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No association of serotonin transporter gene (SLC6A4) with schizophrenia and bipolar disorder in Japanese patients: association analysis based on linkage disequilibrium

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Summary. Serotonin transporter gene (SLC6A4) is one of the most promising candidate genes for psychiatric disorders such as schizophrenia (SCZ) and bipolar disorder (BP). Two functional polymorphisms, 5HTTLPR and 5HTTVNTR, have been a focus for genetic association analyses; however, no conclusive results have been obtained. We conducted, 1) a mutation search of SLC6A4, 2) LD mapping to select 'tagging' markers (10 SNPs and 5HTTVNTR, while 5HTTLPR was treated as an independent marker because of its allelic form), and 3) association analysis of these 'tagging' markers and independent markers (5HTTLPR and Asn605Lys) with SCZ and BP in Japanese patients. In this mutation search, a nonsynonymous SNP, Asn605Lys, was detected. No associations of 'tagging' markers and independent markers with such conditions were found. These results indicate that SLC6A4 might not play a major role in SCZ and BP in Japanese patients, a finding that agrees with both the common disease-common variant hypothesis and common disease-rare variant hypothesis.

Keywords: 5HTTLPR, 5HTTVNTR, linkage disequilibrium, single nucleotide polymorphism.

Introduction

Serotonin transporter gene (5HTT, SLC6A4) is one of the most promising candidate genes for psychiatric disorders, such as schizophrenia (SCZ) and bipolar disorder (BP), based on its import roles in serotonin transmission and the pharmacological mechanism of various antidepressants (OMIM: *182138). Several genetic association analyses of SLC6A4 have been performed in different population samples. These association analyses have focused on two functional polymorphisms, 5HTTLPR (5HTT "Long/Short", a 44-bp insertion/deletion, Polymorphism) and 5HTTVNTR (5HTT a 17-base-pair (bp) variable-number tandem-repeat (VNTR) in intron 2). For SCZ, results from several association studies have been inconsistent (e.g. Serretti et al., 2002; Tsai et al., 2002). On the contrary, for BP, two recent meta-analyses of 5HTTLPR and/or 5HTTVNTR showed significant

association with affective disorder (Anguelova et al., 2003; Lasky-Su et al., 2005). However, while meta-analysis is unquestionably a powerful tool for genetic association analysis, it has several limitations based on publication bias, ethnic diversity and other factors.

A popular hypothesis about allelic architecture proposes that most genetic risk for common, complex diseases including SCZ and BP is due to disease loci where there is one common variant (Chakravarti, 1999). If true, this common disease-common variant (CD-CV) hypothesis implies that linkage disequilibrium (LD) mapping is an important concept in order to narrow the predisposing polymorphisms for complex diseases in association analysis, rather than focusing on specific polymorphisms. To represent LD properties, single nucleotide polymorphisms (SNPs) are often used as haplotype tagging markers, because SNPs are abundant and amenable to genotyping. Applying these concepts, LD-based analysis could provide different insights from previous analyses using only independent markers such as 5HTTLPR and 5HTTVNTR.

To date, only one association study based on LD, which examined the association of SLC6A4 with BP (not with SCZ), has been reported (Sun et al., 2004).

Alternatively, a common disease-rare variant (CD-RV) hypothesis (Pritchard and Cox, 2002) was proposed recently, stemming from criticisms of the CD-CV hypothesis from the viewpoints of allelic heterogeneity and disease heterogeneity. According to the CD-RV hypothesis, it is important to search for rare and functional variants as possible disease-related variants.

In the present study, we conducted 1) a mutation search of all exons and possible promoter regions (2064 bp around 5HTTLPR), 2) LD mapping (10SNPs and 5HTTVNTR) and selection of 'tagging' markers, and 3) a case-control association analysis of SCZ and BP using these 'tagging' markers, 5HTTLPR and a rare variant detected in our mutation search.

Material and methods

Subjects

The subjects for the mutation search were 37 and 27 patients with SCZ and bipolar I disorder, respectively. LD mapping was performed in 96 controls. In the following association analysis, 287 patients with SCZ (148 male and 139 female; mean age ± standard deviation (SD) 42.3 ± 14.7 years), 109 patients with BP (51 male and 58 female; 61 patients with Bipolar I disorder and 48 patients with bipolar II disorder; mean age ± SD 48.3 ± 13.2 years) and 288 controls (150 male and 138 female; mean age ± SD 33.6 ± 12.9 years) were genotyped. They were diagnosed according to DSM-IV criteria with the consensus of at least two experienced psychiatrists on the basis of unstructured interviews and review of the medical records. All healthy controls were also psychiatrically screened based on unstructured interviews. All subjects were unrelated each other and ethnically Japanese. More detailed characterization of these subjects and description of their psychiatric assessment are identical to those published elsewhere (Suzuki et al., 2003). After explanation of the study, written informed consent was obtained from each subject. This study was approved by the Ethics Committee at Fujita Health University and Nagoya University Graduate School of Medicine.

SNP identification

Genomic DNA was extracted from peripheral blood of all subjects. For mutation search, primer pairs were designed using information from the GenBank sequence (accession number: NT-01799.13) and 22 amplified regions, which covered all of the coding regions and introns including the branch sites and 2064 bp around 5HTTLPR. We also developed a denaturing high-performance liquid chromatography (dHPLC) analysis. A more detailed description can be seen in a previous paper (Suzuki et al., 2003). Sequences of primer pairs are available on request.

LD mapping and SNP genotyping

For LD mapping, we included SNPs from databases (dbSNP; http://www.ncbi.nlm.nih.gov/SNP/ and Celera Discovery Systems; http://www.celeradiscoverysystem.com/) and other papers (Battersby et al., 1999), so that the SNPs were evenly distributed (Table 1). First we determined 'LD blocks'

Table 1. SNPs in LD mapping

ID		Distance to next marker	Method of genotyping	MAF of controls[1] (%)
Private IDs	Database IDs			
M1	rs1050565	–	TaqMan	15.1
–	5HTTLPR[2]	11563	PCR	–
2	rs2020934	3053	PCR-RFLP	15.1
3	rs2066713	9795	direct sequencing	6.25
4	rs2020936	851	PCR-RFLP	13.0
5	rs2020937	57	direct sequencing	6.78
6	rs2020938	7	direct sequencing	14.3
7	rs2020939	18	direct sequencing	14.6
8	5HTTVNTR	2104	PCR	5.73
9	rs140701	10096	direct sequencing	16.7
10	rs3794808	6739	PCR-RFLP	15.1
–	Asn605Lys (rs6352)[2]	1600	PCR-RFLP	–
11	rs3813034	5389	PCR-RFLP	16.1

[1]MAF = minor allele frequency of 96 controls used in LD mapping. [2]These variants were not included in LD mapping (see text)

with reasonable criteria based on 95% confidential bounds on D′ values using the Haploview ver. 2.05 software (Barrett et al., 2005). Next, 'tagging' markers were selected within each 'LD block' for 90% haplotype coverage using SNPtagger software (Ke and Cardon, 2003). In this case, we preferred to select possible markers with functional relevance rather than just intronic markers.

Genotyping of 5HTTLPR, M8 (5HTTVNTR), and M11 (rs3813034, 3′ UTR G>T) was as described in other papers (Battersby et al., 1999; Lesch et al., 1996; Ogilvie et al., 1996). For rapid genotyping of the other markers used in our LD mapping, we used TaqMan assays, restriction fragment length polymorphism (RFLP) assays, primer extension methods using dHPLC, and a direct sequencing method (Table 1). TaqMan probes and Universal PCR Master Mix were obtained from Applied Biosystems. A 5-μl total reaction volume was used and, after PCR, the allelic specific fluorescence was measured on ABI PRISM 7900 Sequence Detector Systems (Applied Biosystems). RFLP assays and primer extension methods were described in greater detail previously (Suzuki et al., 2003). Detailed information including primer sequences is also available on request.

Statistical analysis

Genotype deviation from the Hardy-Weinberg equilibrium (HWE) was evaluated by χ^2 test (SAS/genetics, release 8.2, SAS Institute Japan Inc, Japan).

Marker-trait association was evaluated allele/genotype-wise with conventional χ^2 test or Fisher's exact test (SPSS 10.0J, SPSS Japan Inc, Japan) and haplotype-wise with the program COCAPHASE 2.403 (Dudbridge, 2003). The COCAPHASE program performs log-likelihood ratio tests under a log-linear model for global P-value. To estimate haplotype frequencies of 'tagging' markers, the expectation-maximization (EM) algorithm was used. Rare haplotypes found in less than 3% of both cases and controls were excluded from association analyses to provide greater sensitivity and accuracy when the effect is seen in common haplotypes. And for haplotype-wise analyses, we calculated global P-values in sliding-window fashion.

Power calculation was performed using a statistical program prepared by Ohashi et al. (2001). We estimated the power for our sample size under a multiplicative model of inheritance assuming a population susceptibility allele frequency of each value in our screened control samples (type I error rate = 0.05).

The significance level for all statistical tests was 0.05.

Results

In our mutation search, only a nonsynonymous single nucleotide polymorphism (SNP), Asn605Lys (rs6352), was detected in one SCZ; no mutation was found in BP. Due to its low minor allele frequency (MAF), we did not

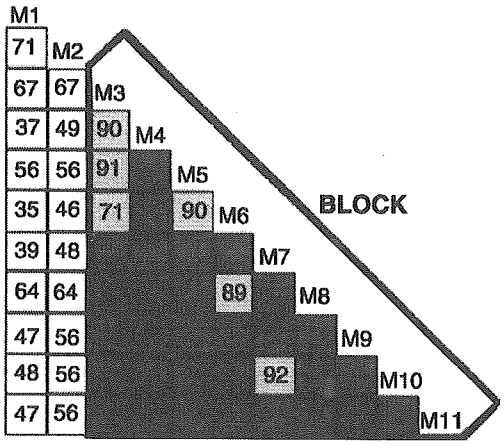

Fig. 1. Linkage disequilibrium mapping. Numbers in box represents D′ values after decimal point. D′ values of 1.0 are not shown. Color schemes represent the evidence of LD or recombination (strong evidence of LD: dark gray, uninformative: light gray, strong evidence of recombination: white). The other information is described at Haploview's website

include this SNP in the following LD mapping. Next, LD mapping of controls showed one LD block covering all of the coding exons (Fig. 1), and M11 and M8 (5HTTVNTR) were selected as 'tagging' markers from this block. Consequently, four markers (M1, M2, M8 and M11) were selected as those of SLC6A4 (Table1). In this case-control association analysis, we treated 5HTTLPR and Asn605Lys as independent markers, because 5HTTLPR is a complex multi-allelic variant (Nakamura et al., 2000) and MAF of Asn605Lys was too low, as described above.

We first evaluated the deviations from HWE for all markers, and found that genotype frequencies were consistent with HWE (for M8, 5HTTVNTR, 9 repeat allele was not found in either our case and control samples). No associations of 'tagging' markers and 5HTTLPR with SCZ and BP were found (Table 2). However, because Asn605Lys

Table 2. Allele/genotype-wise association analysis of 'tagging' markers with SCZ and BP

Phenotype[1]	ID	Number	Genotype[2]			P-values		Power calculation[3]
			M/M	M/m	m/m	Genotype	Allele	GRR
SCZ	M1	287	196	79	12	0.139	0.343	1.47
	2	287	208	68	11	0.161	0.594	1.51
	8[4]	287	237	49	1	0.350	0.583	1.78
	11	287	196	83	8	0.781	0.557	1.49
	5HTTLPR	287	189	82	16	0.214	0.441	1.46
	Asn605Lys	381	353	28	2	0.911	0.541	1.95
BP	M1	109	75	30	4	0.377	0.389	1.67
	2	109	76	30	3	0.867	0.912	1.71
	8[4]	109	93	15	1	0.993	0.930	1.99
	11	109	70	33	6	0.420	0.509	1.68
	5HTTLPR	109	73	30	6	0.339	0.452	1.65
	Asn605Lys	109	100	8	1	0.515	0.489	2.55
Controls	M1	288	180	100	8			
	2	288	197	85	6			
	8[4]	288	245	40	3			
	11	288	189	91	8			
	5HTTLPR	288	175	101	12			
	Asn605Lys	351	327	23	1			

[1]*SCZ* schizophrenia, *BP* bipolar disorder. [2]*M* major allele, *m* minor allele. [3]*GRR* genotype relative risk to obtain 80% power. [4]5HTTVNTR: M (major allele) = 12 repeat, m (minor allele) = 10 repeat

Table 3. Haplotype-wise association analyses

Phenotype[1]	ID	Global P-values in sliding-window fasion		
		2SNP	3SNPs	4SNPs
SCZ	M1	0.651	0.376	0.715
	2	0.701	0.545	
	8	0.151		
	11			
BP	M1	0.675	0.847	0.927
	2	0.910	0.903	
	8	0.878		
	11			

[1]*SCZ* schizophrenia, *BP* bipolar disorder

showed weak association with SCZ ($P = 0.0338$), we expanded the search in a further 96 schizophrenics and 64 control samples (total schizophrenia = 383, control = 351) for conclusive results. In this additional analysis, we could not confirm the positive association of Asn605Lys with SCZ ($P = 0.541$: Table 2). In addition, we performed a haplotype-wise association analysis of sliding-window fashion using these four 'tagging' markers. Again, we found no association with SCZ and BP (Table 3).

The Power calculations are shown in Table 2. We obtained more than 80% power to detect association when we set the genotype relative risk (GRR) at each value as shown in Table 2.

Discussion

In this study, no associations of SLC6A4 with SCZ and BP in Japanese patients were found in accordance with either the common disease-common variant (CD-CV) hypothesis or common disease-rare variant (CD-RV) hypothesis (Pritchard and Cox, 2002).

The association analysis strategy we have adopted is reasonable for genetic association study of SLC6A4 for the following reasons. 1) Mutation search with relatively high power can detect SNPs that are rare but have functional effect (Collins and Schwartz, 2002), and avoid overlooking associations in accordance with the CD-RV hypothesis. In fact, one of us reported that a rare but functional SNP of SLC6A4 (Ile425Val) was associated with severe familial obsessive-compulsive disorder, and suggested that such a rare variant of SLC6A4 is also important in terms of susceptibility genes for complex disorders (Ozaki et al., 2003). We included the mutation scan of this SNP using 500 schizophrenics, however, no mutation was found in our samples. And another nonsynonymous SNP from database, Ala56Gly (rs6355), was also searched for 96 schizophrenics, again no mutation was found (data not shown). 2) Our association analyses using 'tagging' markers were more sensitive and useful than those using randomly selected markers. Moreover, our 'tagging' markers could reflect the haplotype background of SLC6A4 in the Japanese population, especially in coding regions. And it is reasonable to treat 5HTTLPR as an independent marker, because 5HTTLPR is a multi-allelic variant and the arbitrary division ('L' or 'S' allele) cannot estimate the exact LD structure of SLC6A4.

A recent association study based on LD of Taiwanese BP found an association with the fifth commonest haplotype (Sun et al., 2004). However, our haplotypic analysis did not show significance either in global haplotypic analysis or even in individual haplotypic analyses (data not shown). This discrepancy between our results and Sun's might be due to a difference in the study populations.

We also included an explorative analysis of 5HTTLPR and 5HTTVNTR, because a recent expression study reported that allelic combination of 5HTTLPR and 5HTTVNTR showed weak but significant differences in serotonin transporter mRNA levels (Hranilovic et al., 2004). They reported that genotypes were separated into three groups, no 'low-expressing' at either of the loci (L/L, 12/12), 'low-expressing' at one locus (L/L, '10' allele and

'S' allele, 12/12), and 'low-expressing' at both loci ('S' allele, '10' allele), since the low-expressing alleles ('S' allele of 5HTTLPR and '10' allele of 5HTTVNTR) appeared to act dominantly. To detect this combined effect, we divided samples the same those authors. Consequently, we were also unable to find an association of the allelic combination of these polymorphisms with SCZ or BP (SCZ: χ^2 value = 1.097, degree of freedom = 2, P = 0.578, BP: χ^2 value = 1.949, degree of freedom = 2, P = 0.377).

Although several findings have been obtained in transfection, binding, and expression studies, those regarding the functional consequences of 5HTTLPR and 5HTTVNTR have been inconsistent (see introduction of Hranilovic et al., 2004). Taken together with our results, the possibility is suggested that only specific alleles of 5HTTLPR have functional effect and are associated with SCZ or BP, because even the alleles of 5HTTVNTR, for which the LD pattern is easy to evaluate on SLC6A4, can reflect most of the haplotype background around the coding regions.

A few points of caution should be stressed. First, the lack of association may be due to biased samples, such as unmatched aged samples. Second, it is difficult to evaluate the association of an extremely rare variant (e.g. MAF < 0.01) from viewpoint of power. Only a larger sample size will be required in mutation search and association analysis for conclusive results. Third, we could not detect the boundaries of haplotype blocks in 5′-flanking regions. Further LD evaluation will be required.

The powers of our analysis were quite high, especially for SCZ. Thus, we concluded that SLC6A4 is unlikely to be a SCZ and BP susceptibility gene, considering both the CD-CV hypothesis and CD-RV hypothesis. The strategy we have adopted for association analysis is suitable; however, further investigations, especially detection of complete 5HTTLPR alleles, will be necessary for conclusive results.

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The effect of 5-hydroxytryptamine 3A and 3B receptor genes on nausea induced by paroxetine

Running title: The relation between the *HTR3* gene and nausea

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Key words: HTR3A, HTR3B, paroxetine, nausea, gene polymorphism

Abstract

We investigated the effect of 5-hydroxytryptamine 3A and 3B receptor (*HTR3A* and *HTR3B*) gene polymorphisms on nausea induced by paroxetine in Japanese psychiatric patients. Blood samples were collected from 78 individuals after at least 2 weeks treatment with the same daily dose of paroxetine. The patients visited every 2 weeks and the paroxetine dose was changed in response to their clinical symptoms. Nausea was assessed at each visit. The Tyr129Ser polymorphism of the *HTR3B* gene had a significant effect on the incidence of nausea (P=0.038). Logistic regression analysis also showed that patients with the Tyr/Tyr genotype had a 3.95-fold (P=0.048) higher risk of developing nausea than patients with the Ser allele. *HTR3A* gene polymorphisms and the *CYP2D6* gene polymorphisms had no significant effect on the incidence of nausea. The mean score of nausea severity was corrected by the Bonferroni test. *HTR3B* gene polymorphisms are significant predictors of paroxetine-induced nausea.

Introduction

Nausea is a severe side effect induced by selective serotonin reuptake inhibitors (SSRIs). In general, SSRIs are better tolerated than tricyclic antidepressants, although gastrointestinal side effects can have an incidence of up to 40%, which can be severe enough to lead to early treatment discontinuation [1, 2]. Recently, 5-hydroxytryptamine 3 receptors (HTR3) have been considered to have an important role in SSRI-induced gastrointestinal side effects, since HTR3 antagonists cisapride and ondansetron were reported to reduce SSRI-induced gastrointestinal side effects [3].

HTR3 is a ligand-gated ion channel that mediates fast synaptic neurotransmission [4]. Central and peripheral HTR3 have different structures and different properties [5]. HTR3 exist in the area called the chemoreceptor trigger zone of the medulla oblongata, and are thought to be involved with the vomiting reflex [6]. HTR3 are also distributed in the autonomic, enteric and sensory nervous systems [7]. They also regulate the control pain sensation, movement of the digestive tract and vomiting by prompting nerve depolarization [8]. In particular, modification of HTR3 on the small intestinal mucosa is one of the mechanisms for regulation of antineoplastic-induced nausea and vomiting [9]. We do not have any data about the binding affinity of paroxetine for the 5-HT3 receptors. However, HTR3 antagonists such as cisapride and ondansetron were used

for the therapy of nausea induced by SSRIs, and SSRIs such as fluvoxamine display a relatively high affinity for HTR2A. Thus, we considered that there might be some relationship between nausea induced by SSRIs such as paroxetine and the function of HTR3.

Murphy *et al.* [10] report that the T102C polymorphism of the *HTR2A* gene may predict the treatment discontinuation caused by paroxetine-induced side effects in older patients with depression. *HTR3A* and *3B* genes have been assigned to chromosome 11q23.1–q23.3 [11], and several genetic variations have been reported. Tremblay *et al.* [12] report that variations in the *HTR3B* gene predict the efficacy of antiemetic treatment in cancer patients. However, no previous studies have investigated the effects of *HTR3A* and *3B* gene polymorphisms on the occurrence of SSRI-induced nausea.

Cytochrome P450 (CYP) 2D6 has been shown to be involved in the metabolism of paroxetine, and CYP2D6 is known to have genetic polymorphisms that affect enzyme activity [13]. These observations suggest that the *CYP2D6* gene polymorphism may be a predictor for paroxetine-induced side effects. On the other hand, Gerstenberg *et al.* [14] showed that steady-state plasma concentrations of fluvoxamine are not associated with incidence of nausea, and that *CYP2D6* genotype does not affect nausea development. Therefore, further studies are needed to clarify whether *CYP2D6* gene polymorphisms

affect SSRI-induced side effects.

In this study, we investigated the effects of pharmacodynamic factors, such as *HTR3A* and *3B* gene polymorphisms, and the effects of pharmacokinetic factors, such as *CYP2D6* genotype, on the occurrence of paroxetine-induced nausea in Japanese psychiatric patients.

Materials and Methods

Subjects

This study was conducted at Niigata University Medical Hospital, Japan, and the study protocol was approved by the Hospital Ethics Committee. Each subject provided written informed consent before enrolment. The subjects comprised 78 Japanese psychiatric outpatients (28 male, 50 female) aged 38.4±13.8 years (mean±S.D., range 18–70 years.). Thirty-nine subjects had major depressive disorder, 25 had anxiety disorders, six had adjustment disorder, seven had a depressive disorder not otherwise specified, and one had other mood disorders. All patients were diagnosed according to DSM-IV-TR. The exclusion criteria were additional diagnoses of Axis I or II of DSM-IV-TR. Demographic data, medical history and laboratory data, including hematology, serology, electrolytes and urine analysis, were collected for each patient. Patients with obvious physical illnesses were excluded from the study. All patients were orally treated with paroxetine for their psychiatric illness. No patients were being treated with antiemetic medication during our study.

Study design

The patients visited the hospital every 2 weeks and side effects, including nausea, were assessed at each visit. The paroxetine dose was increased from 10 or 20 mg/day

to 30 and 40 mg/day in response to clinical symptoms. We rated the side effects during the last 2 weeks and evaluated the severity of nausea according to our original scale which included five graded items: 0, no nausea; 1, mild nausea for less than during the last 2 weeks; 2, mild nausea for more than 1 week during the last 2 weeks; 3, continuous, moderate nausea during the last 2 weeks; 4, continuous, severe nausea and vomiting during the last 2 weeks. Subjects with a score of 0 or 1 were defined as subjects without nausea, and those with a score or 2, 3 or 4 were defined as having nausea. The side effect raters were blind to the patients' genotypes.

Blood sampling

Blood sampling was performed using a Venoject® tube containing EDTA-Na (Terumo Japan, Tokyo, Japan) at week 1 for genotype detection and subsequently at the first appearance of nausea to measure the concentration of paroxetine. Blood samples were taken at 12 h after the final ingestion of paroxetine. Seven milliliters of venous blood were collected, and genomic DNA was extracted from the peripheral leukocytes by utilizing a QIAamp Blood Kit (QIAGEN, Valencia, CA, USA) within 2h of collection.

Genotyping and determination of plasma concentration

Polymerase chain reaction (PCR) was used to determine the C195T and Pro16Ser genotypes of *HTR3A* gene according to the method of Niesler *et al.* [15] and the Tyr129Ser

genotype of *HTR3B* gene according to the method of Tremblay *et al.* [12]. *CYP2D6*10* alleles causing decreased enzyme activity were identified by the C188T mutation using a two-step PCR as described by Johansson *et al.*[16]. A long-PCR analysis was used to detect the **5* allele causing a lack of enzyme activity as described by Steen *et al.* [17].

The plasma concentration of paroxetine was measured using column-switching high-performance liquid chromatography (HPLC) with ultraviolet detection. Paroxetine was extracted from plasma, to which cisapride had been added as an internal standard, with hexane–chloroform, and the extract was subjected to automated column-switching HPLC using a TSK BSA-C8 precolumn (Tosoh, Tokyo, Japan) for sample clean-up, and a TSK gel ODS-80TS column (Tosoh) for separation.

Statistical analysis

Statistical analysis was performed using SPSSII for Windows. Genotype and allele distributions were analyzed by the χ^2-test. The clinical and demographic characteristics, sex, age, daily dose and paroxetine concentration were compared among groups by the unpaired *t*-test. The mean score of nausea severity was compared among each genotype group by one-way analysis of variance, and post-hoc analysis of the mean score of nausea severity was carried out by using Bonferroni's test. Logistic regression analysis was used to compare the probability of the incidence of nausea. The level of significance was set at $P<0.05$.

Results

The genotype frequencies of the *HTR3A* , *HTR3B* and *CYP2D6* genes are shown in Tables 1–3. All of these genetic variations were in Hardy–Weinberg equilibrium. *HTR3A* C195T polymorphism genotype was not detected in four patients. There were no differences in nausea between each diagnostic groups (e.g. major depressive disorders and anxiety disorders (P=0.12)). Therefore, we consider that the nausea observed in the present study was induced only by paroxetine. There were also no differences in *HTR3A*, *HTR3B* and *CYP2D6* polymorphisms between major depressive disorders and anxiety disorders (P= 0.341).

Effects of HTR3A and HTR3B gene polymorphisms

The genotype distribution of *HTR3A* and *HTR3B* gene polymorphisms are shown in Tables 1 and 2. No significant differences were demonstrated for sex, age, and paroxetine daily dose and plasma concentration among each genotype group. Association analysis revealed that genotype frequencies of *HTR3A* Pro16Ser and *HTR3B* C195T polymorphisms did not significantly differ between subjects with and without nausea (Pro16Ser genotype: χ^2=0.912, df=2, P=0.634; C195T genotype: χ^2=2.128, df=2, P=0.546). There was a significant difference in genotypic distribution associated with *HTR3B* Tyr129Ser polymorphism between patients with

and without nausea (χ^2=6.547, *df*=2, *P*=0.038). The proportion of Ser allele carriers (i.e., patients with either Tyr/Ser or Ser/Ser) was significantly higher in the group without nausea (χ^2=6.082, *df*=1, *P*=0.014). There were significant differences in the severity score of nausea among the three genotypes (score: 0.54±0.91, 0.14±0.49 and 0±0, *df*=2, *P*=0.03).

The results of logistic regression analysis are shown in Table 4. The incidence with or without nausea was used in the analysis as an independent variable, and sex, age, daily dose of paroxetine and the genotypes of *HTR3B* Tyr129Ser were added as potential confounders. This analysis also showed that there was a significant association between nausea and *HTR3B* Tyr129Ser genotype. (*P*=0.048; *OR*=3.95; 95% *CI*=1.009–15.455).

Effect of CYP2D6 gene polymorphism

Five CYP2D6 genotypes were identified: **1/*1* (*n*=51), **1/*5* (*n*=1), **1/*10* (*n*=11), **5/*10* (*n*=3) and **10/*10* (*n*=12). The allele frequencies of the **5* and **10* alleles were 2.6 and 24.4%, respectively. Patients were divided into three genotype groups according to the number of mutated alleles: 51 patients with the **1/*1* genotype, 12 with the **1/*10* and **1/*5* genotypes and 15 with the **5/*10* and **10/*10* genotypes. No significant differences were demonstrated for sex, age, and paroxetine daily dose

and plasma concentration between those three genotype groups (Table 3).

There were no significant differences in the incidence of nausea between the three genotype groups (χ^2=1.029, *df*=2, *P*= 0.716). We also divided patients into two genotype groups: 61 patients with the **1/*1* or **1/*10* genotype were termed normal metabolizers, and 17 patients with the **10/*10*, **1/*5* or **5/*10* genotype were termed low metabolizers. However, there were also no significant differences in the incidence of nausea between the two genotype groups.

Discussion

We screened for two polymorphisms in the *HTR3A* gene and one variant in the *HTR3B* gene as a pharmacodynamic factor, and *CYP2D6* gene polymorphisms (**1*, **5*, **10* alleles) as a pharmacokinetic factor. To our knowledge, the present study is the first demonstration that the *HTR3B* gene may predict the incidence of paroxetine-induced nausea in Japanese psychiatric patients.

Kaiser *et al.* [18] reported that polymorphisms of the *HTR3A* gene may not serve as pharmacogenetic predictors of antiemetic treatment with HTR3 antagonists in cancer patients. We also found no relationship between *HTR3A* gene polymorphism and paroxetine-induced nausea. Tremblay *et al.* [12] reported that the Tyr129Ser polymorphism of the *HTR3B* gene did not alter the incidence of nausea and vomiting.

However, in the present study, there was a significant relationship between the Tyr129Ser polymorphism of the *HTR3B* gene and paroxetine-induced nausea. On the other hand, Tremblay *et al.* also reported that an insertion/deletion polymorphism in the promoter region of the *HTR3B* gene had a significant effect on the incidence of nausea and vomiting induced by cancer chemotherapy, although we did not examine this insertion/deletion polymorphism. This discrepancy may occur because of the difference in medication, i.e., cancer chemotherapy versus paroxetine. We can not

account for this discrepancy between the previous and the present study because the function of the *HTR3B* gene polymorphism still remains unclear. To date, there are no *in vitro* data about the functional effects of the Tyr129Ser polymorphism in *HTR3B* gene. With regard to the functional effects of the other polymorphism in *HTR3B* gene, Cazzola *et al.* [19] report that a 6-bp deletion in the 5'UTR of L-ferritin mRNA is a cause of hereditary hyperferritinanemia-cataract syndrome, and Frank *et al.* [20] report that the deletion -100_-102delAAG polymorphism may change the structure of mRNA compared to the wild type. However, the Tyr129Ser polymorphism of *HTR3B* gene had an amino acid substitution in the coding region, and it was possible that the Tyr129Ser polymorphism of *HTR3B* gene affected the expression level of the B subunit either by itself, or because of linkage disequilibrium with other yet unknown functional variants, and this polymorphism of *HTR3B* gene may affect the occurrence of nausea by itself, or indirectly. Meanwhile, Murphy *et al.* [10] reported that the T102C polymorphism of the *HTR2A*gene could predict the treatment iscontinuation caused by paroxetine-induced side effects in older patients with depression. In future studies, we should also examine the relationship between *HTR2A* gene polymorphism and paroxetine-induced nausea.

In our study, the *CYP2D6* gene polymorphism had no significant effect on the