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

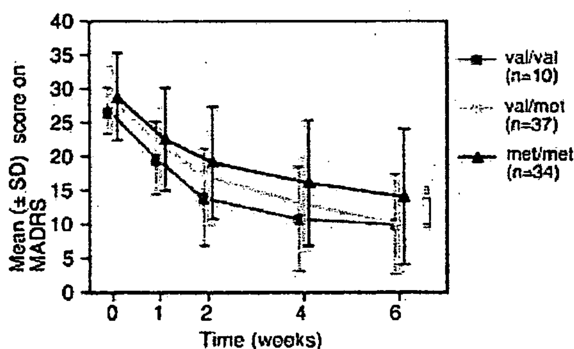

Figure 1. MADRS scores during 6 weeks of the treatment in three TH val81met genotype groups. (Each point represents the mean score ± SD. Differences in the MADRS scores during this study were examined with the use of repeated-measures ANOVA.) [a]There was no significant genotype × time interaction among all three genotype groups ($F = 0.99$, $df = 8$, $p = 0.44$)

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

	Genotype distribution[b,c]			Allele frequency[d,e]	
	val/val	val/met	met/met	val	met
Responder	7 (13.7%)	27 (52.9%)	17 (33.3%)	41 (40.2%)	61 (59.8%)
Nonresponder	3 (10.0%)	10 (33.3%)	17 (56.7%)	16 (26.7%)	44 (73.3%)
Remitter	5 (11.4%)	23 (52.3%)	16 (36.3%)	33 (37.5%)	55 (62.5%)
Nonremitter	5 (13.5%)	14 (37.8%)	18 (48.7%)	24 (32.4%)	50 (67.6%)

[a]Analysis performed with the use of the χ^2 test.
[b]No significant difference between responders and nonresponders (χ^2 = 4.25, df = 2, p = 0.12).
[c]No significant difference between remitters and nonremitters (χ^2 = 1.72, df = 2, p = 0.42).
[d]No significant difference between responders and nonresponders (χ^2 = 3.03, df = 1, p = 0.08).
[e]No significant difference between remitters and nonremitters (χ^2 = 0.45, df = 1, p = 0.50).

distribution (χ^2 = 4.25, df = 2, p = 0.12) and allele frequencies (χ^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 (χ^2 = 1.72, df = 2, p = 0.42) and allele frequencies (χ^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/val and met/met groups (F = 3.19, df = 4, p = 0.011). The MADRS score of the val/val 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/val and val/met genotype groups (F = 0.49, df = 4, p = 0.74). There was no significant difference in the MADRS score at any evaluation point between the val/val 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 the 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 polymorph-

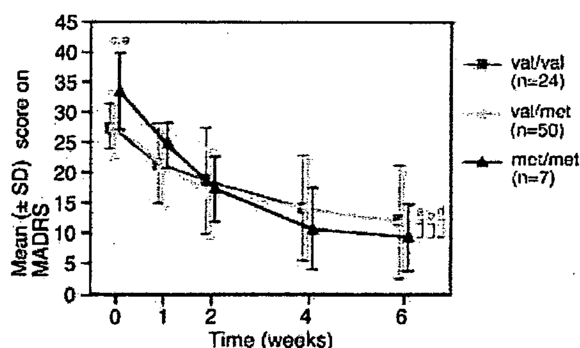

Figure 2. MADRS scores during 6 weeks of the treatment in three COMT val158met genotype groups. (Each point represents the mean score SD. Differences in the MADRS scores during this study were examined with the use of repeated-measures ANOVA. Differences in the MADRS scores at each evaluation point were examined with the use of one-way factorial ANOVA followed by Fisher's PLSD test.) [a]Significant genotype time interaction among all three genotype groups (F = 2.00, df = 8, p = 0.046). [b]Significant genotype time interaction between the val/met and met/met groups (F = 3.31, df = 4, p = 0.011). [c]Significant difference at the 0 week between the val/met and met/met groups (p = 0.0098). [d]Significant genotype time interaction between the val/val and met/met groups (F = 3.19, df = 4, p = 0.011). [e]Significant difference at the 0 week between the val/val and met/met groups (p = 0.013)

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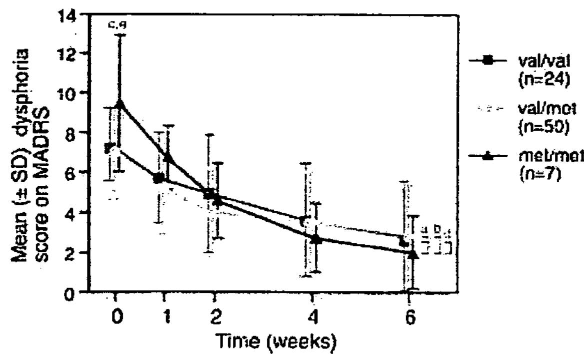

Figure 3. MADRS dysphoria scores during 6 weeks of the treatment in three COMT val158met genotype groups. (Each point represents the mean score SD. Differences in the MADRS dysphoria scores during this study were examined with the use of repeated-measures ANOVA. Differences in the MADRS dysphoria scores at each evaluation point were examined with the use of one-way factorial ANOVA followed by Fisher's PLSD test.) [a]Significant genotype time interaction among all three genotype groups (F ¼ 2.68, df ¼ 8, p ¼ 0.0074). [b]Significant genotype time interaction between the val/met and met/met groups (F ¼ 4.43, df ¼ 4, p ¼ 0.0017). [c]Significant difference at the 0 week between the val/met and met/met groups (p ¼ 0.016). [d]Significant genotype time interaction between the val/val and met/met groups (F ¼ 3.23, df ¼ 4, p ¼ 0.013). [e]Significant difference at the 0 week between the val/val and met/met groups (p ¼ 0.049)

ism. Two-way repeated-measures ANOVA for the dysphoria scores including all three genotype groups indicated a significant genotype time interaction (F ¼ 2.68, df ¼ 8, p ¼ 0.0074). As in the case of overall results, contrast analysis indicated a significant genotype time interaction between the val/met and met/met genotype groups (F ¼ 4.43, df ¼ 4, p ¼ 0.0017), and between the val/val and met/met genotype groups (F ¼ 3.23, df ¼ 4, p ¼ 0.013). Contrast analysis indicated no significant genotype time interaction between the val/val and val/met genotype groups (F ¼ 1.10, df ¼ 4, p ¼ 0.36). 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 ¼ 3.24, df ¼ 8, p ¼ 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 ¼ 5.97, df ¼ 4, p ¼ 0.0001), and between the val/val and met/met genotype groups (F ¼ 4.47, df ¼ 4, p ¼ 0.016). Contrast analysis indicated no significant genotype time interaction between the val/val and val/met genotype groups (F ¼ 0.63, df ¼ 4, p ¼ 0.64). 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 ¼ 0.97, df ¼ 2, p ¼ 0.38). No significant differences in the genotype distribution (χ^2 ¼ 1.79, df ¼ 2, p ¼ 0.41) and allele frequencies (χ^2 ¼ 0.81, df ¼ 1, p ¼ 0.37) were noted between responders and nonresponders. When remitters and nonremitters were compared, there was also no significant difference in the genotype distribution (χ^2 ¼ 0.93, df ¼ 2, p ¼ 0.63) and allele frequencies (χ^2 ¼ 0.16, df ¼ 1, p ¼ 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

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

	Genotype distribution[b,c]			Allele frequency[d,e]	
	val/val	val/met	met/met	val	met
Responder	14 (27.5%)	31 (60.8%)	6 (11.8%)	59 (57.8%)	43 (42.2%)
Nonresponder	10 (33.3%)	19 (63.3%)	1 (3.3%)	39 (65.0%)	21 (35.0%)
Remitter	13 (29.5%)	26 (59.1%)	5 (11.4%)	52 (59.1%)	36 (40.9%)
Nonremitter	11 (29.7%)	24 (64.9%)	2 (5.4%)	46 (62.2%)	28 (37.8%)

[a]Analysis performed with the use of the χ^2 test.
[b]No significant difference between responders and nonresponders (χ^2 ¼ 1.79, df ¼ 2, p ¼ 0.41).
[c]No significant difference between remitters and nonremitters (χ^2 ¼ 0.93, df ¼ 2, p ¼ 0.63).
[d]No significant difference between responders and nonresponders (χ^2 ¼ 0.81, df ¼ 1, p ¼ 0.37).
[e]No significant difference between remitters and nonremitters (χ^2 ¼ 0.16, df ¼ 1, p ¼ 0.69).

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large effect in the genotype distribution (n ¼ 81). For the allele frequency analysis (n ¼ 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 ¼ 0.10, medium effect size ¼ 0.30 and large effect size ¼ 0.50 (alpha ¼ 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 a_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.

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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 (p¼0.08), 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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Relationship between three serotonin receptor subtypes (*HTR3A, HTR2A* and *HTR4*) and treatment-resistant schizophrenia in the Japanese population

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Abstract
The proportion of treatment resistant schizophrenia (TRS) has been estimated as 20 to 40% in the schizophrenic patients. Genetic factors are considered to be involved in the development of this condition. Serotonin subtypes are hypothesized to be the candidate genes. In the present study, single marker and haplotype analyses between several mutations of serotonin receptor subtypes (*HTR2A, HTR3A* and *HTR4*) and TRS (TRS=101, Non-TRS=239) were performed to determine a possible relationship with the development o f TRS. Additionally, we also compared the daily neuroleptic dosage among each genotype. No significant association was observed between TRS and each allele, genotype, and haplotype. However, the daily neuroleptic dosage that patients had been receiving during their maintenance therapy was significantly higher in patients with the T/T genotype of *HTR3A* polymorphism (rs1062613, p=0.041). The present results support further research to examine the relationship between *HTR3A* polymorphism and the development of TRS in the Japanese population.
Keyword: *HTR*, serotonin, antipsychotic drug, refractory, gene, haplotype

Introduction
The proportion of treatment resistant schizophrenia (TRS) has been estimated as 20 to 40 % in the schizophrenic patients, and this unfortunate situation in the clinical psychiatric field still remain unchanged even after the introduction of several atypical antipsychotic agents [1]. Among the atypical antipsychotics, only clozapine has been reported to be effective for 30-60 % of schizophrenic patients refractory to typical and atypical antipsychotics [2, 3]. Clozapine is known to provide antipsychotic effects through binding to the several serotonin receptor subtypes (5-HT) [4]
although the actual mechanism of clozapine for TRS has not been elucidated yet. In order to clarify this mechanism several researches investigated the predictable genetic factors for the clinical response to clozapine, as a result a significant association with the 5-HT receptor subtypes has been reported in a number of studies as follows.
Clozapine has a high affinity for 5-HT2A receptor [5] and produces a significant downregulation of cortical 5-HT2A receptor in the radioligand binding studies [6]. In addition, two PET studies have shown that the systemic administration of clozapine to schizophrenic patients produces an 84-90% occupation of cortical 5-HT2A receptor [7, 8]. A couple of researches have reported the association between 5-HT2A receptor gene (*HTR2A)* polymorphism and TRS [9, 10] or response to clozapine [11], although no association study has been reported in the Japanese subjects with TRS. Since the 5-HT3A receptor has been reported to have potential anxiolytic and anti-psychotic properties from animal studies, 5-HT3A receptor antagonists are being explored as therapeutic agents for a variety of behavioral disorders [12]. Additionally, 5-HT3A receptor gene (*HTR3)* is located on 11q23.1, where linkage with schizophrenia has been suggested in several studies [13, 14]. These results suggest that *HTR3* may be related to the treatment response in the schizophrenic patients. Gutierrez have reported no association between *HTR3A* polymorphism and clozapine response [15], however, this study did not take haplotype block structure into consideration and did not cover whole genomic region of *HTR3A*.
5-HT4 receptor gene (*HTR4)* also has been reported to be associated with schizophrenia in the Japanese population [16]. Therefore, this gene could also be a candidate gene for TRS.
Thus, the *HTR2A, 3A, 4* could be considered as plausible genes related to the development of the TRS. Therefore, in the present study, we performed Linkage disequilibrium (LD) analysis of *HTR3A*, followed by the case-control association studies between *HTR3A* polymorphisms and TRS using

single-marker association analyses and haplotype analyses. In addition, the association was also examined between *HTR2A* polymorphism, *HTR4* polymorphism and TRS.

Materials and Methods

Subjects

This study was initiated after the approval by the Ethics Committee of the Nagoya University School of Medicine. Written informed consent was obtained from all subjects at study entry.

A total of 340 patients with schizophrenia (male = 200, female = 140, age: 54 ± 12.8, duration of illness: 33.6 ± 12.4 years, daily neuroleptic dosage: 1021 ±1857 mg/day) who had been diagnosed using the criteria of DSM-IIIR (American psychiatric association, 1987) were selected in this study. All patients were Japanese descent and had been hospitalized and receiving antipsychotic drugs for more than one year.

Definition of TRS

The definition of TRS is described elsewhere in the previous study [17]. Briefly, information about the neuroleptic therapy that the schizophrenic patients had been receiving was obtained from their clinical records. The daily neuroleptic dosage was calculated from the recent one year neuroleptic prescription history. Schizophrenic patients were diagnosed as having TRS when they had been hospitalized for more than 1 year and had been receiving antipsychotic therapy at dosages of at least 1,000 mg/day chlorpromazine equivalents for more than one year.

SNP selection and Genotyping

Using the information obtained from the HapMap Database and the dbSNP Database, two single nucleotide polymorphisms— rs1062613 and rs1176713—were selected as haplotype tag SNPs (htSNPs) that covered the whole coding region, 5' flanking region upstream 500bp, and 3' UTR region downstream 500bp of *HTR3A*. The LD block was defined using HAPLOVIEW version 3.0 (http://www.broad.mit.edu/mpg/haploview/) as a region of D'>0.8. In each LD block, haplotype frequency was estimated by the expectation-maximization (EM) algorithm and htSNPs were selected using the same program. Additionally, a SNP (rs6313) of *HTR2A* and two SNPs (rs2278392, rs3734119) of *HTR4* which have been reported to be associated with schizophrenia in the previous study [16] were selected. Genotyping was carried out using polymerase chain reaction-restriction fragment length polymorphism assays or direct sequence assays for each SNP. Sequences of each primer pairs are available on request.

Statistics

Genotype deviation from the Hardy-Weinberg equilibrium (HWE) was evaluated by Chi square test. Single-marker and haplotype analyses were performed using SPSS version 11.0J (Tokyo, Japan) and Cocaphase 2.403 (http://www.rfcgr.mrc.ac.uk/~fdudbrid/software/unphased/), respectively.

Comparison of the daily neuroleptic dosage among each genotype was performed using Mann-Whitney *U* test. Power calculation was performed by Power Caluculator (http://calculators.stat.ucla.edu/powercalc/). The level of significance for all statistical tests was set at 0.05.

Results

A total of 101 schizophrenic patients were identified as the TRS (TRS: male=67, female=34, age=50±10.5, onset age=20±5.3; NON-TRS: male=133, female=106, age=56±13.1, onset age=23.5±8.2). The male ratio tended to be higher in the TRS patients ($p<0.1$), and the age at onset was significantly younger in this group ($p=0.009$). However, no significant difference was observed in the incidence of any psychiatric symptom between the two groups, such as delusion and hallucination, bizarre behavior, disorganization, and negative symptoms at their first episode, as reported in our previous report [18]. The genotype distributions of the polymorphisms did not deviate significantly from the HWE in each study group for any polymorphism. The genotype and allele frequencies of 3 kinds of serotonin receptor genes in TRS and NON-TRS groups are shown in Table1.

No significant association was observed in the single marker analysis of *HTR2A*, *HTR3A*, and *HTR4*, and in haplotype analysis of *HTR3A* and *HTR4* (Table1).

The characteristics of neuroleptic treatment among the three subgroups showing each SNP polymorphism are shown in Table2. In rs1062613 of *HTR3A*, the daily neuroleptic dosage during maintenance therapy was significantly higher in patients with the T/T genotype than the others ($p = 0.041$).

When the proportion of TRS was set to be 30% [19], we obtained more than 80% power to detect an association with the SNPs of which the minor frequency is more than 10%.

Discussion

The results presented here suggest that *HTR3A* may be involved in the development of TRS in the Japanese population. In this study, significant difference in the daily neuroleptic dosage received during maintenance therapy was observed in schizophrenic patients with the T/T genotype of *HTR3A* polymorphism (rs1062613).

The SNP rs1062613 is located on the promoter

region of *HTR3A* and has been reported to regulate the expression of this gene [20]. Since presynaptic 5-HT3A receptors modulate the release of several neurotransmitters in various brain regions [21, 22], the abnormal expression of *HTR3A* might increase the dopamine concentration in the synaptic cleft. This may lead to increase the therapeutic antipsychotic doses in the schizophrenic patients with this mutation.

Additionally, several antipsychotic drugs reduce the dopaminergic neurotransmission by antagonizing the 5-HT3A receptor[23]. Therefore, reduction in the expression of 5-HT3A receptor may weaken the therapeutic effect of antipsychotics through this pathway; even higher dose of most antipsychotic drugs may not reduce the dopaminergic neurotransmission.

Furthermore, this SNP has been reported to have a critical role in the amygdala activity leading to the facial expression recognition [24], and the defect of facial expression recognition has been reported to be a specific symptom to the schizophrenia including TRS [25, 26]. Therefore, this SNP may have a role in the development of TRS based on the effect of the SNP on the impairment of facial expression recognition.

The definition of TRS in the present study is different form that proposed by Kane et al (27).

Since the polypharmacy is widely prevalent in the antipsychotic treatment of schizophrenia in Japan. In the present study, the psychopathology of TRS was defined by the total antipsychotic doses that the schizophrenic patients had been receiving during the recent 1 year, that is, the severity of illness was extrapolated by the total antipsychotic doses. In addition, they had been hospitalized for more than 1 year, indicating that they had been no good level of functioning over this period. In fact, age at disease onset had been observed to be significantly younger in the TRS subjects, suggesting that the younger onset patients tend to less response to the antipsychotic therapy. Therefore, we consider that virtually no essential difference exists between the present definition of TRS enrolled in Japan and that proposed by Kane et al (27).

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Table

Table 1: Genotype and allele frequencies of *HTR2A*, *HTR3A* and *HTR4* polymorphisms in TRS and NON-TRS

		Genotype		P value	Allele frequency (%)		P value	Global P-value
HTR2A								
rs6313	C/C	C/T	T/T		C	T		
TRS	19	58	23	0.500	48	52	0.777	
NON-TRS	48	123	68		46	54		
HTR3A								
rs1062613	C/C	C/T	T/T		C	T		
TRS	75	21	5	0.117	85	15	0.400	
NON-TRS	189	47	3		89	11		0.576
rs1176713	A/A	A/G	G/G		A	G		
TRS	49	38	14	0.744	67	33	0.648	
NON-TRS	124	86	27		70	30		
HTR4								
rs2278392	G/G	G/A	A/A		G	A		
TRS	59	36	7	0.867	76	24	0.868	
NON-TRS	148	80	15		77	23		0.863
rs3734119	T/T	T/C	C/C		T	C		
TRS	59	36	8	0.891	75	25	0.869	
NON-TRS	148	80	19		76	24		

Table

Table 2: Characteristics of NLP treatment among three subgroups showing *HTR2A*, *HTR3A* and *HTR4* polymorphisms

	Genotype		
HTR2A			
rs6313	C/C	C/T	T/T
Daily NLP	575 (2-4042)	603 (4-12893)	372 (3-6283)
HTR3A			
rs1062613	C/C	C/T	T/T
Daily NLP	496 (2-12893)	568 (5-12850)	1179 (281-3048)[a]
rs1176713	A/A	A/G	G/G
Daily NLP	559 (3-8337)	417 (2-12893)	710 (42-4226)
HTR4			
rs2278392	G/G	G/A	A/A
Daily NLP	491 (2-12893)	600 (4-6283)	460 (50-2262)
rs3734119	T/T	T/C	C/C
Daily NLP	491 (2-12893)	605 (4-6283)	439 (30-2262)

Data are expressed as median (Min-Max).

[a] **P=0.041** when compared to the (C/C+C/T) subgroup.

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BRIEF REPORT

Genetic polymorphisms in the 5-hydroxytryptamine type 3B receptor gene and paroxetine-induced nausea

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Abstract

Selective serotonin reuptake inhibitor (SSRI)-induced nausea can be severe enough to lead to early treatment discontinuation. However, it is currently not possible to predict the occurrence of nausea before the initiation of SSRI treatment. In this study, we investigated the effect of genetic polymorphisms in the 5-hydroxytryptamine type 2A, 3A, and 3B ($5\text{-}HT_{3B}$) receptors, 5-HT transporter, and *CYP2D6* genes on the incidence of paroxetine-induced nausea. A consecutive series of 72 Japanese patients with depressive or anxiety disorders were treated with paroxetine. Paroxetine-induced nausea was assessed by a pharmacist and was observed in 29.2% of the patients. A significant (nominal $p=0.00286$) association was found between the incidence of nausea and the -100_-102AAG insertion/deletion polymorphism of the $5\text{-}HT_{3B}$ receptor gene. No significant associations were observed between the other genetic polymorphisms and the incidence of nausea. The -100_-102AAG deletion variant of the $5\text{-}HT_{3B}$ receptor gene may affect paroxetine-induced nausea.

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Key words: Genetic polymorphism, nausea, paroxetine, selective serotonin reuptake inhibitor, 5-hydroxytryptamine type 3B receptor.

Introduction

Paroxetine, a selective serotonin reuptake inhibitor (SSRI), has been widely and successfully used in the treatment of major depressive disorder, panic disorder, obsessive–compulsive disorder, generalized anxiety disorder and social phobia (Wagstaff et al., 2002). SSRIs, including paroxetine, have now become the first-line treatment for depression, especially in elderly patients, replacing the well-established tricyclic antidepressants, probably because of their better adverse-effect profile and safety. However, SSRIs induce adverse effects such as gastrointestinal symptoms, headache, anxiety, and sexual dysfunction. Of the gastrointestinal side-effects, nausea is the most problematic. It has been reported to be the most frequent adverse effect induced by SSRIs, occurring in about 15–30% of treated patients (Ottevanger, 1994; Takahashi et al., 2002; Wagner et al., 1992), and the nausea can often be severe enough to lead to early discontinuation of treatment (Murphy et al., 2003;

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Ottevanger, 1994; Wagner et al., 1992). The four main SSRIs (sertraline, fluvoxamine, fluoxetine and paroxetine) have been shown to be similar in this regard (Ottevanger, 1994). Therefore, it would be of great benefit to be able to predict the occurrence of nausea before SSRI treatment is started.

Bergeron and Blier (1994) reported that a low dose of cisapride, which is not available now because of its cardiotoxic effects, with the 5-hydroxytryptamine type 3 ($5\text{-}HT_3$) antagonistic and $5\text{-}HT_4$ agonistic property produced rapid relief from nausea elicited by SSRIs. Bailey et al. (1995) showed in a double-blind, placebo-controlled study that the co-administration of ondansetron, a selective $5\text{-}HT_3$ receptor antagonist, significantly attenuated the nausea induced by fluvoxamine in healthy volunteers. Thus, nausea induced by SSRIs seems to be elicited by serotonin release and the consecutive activation of the $5\text{-}HT_3$ receptors on peripheral vagal afferent terminals in the gastrointestinal mucosa and central structures such as the area postrema and nucleus tractus solitarii (Miller and Leslie, 1994; Tyers and Freeman, 1992). A few studies using the whole-cell patch-clamp technique have shown that fluoxetine inhibited the $5\text{-}HT_3$ receptor-mediated currents (Choi et al., 2003; Fan, 1994) and indicate that the $5\text{-}HT_3$ receptor may contribute to the pharmacological actions of SSRIs.

Tremblay et al. (2003) reported an interesting and notable study that investigated the efficacy of $5\text{-}HT_3$ receptor antagonists for chemotherapy-induced nausea and vomiting in cancer patients and showed that patients who underwent anti-emetic treatment and who were homozygous for the -100_-102AAG deletion allele of the $5\text{-}HT_{3B}$ receptor gene had the highest nausea and vomiting score, whereas patients homozygous for the -100_-102AAG insertion allele had the lowest nausea and vomiting score.

Based on the reports described above, we hypothesized that polymorphisms of the $5\text{-}HT_3$ receptor gene might be also associated with the incidence of paroxetine-induced nausea.

Paroxetine is metabolized extensively by cytochrome P450 2D6 (CYP2D6) (Sindrup et al., 1992), and CYP2D6 is known to have genetic polymorphisms that affect enzymatic activity (Zanger et al., 2004). These observations suggest that the *CYP2D6* gene polymorphism may influence paroxetine-induced nausea.

Although, several studies have investigated the relationship between serotonergic genetic polymorphisms such as $5\text{-}HT_{2A}$ receptor and 5-HT transporter (*5-HTT*) genes and SSRI-induced nausea (Murphy et al., 2003, 2004; Sugai et al., 2006; Suzuki et al., 2006; Takahashi et al., 2002), the association of the variants with individual differences in the occurrence of SSRI-induced nausea remains controversial. At present, it is not possible to predict the occurrence of nausea before the initiation of SSRI treatment.

The present study was done to elucidate the effects of genetic polymorphisms in the serotonergic receptors, *5-HTT* and *CYP2D6* genes of Japanese patients on the incidence of paroxetine-induced nausea.

Methods

Subjects

The subjects were a series of 81 consecutive Japanese outpatients who were treated with paroxetine and who provided written informed consent after the procedure was fully explained. All of the patients had DSM-IV diagnoses (APA, 1994) of depressive disorders or anxiety disorders, and were treated at the Department of Psychosomatic Medicine, Kyushu University Hospital. Patients with severe physical illness were excluded from the study. No patients were being treated with anti-emetic medication during the present study. The patients visited the hospital once each week during the first 2 wk of treatment, and then visited every 2 wk for the treatment and assessment of adverse effects, including nausea. The paroxetine dose was increased from 10 mg/d to 20, 30, or 40 mg/d in response to clinical symptoms. Patients taking benzodiazepines, zolpidem or tandospirone were included. No other psychotropic drugs were administered during the study. The study was approved by the Ethics Committee of the Kyushu University Hospital.

Assessment of nausea induced by paroxetine

The severity of adverse effects after the administration of paroxetine was assessed according to the Udvalg for Kliniske Undersogelser (UKU) side-effects rating scale (Lingjaerde et al., 1987). For the assessment, a pharmacist interviewed the patient before and 1, 2, 4, 8, and 12 wk after the start of treatment. Because nausea and vomiting are not only adverse effects of paroxetine treatment but also symptoms of depressive disorders, subjects who had nausea before treatment were excluded.

DNA analysis

Genomic DNA was isolated from peripheral blood using the QIAamp DNA Blood Mini kit (Qiagen, Hilden, Germany). For the identification of polymorphisms in the protein coding exons including the

exon–intron junctions and the 5′-flanking region of the *5-HT*$_{3B}$ receptor gene, polymerase chain reaction–single strand conformational polymorphism (PCR–SSCP) analysis was performed with genomic DNA obtained from 81 unrelated patients. The primer design was based on published sequences (GenBank accession number NM006028). The primer sequences are presented in Supplementary Table S1 (available in the online version of the paper). PCR products were sequenced either directly or after subcloning on an ABI 310 automatic sequencer (Applied Biosystems, Foster City, CA, USA). For genotyping of the *5-HT*$_{3A}$ receptor gene, PCR–restriction fragment length polymorphism assays were developed; *Hpy*188III for C-42T (dbSNP database identification number, rs1062613), *Dra*I for G2423A (rs1176722), and *Bfa*I for A14371G (rs1176713). The other gene polymorphisms were genotyped according to the previously described methods for T102C (rs6313) of the *5-HT*$_{2A}$ receptor gene (Ozaki et al., 1996), *5-HTT* gene-linked polymorphic region (5-HTTLPR) (Takahashi et al., 2002), and *CYP2D6* (Zanger et al., 2004).

Determination of plasma concentration of paroxetine

Blood samples were obtained after at least 2 wk of the same daily dose of paroxetine was administered to ensure that patients had a steady-state plasma concentration of paroxetine. Blood was collected into a heparinized tube approximately 15.7±2.4 h (0.5–20.2) after the last dose of paroxetine. The plasma level of paroxetine was determined using a gas chromatography–mass spectrometry method described by Eap et al. (1998).

Statistical analysis

The significance of frequency differences of the different genotypes was assessed by Fisher's exact test. The scores of nausea and vomiting of the genotype groups were compared by the Mann–Whitney *U* test. The limit of significance was set to 0.05. Bonferroni correction was performed for multiple comparisons and the nominal p value was considered significant at <0.00294 (0.05 divided by 17 genetic polymorphisms). The plasma level of paroxetine was compared by one-way ANOVA.

Results

Of the 81 patients, nine who had nausea before the administration of paroxetine were excluded, leaving 35 male and 37 female patients (57 depressive disorder, 15 anxiety disorder; age range 17–78 yr; mean age ± S.D. 47.0±17.5 yr; mean body weight ± S.D. 62.2±11.4 kg) for analysis of the effect of genetic polymorphisms on nausea induced by paroxetine. Of these 72 patients, 21 (29.2%) experienced nausea within the first week after administration of paroxetine, and two (2.8%) discontinued the treatment with paroxetine due to severe paroxetine-induced nausea.

Among the 81 unrelated patients, eleven polymorphisms were detected in the *5-HT*$_{3B}$ receptor gene by SSCP analysis and identified by subsequent sequencing (Table 1). In the coding region, A27373G was detected with an amino-acid substitution from Tyr to Ser at codon 129 (Tyr129Ser). G27449A (Ala154Ala) was observed as a synonymous polymorphism and G26943A, A26946G, and A28232G were found as intronic polymorphisms. In the 5′-flanking region, six polymorphisms were identified: C-1710T, T-833C, G-761A, T-381C, C-206T and an AAG deletion at -100 to -102. The genotype distributions of these 11 variants were in Hardy–Weinberg equilibrium. A screening of the dbSNP database showed T-833C, C-206T and G26943A to be novel variants.

As shown in Table 2, a significant (nominal $p=0.00286$, Fisher's exact test) difference in genotypic distribution associated with the -100_-102AAG insertion/deletion polymorphism of the *5-HT*$_{3B}$ receptor gene was found between patients with and without nausea, and the -100_-102AAG deletion allele carriers (i.e. patients with either insertion/deletion or deletion/deletion) showed a significantly higher incidence of nausea than patients who were homozygous for the -100_-102AAG insertion allele. The Tyr129Ser polymorphism of the *5-HT*$_{3B}$ receptor gene had no significant effect on the incidence of paroxetine-induced nausea (nominal $p=0.0680$, Fisher's exact test) nor did the other polymorphisms of the *5-HT*$_{3B}$ receptor gene [data are presented in Supplementary Table S2 (available online)].

No significant differences between patients with and without nausea were found in the genotype distribution of C-42T, G2423A, and A14371G of the *5-HT*$_{3A}$ receptor gene, T102C of the *5-HT*$_{2A}$ receptor gene, 5-HTTLPR of the *5-HTT* gene and *CYP2D6* gene polymorphisms (Supplementary Table S2).

The nausea score was significantly higher in the -100_-102AAG deletion allele carriers than in patients homozygous for the -100_-102AAG insertion allele [median (25%, 75%): 1 (0, 1) vs. 0 (0, 0), nominal $p=0.0028$, Mann–Whitney *U* test). No significant associations were observed between the other genetic polymorphisms and the nausea score (data not shown).

Table 1. Genetic polymorphisms of the *5-HT$_{3B}$* receptor gene in 81 Japanese patients (36 males and 45 females)

Location	Position[a]	Reference allele[b]	Variant allele	Amino acid substitution	rs number[c]	Genotype[d] R/R	R/V	V/V	Frequency of variant allele
5'-flanking region	-1710	gccaCtgtc	gccaTtgtc	–	rs10789970	11	39	31	0.623 (0.544–0.702)
	-833	ggggTgtct	ggggCgtct	–	–	76	5	0	0.031 (0.003–0.059)
	-761	atgcGtatt	atgcAtatt	–	rs11214763	61	20	0	0.123 (0.070–0.176)
	-381	agagTactg	agagCactg	–	rs3758987	41	33	7	0.290 (0.216–0.364)
	-206	atgaCggca	atgaTggca	–	–	77	4	0	0.025 (0.000–0.050)
	-100_-102	ggagAAGgagg	ggag—gagg	–	rs3831455[e] rs35312182	51	28	2	0.196 (0.132–0.260)
Intron 4	26943	gtgtGccag	gtgtAccag	–	–	78	3	0	0.019 (0.000–0.041)
	26946	tgccAgtgt	tgccGgtgt	–	rs1176746	6	29	46	0.747 (0.676–0.818)
Exon 5	27373	agatAccct	agatGccct	Tyr129Ser	rs1176744	44	31	6	0.265 (0.193–0.337)
	27449	ctgcGtgca	ctgcAtgca	Ala154Ala	rs2276305	56	25	0	0.154 (0.095–0.213)
Intron 6	28232	aggaAtttc	aggaGtttc	–	rs2276307	44	32	5	0.259 (0.188–0.330)

Values in parentheses indicate 95% confidence intervals.
[a] Position is in respect to the translation start site of the *5-HT$_{3B}$* receptor gene; the A in the ATG is +1 and the base immediately 5' is -1.
[b] Reference allele: GenBank accession no. NM006028.
[c] NCBI dbSNP database identification number.
[d] R, reference allele; V, variant allele.
[e] -104_-106GGA deletion (rs3831455) is identical to -100_-102AAG deletion when A-102G (rs35312182) is the variant allele.

For the determination of the plasma levels of paroxetine, 63 blood samples from 50 patients who met the blood sampling conditions were tested. The mean plasma levels of paroxetine were 76.3±77.2 and 60.0±48.9 ng/ml in patients with and without nausea, respectively, and there was no significant difference ($p = 0.4009$, one-way ANOVA).

Discussion

SSRIs frequently induce nausea as an adverse effect. In the present study, nausea was observed in approximately 30% of patients within the first week after the administration of paroxetine. These results are in keeping with previous reports in which the percentage of patients treated with SSRIs who experienced nausea ranged from 14% to 30% (Ottevanger, 1994; Takahashi et al., 2002). Nausea is also the most common reason for early discontinuation of SSRI treatment (Murphy et al., 2003; Ottevanger, 1994; Wagner et al., 1992).

In the present study, we showed that patients who had the -100_-102AAG deletion allele of the *5-HT$_{3B}$* receptor gene experienced paroxetine-induced nausea more frequently than other patients. In other words, patients homozygous for the -100_-102AAG insertion allele had a lower risk of developing nausea than did other patients.

The way in which SSRIs cause nausea has not been fully elucidated. Bergeron and Blier (1994) reported in an open study that the non selective 5-HT$_3$ antagonist cisapride reduced SSRI-induced nausea in most patients. Bailey et al. (1995) showed that coadministration of the 5-HT$_3$ antagonist ondansetron significantly reduced nausea induced by fluvoxamine

Table 2. The genotype distribution of -100_-102AAG insertion/deletion polymorphism of the *5-HT$_{3B}$* receptor gene in 72 patients with and without paroxetine-induced nausea

	Genotype distribution, *n* (%)				
	Ins/Ins	Ins/Del	Del/Del	Ins/Del+Del/Del	*p* value[a]
Nausea (+)	8 (38.1)	12 (57.1)	1 (4.8)	13 (61.9)	0.00286*
Nausea (−)	39 (76.5)	11 (21.6)	1 (2.0)	12 (23.5)	

Ins, Insertion; Del, deletion.
The severity of nausea and vomiting was scored as follows: Degree 0, no or doubtful nausea; Degree 1, slight nausea; Degree 2, disturbing nausea but without vomiting; Degree 3, nausea with vomiting. Subjects with a score of 1, 2, or 3 were defined as patients with nausea, whereas those with a score of 0 were defined as patients without nausea.
[a] Nominal *p* value by Fisher's exact test.
*5% statistical significant with Bonferroni correction for multiple comparison.

in volunteers in a double-blind, placebo-controlled study. The above studies strongly suggest that nausea induced by SSRIs might be mediated by the activation of 5-HT$_3$ receptors.

On the other hand, it has been shown that 5-HT$_3$ receptor antagonists can prevent chemotherapy-induced nausea in cancer patients (Kris et al., 2006). However, there are individual differences in the efficacy of anti-emetic treatment with 5-HT$_3$ receptor antagonists in cancer patients suffering from chemotherapy-induced nausea. Tremblay et al. (2003) reported that genetic polymorphism of the *5-HT$_{3B}$* receptor gene might be a pharmacogenetic predictor of the effect of anti-emetic treatment with 5-HT$_3$ receptor antagonists in cancer patients receiving chemotherapy. Interestingly, they showed that patients who were homozygous for the -100_-102AAG deletion allele of the *5-HT$_{3B}$* receptor gene, which is the same allele of the *5-HT$_{3B}$* receptor gene found in our study in relation to paroxetine-induced nausea, experienced vomiting more frequently than did other patients. Frank et al. (2004) also reported that the prevalence of the -100_-102AAG deletion of the *5-HT$_{3B}$* receptor gene was much lower in patients with bipolar affective disorder compared with controls and that the -100_-102AAG deletion variant may alter the structure of the 5′-untranslational region of mRNA, leading to changes in the translational efficiency of the 5-HT$_{3B}$ receptor.

To our knowledge, there are no in-vitro studies with regard to the functional effects of the -100_-102AAG deletion variant of the *5-HT$_{3B}$* receptor gene. We speculate that the -100_-102AAG deletion variant might regulate the mRNA and protein expression levels of the 5-HT$_{3B}$ receptor, because this variant is located in the 5′-flanking region of the *5-HT$_{3B}$* receptor gene. Otherwise, the possibility that the -100_-102AAG deletion variant is linked with other, yet unknown variants which change the 5-HT$_3$ receptor function could not be excluded. Barrera et al. (2005) showed that the subunit stoichiometry of the heteromeric 5-HT$_{3A}$ and 5-HT$_{3B}$ receptors is 2A:3B and that the subunit arrangement is B-B-A-B-A. The 5-HT$_{3B}$ subunit is thought to modify the 5-HT$_{3A}$ receptor and seems to be a major determinant of 5-HT$_3$ receptor function (Davies et al., 1999; Dubin et al., 1999). However, a functional explanation of the relationship between the -100_-102AAG deletion variant of the *5-HT$_{3B}$* receptor gene and nausea remains unaccounted for.

Sugai et al. (2006) reported that the 129Tyr/Tyr genotype of the *5-HT$_{3B}$* receptor gene had a significant effect on paroxetine-induced nausea. In the present study, we screened for genomic DNA polymorphisms in the protein coding exons, including the exon–intron junctions and the 5′-flanking region of the *5-HT$_{3B}$* receptor gene, and did not find a significant association between Tyr129Ser polymorphism and paroxetine-induced nausea. To resolve this discrepancy, we determined haplotypes for the -100_-102AAG insertion/deletion and Tyr129Ser polymorphisms, and three major haplotypes were identified: AAG(insertion)-Tyr, AAG(insertion)-Ser, and AAG(deletion)-Tyr. Because the Tyr129 allele was in linkage disequilibrium with the -100_-102AAG insertion and deletion alleles, there may be differences in the results of the two studies. Because Sugai et al. (2006) investigated only one variant of the *5-HT$_{3B}$* receptor gene and did not

examine the -100_-102AAG insertion/deletion polymorphism, the association between this polymorphism and paroxetine-induced nausea in the same subjects of their study remains unclear. The functional effects of the Tyr129Ser variant have not been clarified, thus further in-vivo and in-vitro studies with regard to haplotypic considerations are needed to elucidate the functional properties of these polymorphisms of the *5-HT$_{3B}$* receptor gene.

The sample size in the present study was too small. Therefore, in order to elucidate the associations between paroxetine-induced nausea and polymorphisms of the *5-HT$_{2A}$* receptor, *5-HT$_{3A}$* receptor, *5-HTT* and *CYP2D6* genes, further studies with larger sample sizes are warranted.

In conclusion, the -100_-102AAG insertion/deletion polymorphism of the *5-HT$_{3B}$* receptor gene may affect the incidence of paroxetine-induced nausea in Japanese patients with depressive and anxiety disorders. If our findings are replicated in further studies with larger sample sizes, genotyping of the *5-HT$_{3B}$* receptor gene before the initiation of treatment with paroxetine might provide a substantial improvement in paroxetine treatment of patients with depressive and anxiety disorders.

Note

Supplementary information accompanies this paper on the Journal's website (http://journals.cambridge.org).

Acknowledgements

None.

Statement of Interest

None.

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A possible association between missense polymorphism of the breakpoint cluster region gene and lithium prophylaxis in bipolar disorder

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Abstract

Lithium is one of the most commonly used drugs for the treatment of bipolar disorder. To prescribe lithium appropriately to patients, predictors of response to this drug were explored, and several genetic markers are considered to be good candidates. We previously reported a significant association between genetic variations in the breakpoint cluster region (BCR) gene and bipolar disorder. In this study, we examined a possible relationship between response to maintenance treatment of lithium and Asn796Ser single-nucleotide polymorphism in the BCR gene. Genotyping was performed in 161 bipolar patients who had been taking lithium for at least 1 year, and they were classified into responders for lithium monotherapy and non-responders. We found that the allele frequency of Ser796 was significantly higher in non-responders than in responders. Further investigation is warranted to confirm our findings.
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Keywords: BCR (breakpoint cluster region); Bipolar disorder; Lithium; SNP (single-nucleotide polymorphism)

1. Introduction

Bipolar disorder (BPD) is one of the most distinct syndromes in psychiatry, which is characterized by recurrent episodes of mania and depression. Three representative mood stabilizers, lithium, valproate and carbamazepine, are used worldwide for its treatment, and American Psychiatric Association guideline listed lithium as a first line agent (American Psychiatric Association, 2002). However, these treatments are associated with variable rates of efficacy and often with intolerable side effects. Therefore, many researchers explored psychopathological and biological markers for good response to lithium treatment (Gelenberg and Pies, 2003; Ikeda and Kato, 2003). To date, several studies investigated possible molecular predictors of lithium efficacy. The functional polymorphism in the upstream regulatory region of the serotonin transporter gene (5-HTTLPR) has been associated with lithium efficacy in two independent studies (Serretti et al., 2001;

Abbreviations: ANOVA, analysis of variance; BCR, breakpoint cluster region; BDNF, brain-derived neurotrophic factor; BPD, bipolar disorder; BP I, bipolar I disorder; BP II, bipolar II disorder; PH domain, pleckstrin homology domain; SNP, single-nucleotide polymorphism.

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