Influence of the tyrosine hydroxylase val81met polymorphism and catechol-\textit{O}-methyltransferase val158met polymorphism on the antidepressant effect of milnacipran

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Objective Genetic polymorphisms of the noradrenergic pathway can be factors to predict the effect of antidepressants when their pharmacological mechanisms of action include the noradrenergic system. The purpose of the present study was to determine whether the tyrosine hydroxylase (TH) val81met and catechol-\textit{O}-methyltransferase (COMT) val158met polymorphisms are associated with the antidepressant effect of milnacipran, a serotonin/noradrenaline reuptake inhibitor.

Method Eighty-one Japanese patients with major depressive disorder were treated with milnacipran for 6 weeks. Severity of depression was assessed with the Montgomery and Åberg Depression Rating Scale (MADRS). Assessments were carried out at baseline and at 1, 2, 4 and 6 weeks of treatment. The method of polymerase chain reaction was used to determine allelic variants.

Results The met/met genotype of the COMT val158met polymorphism was associated with a significantly faster therapeutic effect of milnacipran in the MADRS score during this study. No influence of the TH val81met polymorphism on the antidepressant effect of milnacipran was detected.

Conclusion These results suggest that the COMT val158met polymorphism in part determines the antidepressant effect of milnacipran.

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KEY WORDS — catechol-\textit{O}-methyltransferase; major depressive disorder; milnacipran; polymorphism; tyrosine hydroxylase

INTRODUCTION

Individual genetic differences of monoaminergic pathways can have an impact on the effect of antidepressant agents, though the exact mechanism of their action is still unclear. Several lines of evidence have suggested the relationship between genetic polymorphisms of the serotonergic pathway, especially those of the 5-hydroxytryptamine transporter (5-HTT), and the antidepressant effect of selective serotonin reuptake inhibitors (SSRIs) (Binder and Holsboer, 2006).

Genetic polymorphisms of the noradrenergic pathway as well as serotonergic pathway could also affect the effect of antidepressants, especially when their pharmacological mechanisms of action include the noradrenergic system. Tyrosine hydroxylase (TH) is
the initial and rate-limiting enzyme in the biosynthesis of catecholamine neurotransmitters including noradrenaline. The TH val81met polymorphism in exon 2 (Ludecke and Bartholome, 1995) is located in the amino-terminal regulatory domain of the tetrameric enzyme. The regulatory region is reported to have an inhibiting effect on the enzymatic function (Kumer and Vrana, 1996). Catechol-O-methyltransferase (COMT) is an important enzyme involved in degradation of catecholamine neurotransmitters including noradrenaline. The COMT val158met polymorphism located in exon 4 (Lotta et al., 1995) was reported to be associated with variation in COMT enzyme activity (Lachman et al., 1996).

No pharmacogenetic study addressed the relationship between the TH val81met polymorphism and antidepressant response. Only two studies investigated the relationship between the COMT val158met polymorphism and antidepressant response to SSRIs and mirtazapine. One reported its no overall effect on the antidepressant response to SSRIs (Arias et al., 2006), and the other reported its significant effect on the antidepressant response to mirtazapine but not paroxetine (Szegedi et al., 2005).

So far, there has been no study investigating the relationship between the TH val81met polymorphism, the COMT val158met polymorphism and the antidepressant response to serotonin noradrenaline reuptake inhibitors (SNRIs), although noradrenergic genetic factors could be one of the most plausible candidates for pharmacogenetic analysis of SNRIs. The class of SNRIs now comprises of three medications: venlafaxine, duloxetine and milnacipran. Among SNRIs, venlafaxine has a high affinity for the 5-HTT but not the noradrenaline transporter. Duloxetine has a more balanced affinity but is still more selective for the 5-HTT. Milnacipran is the most balanced and may even be slightly more noradrenergic than serotonergic (Stahl et al., 2005). Thus, the authors investigated whether the above two noradrenergic polymorphisms affect the antidepressant effect of milnacipran.

**SUBJECTS AND METHODS**

**Subjects**

For the present study, one subject treated with milnacipran was added to those in our previous study (Yoshida et al., 2007). Detailed inclusion criteria have been described previously (Yoshida et al., 2007). In brief, the subjects were Japanese patients who fulfilled DSM-IV criteria for a diagnosis of major depressive disorder and whose scores on the Montgomery Asberg Depression Rating Scale (MADRS) (Montgomery and Asberg, 1979) were 21 or higher. Patients with other axis I and II disorders determined by clinical interview and those with severe nonpsychiatric medical disorders were excluded. The patients were 25–69 years of age (mean age (±SD) = 51.1 ± 12.3) and had been free of psychotrophic 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 and Nagoya University Graduate School of Medicine. The clinical characteristics of the patients are shown in Table 1. There was no significant difference between responders and nonresponders in regard to sex, age, number of previous episodes and presence of melancholia.

**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 0.25 or 0.5 mg of brotizolam, a benzodiazepine sedative hypnotic, at bedtime. No other psychotrophic drugs were permitted during the study. Of 98 enrolled patients, 10 did not complete the study: five patients because of side effects, one patient because of severe insomnia and four patients without explanation. Of the 88 patients who completed the 6-week study, seven patients were excluded from the

| Table 1. Clinical characteristics of the patients (responders and nonresponders) |
|-----------------|-----------------|-----------------|-----------------|
| **Responders (n = 51)** | **Nonresponders (n = 30)** | **p** |
| Sex (male/female) | 20/31 | 9/21 | \( \chi^2 = 0.70 \) | 0.40\(^a\) |
| Age (year) (±SD) | 50.7 ± 12.4 | 51.8 ± 12.2 | \( t = -0.41 \) | 0.68\(^b\) |
| No. of previous episodes (±SD) | 0.47 ± 1.3 | 0.23 ± 0.6 | \( t = 0.97 \) | 0.34\(^b\) |
| Melancholia (+/−) | 16/35 | 9/21 | \( \chi^2 = 0.017 \) | 0.90\(^a\) |

\(^a\) Analysis performed with the use of the \( \chi^2 \) test.  
\(^b\) Analysis performed with the use of the unpaired \( t \)-test.
current analysis because plasma samples revealed very low milnacipran concentrations, indicative of poor compliance. Patients who completed the study included 52 women and 29 men, 50 outpatients and 31 inpatients.

Data collection
Depression symptom severity was assessed with the use of the MADRS. Assessments were conducted at baseline and at 1, 2, 4 and 6 weeks after initiation of antidepressant treatment. A single rater conducted each of the ratings for each patient. A clinical response was defined as a 50% or greater decrease in the baseline MADRS score. Clinical remission was defined as a final MADRS score less than 10 (Hawley et al., 2002). Collection of blood samples was performed 12 h after drug administration at bedtime, 4 weeks after initiation of antidepressant treatment.

Genotyping
The TH val81met polymorphism was determined by the method of Sharma et al. (1998). The COMT val158met polymorphism was determined by the method of Lachman et al. (1996).

Quantification of plasma milnacipran concentration
Plasma concentrations of milnacipran were measured with high performance liquid chromatography (HPLC). Details of the method have been described previously (Higuchi et al., 2003). Genotyping and measurement of plasma concentrations were performed by laboratory personnel blind to the identity and clinical antidepressant effect of the patients. Moreover, clinicians were unaware of the genotyping results and the plasma milnacipran concentrations of each patient.

Statistical analysis
Differences in patient characteristics were analysed with the use of the unpaired t-test or Chi-square test where appropriate. Differences in the MADRS scores during this study were examined with the use of two-way repeated-measures analysis of variance (ANOVA), with genotype and time as factors. Additional repeated-measures analysis of covariance (ANCOVA) was performed if necessary. When significant interaction between factors was observed, contrasts were used to enable comparisons between each two of the three genotype groups. Differences in the MADRS scores at each evaluation point were examined with the one-way factorial ANOVA followed by the Fisher’s PLSD test. Genotype deviation from the Hardy–Weinberg equilibrium was evaluated by the Chi-square test. Genotype distribution and allele frequencies were analysed with the use of the Chi-square test. Plasma concentrations of milnacipran were analysed with the use of one-way factorial ANOVA in each genotype group; an unpaired t-test was then used to analyse differences between groups who were or were not responsive to milnacipran. Statistical analysis was performed using StatView version 5.0 (SAS Institute, Inc., Cary, NC) and SuperANOVA version 1.11 (Abacus Concepts, Inc., Berkeley, CA). Power analysis was performed with the use of G-Power (Buchner et al., 1996). All tests were two-tailed; alpha was set at 0.05.

RESULTS

TH val81met polymorphism
The observed genotype frequencies of the TH val81met polymorphism were within the distribution expected according to the Hardy–Weinberg equilibrium. Figure 1 shows the MADRS scores over time in relation to the TH val81met polymorphism. Two-way repeated-measures ANOVA including all three genotype groups indicated no significant genotype × time interaction (F = 0.99, df = 8, p = 0.44). Plasma concentrations of milnacipran were not significantly different among each genotype group (val/val: 96.1 ± 32.6 (±SD), val/met: 86.2 ± 30.4, met/met: 92.2 ± 47.9, F = 0.35, df = 2, p = 0.71). No significant differences in the genotype

Figure 1. MADRS scores during 6 weeks of the treatment in three TH val81met genotype groups. (Each point represents the mean score ± SD. Differences in the MADRS scores during this study were examined with the use of repeated-measures ANOVA.) *There was no significant genotype × time interaction among all three genotype groups (F = 0.99, df = 8, p = 0.44)
distribution ($\chi^2 = 4.25$, df = 2, $p = 0.12$) and allele frequencies ($\chi^2 = 3.03$, df = 1, $p = 0.08$) were noted between responders and nonresponders. When remitters and nonremitters were compared, there was also no significant difference in the genotype distribution ($\chi^2 = 1.72$, df = 2, $p = 0.42$) and allele frequencies ($\chi^2 = 0.45$, df = 1, $p = 0.50$) (Table 2).

**COMT val158met polymorphism**

The observed genotype frequencies of the COMT val158met polymorphism were within the distribution expected according to the Hardy–Weinberg equilibrium. Figure 2 shows the MADRS scores over time in relation to the COMT val158met polymorphism. Two-way repeated-measures ANOVA including all three genotype groups indicated a significant genotype × time interaction ($F = 2.00$, df = 8, $p = 0.046$). Contrast analysis indicated a significant genotype × time interaction between the val/met and met/met genotype groups ($F = 3.31$, df = 4, $p = 0.011$). The MADRS score of the val/met genotype group was significantly lower than that of the met/met genotype group at the 0 week ($p = 0.0098$). Contrast analysis indicated a significant genotype × time interaction between the val/met and met/met genotype groups ($F = 3.19$, df = 4, $p = 0.011$). The MADRS score of the val/met genotype group was significantly lower than that of the met/met group at the 0 week ($p = 0.013$). Contrast analysis indicated no significant genotype × time interaction between the val/met and met/met genotype groups ($F = 0.49$, df = 4, $p = 0.74$). There was no significant difference in the MADRS scores at any evaluation point between the val/met and val/met genotype groups. To determine whether the initial difference of the MADRS scores affect the subsequent scores, a repeated measures ANCOVA was performed with the initial MADRS score as a covariate. This analysis revealed no significant time × initial MADRS score interaction ($F = 0.46$, df = 3, $p = 0.71$), indicating that the initial MADRS score was not a significant covariate.

To determine which aspects of depressive symptoms contributed to overall differences over time of the MADRS scores, the results of factor analyses of depression symptomatology using MADRS (Parker et al., 2003; Suzuki et al., 2005) were applied to the present results. Suzuki et al. (2005) identified three factors labelled dysphoria, retardation and vegetative symptoms. Figure 3 shows the dysphoria scores over time in relation to the COMT val158met polymorphism.

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Table 2. Genotype distribution and allele frequencies in responders/nonresponders and remitters/nonremitters in the TH val81met polymorphism

<table>
<thead>
<tr>
<th>Genotype distribution</th>
<th>Allele frequency</th>
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<tbody>
<tr>
<td></td>
<td>val</td>
</tr>
<tr>
<td>Responder</td>
<td>7 (13.7%)</td>
</tr>
<tr>
<td>Nonresponder</td>
<td>3 (10.0%)</td>
</tr>
<tr>
<td>Remitter</td>
<td>5 (11.4%)</td>
</tr>
<tr>
<td>Nonresponder</td>
<td>5 (13.5%)</td>
</tr>
</tbody>
</table>

*aAnalysis performed with the use of the $\chi^2$ test.

*bNo significant difference between responders and nonresponders ($\chi^2 = 4.25$, df = 2, $p = 0.12$).

*cNo significant difference between remitters and nonremitters ($\chi^2 = 1.72$, df = 2, $p = 0.42$).

*dNo significant difference between responders and nonresponders ($\chi^2 = 3.03$, df = 1, $p = 0.08$).

*eNo significant difference between remitters and nonremitters ($\chi^2 = 0.45$, df = 1, $p = 0.50$).
Two-way repeated-measures ANOVA for the scores of retardation and vegetative symptoms did not indicate significant genotype × time interactions (data not shown). Parker et al. (2003) identified three factors labelled dysphoric apathy/retardation, psychic anxiety and vegetative symptoms. Two-way repeated-measures ANOVA for the scores of psychic anxiety including all three genotype groups indicated a significant genotype × time interaction (F = 3.24, df = 8, p = 0.0015). As in the case of overall results and those based on the factor analyses by Suzuki et al. (2005), contrast analysis indicated a significant genotype × time interaction between the val/met and met/met genotype groups (F = 5.97, df = 4, p = 0.0001), and between the val/val and val/met genotype groups (F = 4.47, df = 4, p = 0.064). Two-way repeated-measures ANOVA for the scores of dysphoric apathy/retardation and vegetative symptoms did not indicate significant genotype × time interactions (data not shown).

Plasma concentrations of milnacipran were not significantly different among each genotype group (val/val: 82.7 ± 21.6 (±SD), val/met: 94.7 ± 44.9, met/met: 81.1 ± 34.7, F = 0.97, df = 2, p = 0.38). No significant differences in the genotype distribution (χ² = 1.79, df = 2, p = 0.41) and allele frequencies (χ² = 0.81, df = 1, p = 0.37) were noted between responders and nonresponders. When remitters and nonremitters were compared, there was also no significant difference in the genotype distribution (χ² = 0.93, df = 2, p = 0.63) and allele frequencies (χ² = 0.16, df = 1, p = 0.69) (Table 3).

**Power**

This study had a power of 0.12 to detect a small effect, 0.67 to detect a medium effect and 0.99 to detect a

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**Table 3.** Genotype distribution and allele frequencies in responders/nonresponders and remitters/nonremitters in the COMT val158met polymorphism

<table>
<thead>
<tr>
<th></th>
<th>Genotype distribution</th>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>val/val</td>
<td>val/met</td>
</tr>
<tr>
<td>Responder</td>
<td>14 (27.5%)</td>
<td>31 (60.8%)</td>
</tr>
<tr>
<td>Nonresponder</td>
<td>10 (33.3%)</td>
<td>19 (63.3%)</td>
</tr>
<tr>
<td>Remitter</td>
<td>13 (29.5%)</td>
<td>26 (59.1%)</td>
</tr>
<tr>
<td>Nonresmitter</td>
<td>11 (29.7%)</td>
<td>24 (64.9%)</td>
</tr>
</tbody>
</table>

*aAnalysis performed with the use of the χ² test.
*bNo significant difference between responders and nonresponders (χ² = 1.79, df = 2, p = 0.41).
*cNo significant difference between remitters and nonremitters (χ² = 0.93, df = 2, p = 0.63).
*dNo significant difference between responders and nonresponders (χ² = 0.81, df = 1, p = 0.37).
*eNo significant difference between remitters and nonremitters (χ² = 0.16, df = 1, p = 0.69).
large effect in the genotype distribution \((n = 81)\). For the allele frequency analysis \((n = 162)\), this study had a power of 0.25 to detect a small effect, 0.97 to detect a medium effect and 0.99 to detect a large effect. In the power analysis, effect size conventions were determined according to the method of Buchner et al. (1996) as follows: small effect size \(= 0.10\), medium effect size \(= 0.30\) and large effect size \(= 0.50\) (alpha = 0.05).

DISCUSSION

The present study revealed that the COMT val158met polymorphism affected the antidepressant effect of milnacipran. The met/met genotype of this polymorphism was associated with a significantly faster therapeutic effect in the MADRS scores during this study, although the difference in final therapeutic response was not significant between the met/met and other genotype groups.

Lachman et al. (1996) reported that individuals with the met/met genotype of the COMT val158met polymorphism had a threefold to fourfold reduction in enzymatic activity compared with those with the val/val genotype, and heterozygous individuals had intermediate enzymatic activity between that of homozygous individuals. However, the impact of the COMT val158met polymorphism on the metabolism of catecholamines appears to be minimal in usual physiological condition, even though it is a functional polymorphism. The high-affinity neuronal reuptake is an efficient elimination system for the released catecholamines, being responsible for most of their elimination both in the peripheral tissues and the brain (Mannisto and Kaakkola, 1999).

When exogenous levodopa, a dopamine precursor, is administered, the situation is dramatically altered for dopamine. During the combination therapy of levodopa and dopa decarboxylase inhibitor, the majority of surplus levodopa is preferably metabolised by COMT (Mannisto and Kaakkola, 1999). Individual differences of COMT activity become important for the pharmacological effect of levodopa in this situation.

The similar situation can occur to noradrenaline when its synaptic concentration is pharmacologically increased by the reuptake inhibition induced by milnacipran, though it has not been investigated yet. As the individuals with the met/met genotype of the COMT val158met polymorphism have a lower enzymatic activity, the synaptic concentration of norepinephrine may remain higher in patients with the met/met genotype than those with other genotypes. One possibility to explain the present result is that prolonged higher synaptic concentration of norepinephrine potentiates its neurotransmission particularly in patients with the met/met genotype, resulting in a faster antidepressant effect.

The present result about the COMT val158met polymorphism is not consistent with that of a previous study using an antidepressant mirtazapine (Szegedi et al., 2005). Szegedi et al. (2005) reported that carriers of the val/val and val/met genotype had significantly greater antidepressant effect than those of the met/met genotype. The initial pharmacological action of milnacipran and mirtazapine is not identical: that of the former is blockade of noradrenaline transporters, and that of the latter is blockade of \(\alpha_2\)-adrenergic autoreceptors. However, the discrepancy of the present results and those of Szegedi et al. (2005) cannot be explained by the difference of the initial pharmacological action of milnacipran and mirtazapine, because both drugs commonly result in enhanced noradrenergic transmission. Detailed mechanisms underlying the discrepancy of the present results and those of Szegedi et al. (2005) remain unclear.

Additional analyses based on the results of factor analyses of depression symptomatology revealed that the factor of dysphoria (Suzuki et al., 2005) and psychic anxiety (Parker et al., 2003) contributed to overall differences over time of the MADRS scores among each COMT val158met genotype group. The factor of dysphoria identified by Suzuki et al. (2005) and that of psychic anxiety identified by Parker et al. (2003) shares the symptoms of pessimistic and suicidal thoughts. Although serotonergic dysfunction in brain has been reported to be responsible for these symptoms (Carroll, 1994), this conclusion is not adequately justified by current evidence. For example, Poelinger and Haber (1989) found anxiety ratings decreased more with maprotiline (noradrenaline selective agent) than with fluoxetine (serotonin selective agent). Akkaya et al. (2006) reported that response rate for anxiety of reboxetine (noradrenaline selective agent) group was significantly higher than venlafaxine groups in the middle of treatment in patients with anxious depression, though the final response rate for anxiety was not significantly different. These findings and the present results suggest that the noradrenergic system in brain play a role in improvement of anxious symptoms of depression, and its genetic polymorphisms might affect the onset of therapeutic efficacy of milnacipran for anxiety in depression.
The present study also revealed that the TH val81met polymorphism did not affect the antidepressant effect of milnacipran. The TH val81met polymorphism is reported to be associated with early-onset alcoholism (Dahmen et al., 2005) and the left ventricular structure (Linhart et al., 2002). However, Ishiguro et al. (1998) reported that TH val81met polymorphism was not likely to play a major role in the genetic predisposition to schizophrenia, mood disorders or alcohol dependence. Kunugi et al. (1998) also reported no evidence for involvement of the TH val81met polymorphism in schizophrenia or Parkinson’s disease. The functional effect of the TH val81met polymorphism is still unknown, and the present results indicate no important role of the TH val81met polymorphism on the antidepressant effect of milnacipran.

One major limitation of this study is the relatively small number of subjects. A second limitation is the relatively small endpoint treatment differences in the analysis for the COMT val158met polymorphism. These limitations make it difficult to definitely conclude that the COMT val158met polymorphism is the genetic factor to predict the antidepressant effect of milnacipran. Difference in allele frequencies of the TH val81met polymorphism between responders and nonresponders seems marginal ($p = 0.08$), and increased number of subjects may reveal significant difference. Serotonergic effects of milnacipran cannot be neglected, and are probably independent of genetic differences in enzyme activities affecting catecholamine biosynthesis and elimination. Therefore, genetic polymorphisms of TH and COMT only have limited predictive value, and if any, can be at most partial predictors for the overall response to milnacipran. The authors performed collection of blood samples 4 weeks after initiation of antidepressant treatment. This schedule makes it impossible to perform an intent-to-treat analysis in relation to genetic polymorphisms, because the authors have no information of genotypes of dropout subjects. Further studies with a larger number of subjects are needed not only to confirm the results of this study but also to investigate the interaction of many genes, including the COMT gene, on the mechanisms of antidepressant action.

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