al., 1999]. Phenytoin, a drug biotransformed by CYP2C9 and CYP2C19, increase 5-HT in the brain without enhancing its synthesis [Chadwick et al., 1978; Pratt et al., 1985], suggesting that phenytoin competitively inhibits 5-HT biotransformation. In addition, in the presence of CYP2B6, 2C9, and 2C19, 5-HT relaxed precontracted isolated aortic rings, with or without endothelium, an effect prevented by the addition of methylene blue and an inhibitor of catalase, but not by myoglobin [Fradette et al., 2004]. In the absence of catalase, hydroxylamine was always assayed as a byproduct of 5-HT metabolism. CYP2B6, 2C9, and 2C19 biotransform 5-HT, yielding hydroxylamine, which is converted to nitric oxide in the presence of catalase.

Association between personality and another polymorphic enzyme CYP2D6 has been investigated. Bertilsson et al. [1989] investigated relationship between debrisoquine hydroxylation phenotype and personality in healthy Swedes using the KSP psychoasthenia scale. This study showed that PM for CYP2D6 had lower scores in KSP and a lack of hesitation in comparison to EM. Meanwhile, the second study reported that PM were more prone to anxiety and less successfully socialized than EM using the subjects of Spanish students [LLerena et al., 1993].

TABLE I. Distribution of the Temperament and Character Inventory (TCI) Subitem Scores Among the Number of Mutated Alleles for CYP2C19

| | Number of mutated alleles | | | |
|---|---|---|---|---|
| | 0 (n = 113) | 1 (n = 181) | 2 (n = 58) | Significance |
| Novelty seeking | 21.3 ± 4.0 | 21.6 ± 5.2 | 21.1 ± 4.5 | n.s. |
| Harm avoidance | 18.7 ± 5.8 | 20.5 ± 6.1** | 21.1 ± 6.7* | $P < 0.05$ |
| Reward dependence | 15.2 ± 2.9 | 14.7 ± 3.5 | 14.6 ± 3.2 | n.s. |
| Persistence | 4.5 ± 1.7 | 4.2 ± 1.9 | 4.4 ± 2.0 | n.s. |
| Self-directedness | 27.3 ± 6.7 | 26.0 ± 6.5 | 26.0 ± 6.8 | n.s. |
| Cooperativeness | 28.4 ± 5.3 | 27.8 ± 5.1 | 28.5 ± 5.5 | n.s. |
| Self-transcendence | 11.3 ± 5.6 | 10.0 ± 4.5 | 10.5 ± 4.8 | n.s. |

Data are mean ± SD.
*$P < 0.05$.
**$P < 0.01$, compared with subjects without mutated allele.

TABLE II. Distribution of the Harm Avoidance (HA) Subitem Scores Among the Number of Mutated Alleles for CYP2C19

| | Number of mutated alleles | | | |
|---|---|---|---|---|
| | 0 (n = 113) | 1 (n = 181) | 2 (n = 58) | Significance |
| Total HA | 18.7 ± 5.8 | 20.5 ± 6.1** | 21.1 ± 6.7* | $P < 0.016$ |
| HA1 (anticipatory worry) | 5.2 ± 2.3 | 5.8 ± 2.4 | 6.3 ± 2.8* | $P < 0.020$ |
| HA2 (fear of uncertain) | 5.3 ± 1.5 | 5.5 ± 1.7 | 5.4 ± 1.6 | $P < 0.631$ |
| HA3 (shyness with strangers) | 4.3 ± 1.9 | 5.0 ± 1.7** | 5.1 ± 2.0* | $P < 0.001$ |
| HA4 (fatigability) | 3.8 ± 2.2 | 4.1 ± 2.1 | 4.4 ± 2.4 | $P < 0.218$ |

Data are mean ± SD.
*$P < 0.05$.
**$P < 0.01$, compared with subjects without mutated allele.

The discrepant results between the two studies might be explained by differences in the method for measuring debrisoquine hydroxylation capacity, the age of subjects, and the national character or race between Sweden and Spain. Another study in New Zealand showed that PM for CYP2D6 tends to be more novelty seeking and less harm avoidance than EM, using TCI. They used subjects that were recruited from long-term depressed patients [Roberts et al., 2004]. Although their findings were in accordance with Swedish findings but contrary to Spanish findings, this result should be carefully interpreted because depressed patients have lower scores of harm avoidance than controls.

The association between temperament and genetic variability within the serotonergic and dopaminergic neurotransmitter system has been investigated. For example a 44-bp polymorphism of the serotonin transporter gene (*5-HTT*) implies the relationship with harm avoidance [Lesch et al., 1996]. It is also reported that polymorphism of 48 bp repeat in dopamine D4 receptor gene (*DRD4*) has been associated with the novelty seeking category [Benjamin et al., 1996]. However, some studies have not been able to demonstrate an association between personality and polymorphisms [Ebstein et al., 1997; Blairy et al., 2000; Gebhardt et al., 2000; Kusumi et al., 2002], thus additional investigation that includes genetic polymorphisms, *CYP2D6*, *5-HTT*, and *DRD4* may be necessary to clarify the relationship between temperament and genetic polymorphisms within the serotonergic and dopaminergic neurotransmitter system.

Several studies have suggested that high HA is associated with depression [Farmer et al., 2003; Cloninger et al., 2006]. On the basis of the present result, it is possible that interindividual variation of CYP2C19 activity is related with predisposition to depression. Likewise, a recent study suggests an association between *CYP2C9* gene and susceptibility to major depressive disorder due to an alteration in endogenous metabolism [LLerena et al., 2003]. Further studies are required to clarify the effect of CYP2C19 gene on susceptibility to major depressive disorder.

Our results failed to find association between CYP2C19 polymorphism and characters such as self-directedness, cooperativeness, and self-transcendence. This finding may be reasonable because characters are regarded as a product of learning according to Cloninger's Model.

In conclusion, this study demonstrated a significant association between inferred CYP2C19 activity and HA in Japanese. This result may depend upon the relative implication of CYP2C19 in the biotransformation of 5-HT.

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# The G196A polymorphism of the brain-derived neurotrophic factor gene and the antidepressant effect of milnacipran and fluvoxamine

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## Abstract

Prediction of the response to different classes of antidepressants has been an important matter of concern in the field of psychopharmacology. The purpose of the present study was to investigate whether the G196A polymorphism of the brain-derived neurotrophic factor (BDNF) gene is associated with the antidepressant effect of milnacipran, a serotonin norepinephrine reuptake inhibitor, and fluvoxamine, a selective serotonin reuptake inhibitor. The subjects of our previous study of milnacipran ($n = 80$) and fluvoxamine ($n = 54$) were included in the present study. Severity of depression was assessed with the Montgomery Åsberg depression rating scale (MADRS). Assessments were carried out at baseline and at 1, 2, 4 and 6 weeks of treatment. Polymerase chain reaction was used to determine allelic variants. In all subjects receiving milnacipran or fluvoxamine, the *G/A* genotype of the BDNF G196A polymorphism was associated with a significantly better therapeutic effect in the MADRS scores during this study. When milnacipran and fluvoxamine-treated subjects were analysed independently, the *G/A* genotype group showed greater reduction of MADRS scores than other genotype groups, irrespective of which antidepressant was administered. These results suggest that the BDNF G196A polymorphism in part determines the antidepressant effect of both milnacipran and fluvoxamine.

### Keywords

antidepressant effect, genetic polymorphism, fluvoxamine, major depressive disorder, milnacipran

## Introduction

Prediction of the response to different classes of antidepressants has been an important matter of concern in the field of psychopharmacology. A consistent relationship between the antidepressant effect and the plasma concentrations of selective serotonin (5-HT) reuptake inhibitors (SSRIs) has not been obtained (Burke and Preskorn, 1999), although early pharmacokinetic studies identified significant relationships between the antidepressant effect and plasma concentrations of several tricyclic antidepressants (Perry *et al.*, 1987). In terms of serotonin norepinephrine (NE) reuptake inhibitors (SNRIs), venlafaxine showed a positive association between antidepressant efficacy and plasma concentrations (Charlier *et al.*, 2002), while this relationship was not observed for milnacipran (Higuchi *et al.*, 2003).

Recent progress in pharmacogenetics has facilitated investigation of the relationship between genetic polymorphisms and the antidepressant response. Genetic polymorphisms of the 5-HT and NE transporter have been investigated intensively, because they are believed to be the primary target of SSRIs and SNRIs. As a result,

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several interesting findings have been reported (Malhotra *et al.*, 2004; Yoshida *et al.*, 2004), but there is no consistent evidence to predict the antidepressant response. Thus, further pharmacogenetic studies of antidepressants must be performed in order to predict the antidepressant response adequately.

Recently, it has been proposed that antidepressants eventually cause critical genes to be activated or inactivated, no matter how they act on receptors and enzymes (Stahl, 2000). One of the likeliest candidate genes is brain-derived neurotrophic factor (BDNF), which belongs to a family of neurotrophic factors including neurotrophin-3/4/5 and nerve growth factor and has an important role as a potent modulator of synaptic transmission and plasticity. Substantial evidence supports that BDNF is involved not only in cognitive processes, such as memory and learning, but also in the pathophysiology of mood disorders and in the mechanism of antidepressant action, as follows. Expression of BDNF mRNA is down-regulated by either acute or repeated stressful conditions of immobilization (Smith *et al.*, 1995). An antidepressant effect in both the learned helplessness and the forced swimming tests is observed as early as 3 days after a single infusion of BDNF into the hippocampus (Shirayama *et al.*, 2002). Chronic treatment with tranylcypromine, a monoamine oxidase inhibitor, caused a significant increase in BDNF mRNA in the rat hippocampus (Russo-Neustadt *et al.*, 1999), and chronic administration of amitriptyline, a tricyclic antidepressant, significantly increased BDNF protein levels in the rat hippocampus and prefrontal cortex (Okamoto *et al.*, 2003). Thus, the BDNF gene is a plausible candidate gene for mood disorders and pharmacogenetic studies of the antidepressant response.

The G196A polymorphism in exon IIIA is located within the propeptide region of the BDNF gene. Several association studies have examined the G196A polymorphism and vulnerability for bipolar or major depressive disorders (Hong *et al.*, 2003; Nakata *et al.*, 2003). These studies have found no major role for the polymorphism in the pathophysiology of mood disorders, although Egan *et al.* (2003) reported that it influences human memory and hippocampal function. So far only one pharmacogenetic study of antidepressants and the BDNF G196A polymorphism has been carried out (Tsai *et al.*, 2003); in this study, the response to treatment with fluoxetine was evaluated for only 4 weeks and the response rate was as low as 33.6%.

In the current 6-week study, we examined the effect of the BDNF G196A polymorphism on the antidepressant effect of milnacipran, an SNRI, and fluvoxamine, an SSRI. In addition, we investigated another polymorphism of C132T in the non-coding region of exon V of the BDNF gene, which was detected and named C270T by Kunugi *et al.* (2001). Plasma concentrations of milnacipran and fluvoxamine were investigated to evaluate patients' compliance and an influence on the antidepressant effect.

## Materials and methods

### Subjects

The subjects in our previous studies (Yoshida *et al.*, 2002; Yoshida *et al.*, 2004) were included in the present study. The subjects were Japanese patients who fulfilled DSM-IV criteria for a diagnosis of major depressive disorder and whose scores on the Montgomery Åsberg depression rating scale (MADRS) (Montgomery and Asberg, 1979) were 21 or higher. Patients with other axis I disorders (including dementia, substance abuse, dysthymia, panic disorder, obsessive–compulsive disorder and generalized anxiety disorder) and those with axis II disorders determined by clinical interview were excluded. Patients with a history of childhood disorders were also excluded, as were patients with severe non-psychiatric medical disorders. The patients were 20–69 years of age and had been free of psychotropic drugs at least 14 days before entry into the study. After complete description of the study to the subjects, written informed consent was obtained. This study was approved by the Ethical Committee of Akita University School of Medicine. The clinical characteristics of the patients are shown in Table 1. There was no significant difference between responders and non-responders in regard to sex, age, number of previous episodes and presence of melancholia. There was no significant difference in clinical characteristics when milnacipran and fluvoxamine-treated patients were analysed independently (data not shown). The number of previous depressive episodes was very low. Indeed, most of the patients (milnacipran: 64/80, fluvoxamine: 41/54) were in their first episode.

### Milnacipran treatment

Milnacipran was administered twice daily (the same dose after dinner and at bedtime) for 6 weeks. The initial total daily dose was 50 mg/day, and after a week it was increased to 100 mg/day. Patients with insomnia were prescribed brotizolam, 0.25 or 0.5 mg, a benzodiazepine sedative hypnotic, at bedtime. No other

**Table 1** Clinical characteristics of the patients in the milnacipran and fluvoxamine treatment (responders and non-responders)

| | Responders ($n = 85$) | Nonresponders ($n = 49$) | | $p$ |
|---|---|---|---|---|
| Sex (male/female) | 34/51 | 16/33 | $\chi^2 = 0.72$ | 0.40[a] |
| Age (years) (± SD) | 50.7 ± 12.4 | 52.2 ± 12.8 | $t = -0.68$ | 0.50[b] |
| Number of previous episodes (± SD) | 0.48 ± 1.7 | 0.33 ± 0.7 | $t = 0.77$ | 0.44[b] |
| Melancholia (+/-) | 21/64 | 15/34 | $\chi^2 = 0.55$ | 0.46[a] |

[a] Analysis performed with the use of the $\chi^2$ test.

[b] Analysis performed with the use of the unpaired t test.