incidence of paroxetine-induced nausea. Murphy *et al.* [10] reported that the *CYP2D6* genotype did not influence side effects of paroxetine. Gerstenberg *et al.* [14] also reported that the number of *CYP2D6* mutated alleles was not related to the development of fluvoxamine-induced nausea. Our present results support these previous findings.

Kasper *et al.* [1] reported that there was a relationship between plasma concentration of fluvoxamine and incidence of nausea. Sawamura *et al* [21] showed that plasma paroxetine concentration in patients with *CYP2D6*10* alleles was significantly higher than those without **10* alleles, and plasma paroxetine concentration in patients with **5* alleles also showed a tendency to be higher than those without **5* alleles. Therefore, we hypothesized that the *CYP2D6* gene polymorphism had a significant influence on paroxetine-induced nausea, but it did not affect paroxetine-induced nausea in the present study. Sindrup et al[22] reported that the effect of CYP2D6 on metabolism was less prominent at steady state than after a single dose of paroxetine, since CYP2D6 enzymatic activity seems to be easily saturated upon increasing paroxetine dose. This may be one of the reasons that there was no effect on paroxetine-induced nausea . Furthermore, the groups with two mutated alleles were expected to have the highest concentration among these three genotype groups, but the concentration in groups with two mutated alleles was lower than in groups with one mutated allele, and was the same

as in groups with no mutated allele (Table 3). This result suggests that there are patients who had noncompliance with paroxetine in the group with **5/*10* and **10/*10* alleles, since they discontinued because of various adverse events other than nausea, as a result of a great increase in plasma paroxetine concentration.

We demonstrated that a pharmacodynamic factor such as the Tyr129Ser polymorphism of the *HTR3B* gene may be a predictor of paroxetine-induced nausea in Japanese psychiatric patients. Taking account of these findings, in clinical situations, it may be possible to tailor paroxetine pharmacotherapy based on genetic factors. However, since the results of some previous studies are not consistent with our current results, further study is needed to clarify these discrepancies.

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Table 1. Genotypic distribution and demographic data of the *HTR3A* Pro16Ser and C195T

	HTR3A Pro16Ser			*HTR3A* C195T		
	Pro/Pro	Pro/Ser	Ser/Ser	C/C	C/T	T/T
Sex (M/F)	23/34	5/13	0/3	15/22	11/16	1/9
P		*0.261*			*0.291*	
Age [±SD]	37.8[13.8]	38.2[14.2]	52.0[4.4]	37.8[13.8]	38.2[14.2]	52.0[4.4]
P		*0.411*			*0.370*	
Daily dose of PRX [±SD]	23.0[10.5]	20.0[9.7]	20.0[17.3]	20.5[9.7]	25.9[10.5]	19.0[12.9]
P		*0.408*			*0.205*	
Concentration of PRX [±SD]	66.4[89.3]	28.9[31.3]	22.3[29.8]	42.8[61.1]	65.8[90.6]	52.9[80.6]
P		*0.167*			*0.707*	
Nausea (+) (n=15)	12	3	0	9	5	1
(%)	(21.1)	(16.7)	(0.0)	(24.3)	(18.5)	(10.0)
Nausea (−) (n=63)	45	15	3	28	22	9
(%)	(78.9)	(83.3)	(100.0)	(75.7)	(81.5)	(90.0)
P		*0.634*			*0.546*	
Mean score of severity of nausea	0.21[0.411]	0.17[0.383]	0[0]	0.1[0.316]	0.19[0.396]	0.24[0.435]
P		*0.643*			*0.593*	

[ ] = SD, ( ) = %

Table 2. Genotypic distribution and demographic data of the *HTR3B* Tyr129Ser

	HTR3B Tyr129Ser			
	Tyr/Tyr	Tyr/Ser	Ser/Ser	Tyr/Ser + Ser/Ser
Sex (M/F)	16/19	10/26	2/5	12/31
P		*0.264*		*0.103*
Age [±SD]	36.3[11.5]	38.9[15.1]	46.1[16.6]	40.1[15.4]
P		*0.099*		*0.267*
Daily dose of PRX [±SD]	21.4[10.6]	21.9[10.6]	27.1[9.5]	22.8[10.5]
P		*0.4*		*0.939*
Concentration of PRX [±SD]	37.6[46.9]	66.4[100.2]	95.0[77.2]	71.1[96.6]
P		*0.124*		*0.064*
Nausea (+) (n=15)	11	4	0	4
(%)	(31.4)	(11.1)	(0.0)	(9.3)
Nausea (−) (n=63)	24	32	7	39
(%)	(68.6)	(88.9)	(100.0)	(90.7)
P		*0.038**		*0.014**
Mean score of severity of nausea	0.54[0.919]	0.14[0.487]	0[0]	0.12[0.448]
P		*0.030**		*0.009**

[ ] = SD, ( ) = %

Table 3. Genotypic distribution and demographic data of *CYP2D6* genotype groups

	CYP2D6		
	**1/*1*	**1/*5, *1/*10*	**5/*10, *10/*10*
Sex (M/F)	17/34	5/7	6/9
P		*0.807*	
Age [±SD]	38.6[14.9]	39.7[10.7]	36.7[12.9]
P		*0.126*	
Daily dose of PRX [±SD]	21.4[10.2]	24.2[11.7]	23.3[11.1]
P		*0.925*	
Concentration of PRX [±SD]	50.7[76.3]	89.5[114.4]	47.5[51.2]
P		*0.287*	
Nausea (+) (n=15)	9	2	4
(%)	(17.6)	(16.7)	(26.7)
Nausea (−) (n=63)	42	10	11
(%)	(82.4)	(83.3)	(73.3)
P		*0.716*	
Mean score of severity of nausea	0.18[0.385]	0.17[0.389]	0.27[0.458]
P		*0.725*	

[ ] = SD, ( ) = %

Table 4. Logistic regression analysis of independent valiables to Nausea

Independent valiable	Partial regression coefficients	P	Odd ratio (95% confidence interval)
Sex	-0.040	0.111	1.115 (0.518-3.225)
Age	-0.793	0.041	1.200 (0.810-1.110)
Daily dose of Paroxetine	-0.538	0.320	0.447 (0.112-3.154)
HTR3B Tyr129Ser genotype	-0.148	0.048	3.950 (1.009-15.455)

No association between the *brain-derived neurotrophic factor* gene and schizophrenia in a Japanese population

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Abstract

Brain-derived neurotrophic factor (BDNF) plays important roles in the survival, maintenance and growth of neurons. Several studies have indicated that BDNF is likely to be related to the pathogenesis of schizophrenia. Recent genetic analyses have revealed that *BDNF* gene polymorphisms are associated with schizophrenia, although contradictory negative findings have also been reported. To assess whether three *BDNF* gene polymorphisms (rs988748, C132T and rs6265) could be implicated in vulnerability to schizophrenia, we conducted a case-control association analysis (349 patients and 423 controls) in Japanese subjects. We found no association between these *BDNF* gene polymorphisms and schizophrenia using both single-marker and haplotype analyses. The results of the present study suggest that these three *BDNF* gene polymorphisms do not play major roles in conferring susceptibility to schizophrenia in a Japanese population. However, further studies assessing the associations between these *BDNF* gene polymorphisms and schizophrenia should be performed in several other ethnic populations.

Keywords: Brain-derived neurotrophic factor; Schizophrenia; Case-control study; Single nucleotide polymorphism

1. Introduction

Cytokines and growth factors may be implicated in the etiology or pathology of schizophrenia (for a review: Nawa et al., 2000). However, the pathogenesis of schizophrenia currently remains unclear. Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family, which also includes nerve growth factor and neurotrophin-3. BDNF supports the survival and differentiation of several types of neurons, such as dopaminergic and GABAergic neurons (Knüsel et al., 1991; Mizuno et al., 1994), and has also been implicated in the types of synaptic plasticity associated with long-term potentiation (Korte et al., 1995) and spatial memory (Mizuno et al., 2000). Postmortem studies on brains from schizophrenia patients have revealed changes in the BDNF protein or mRNA expression levels, although these observations remain controversial (Takahashi et al., 2000; Chen et al., 2001; Durany et al., 2001; Iritani et al., 2003; Weickert et al., 2003; Knable et al., 2004; Hashimoto et al., 2005). Furthermore, several studies reported decreased serum BDNF levels in schizophrenia patients (Toyooka et al., 2002; Pırıldar et al., 2004; Tan et al., 2005b, 2005c), whereas other studies failed to find such decreases (Shimizu et al., 2003; Jockers-Scherübl et al.,

2004). Nevertheless, these findings suggest that BDNF is likely to be related to the pathogenesis of schizophrenia.

Genetic variants in the *BDNF* gene, located on chromosome 11p13, have also been tested for their associations with schizophrenia. Specifically, the most extensively investigated *BDNF* gene polymorphisms are as follows: the $(GT)_n$ dinucleotide repeat in the promoter region; rs6265 (G196A) producing an amino acid substitution (valine to methionine) at codon 66; and C132T (formerly designated C270T; Kunugi et al., 2001; Itoh et al., 2005) in the 5'-noncoding region. Muglia et al. (2003) demonstrated biased transmission of the $(GT)_n$ alleles from parents to schizophrenia probands. Neves-Pereira et al. (2005) found that the G allele frequency of rs6265 was significantly higher in schizophrenia patients than in control subjects, while Rosa et al. (2006) reported that the G allele was preferentially transmitted from parents to affected offspring. Interestingly, this polymorphism was associated with episodic memory (Egan et al., 2003; Dempster et al., 2005; Tan et al., 2005a) and hippocampal volume (Szeszko et al., 2005), suggesting that rs6265 may be functional. Recently, Schumacher et al. (2005) demonstrated that the haplotype constructed from

rs988748-$(GT)_n$-rs6265 was associated with schizophrenia. In addition, Nanko et al. (2003) and Szekeres et al. (2003) detected an association between the C132T polymorphism and schizophrenia. However, contradictory negative results have also been reported for $(GT)_n$ (Sasaki et al., 1997; Hawi et al., 1998; Wassink et al., 1999; Krebs et al., 2000; Virgos et al., 2001; Fanous et al., 2004; Neves-Pereira et al., 2005), rs6265 (Egan et al., 2003; Hong et al., 2003; Anttila et al., 2005; de Krom et al., 2005; Dempster et al., 2005; Gourion et al., 2005; Szeszko et al., 2005; Tan et al., 2005a; Chen et al., 2006) and C132T (Anttila et al., 2005; Galderisi et al., 2005; Szczepankiewicz et al., 2005), and these inconsistencies require further investigation. Therefore, we performed a case-control association study in Japanese subjects to assess whether three *BDNF* gene polymorphisms (rs988748, C132T and rs6265) could be implicated in vulnerability to schizophrenia. We did not investigate the $(GT)_n$ dinucleotide repeat, since Sasaki et al. (1997) reported that this repeat was not associated with schizophrenia in Japanese and that the variation was much less polymorphic in Japanese subjects than in Caucasian subjects.

2. Materials and methods

2.1. Subjects

The study population consisted of 349 schizophrenia patients (187 males and 162 females; mean age, 41.4 [SD 15.0] years) and 423 control subjects (217 males and 206 females: mean age, 34.2 [SD 7.7] years). All participants were unrelated Japanese living in Niigata Prefecture or Fukushima Prefecture. Patients meeting the *Diagnostic and Statistical Manual of Mental Disorders* fourth edition (DSM-IV) criteria for schizophrenia were recruited from the following 14 hospitals: Iizuka Hospital, Kohdo Hospital, Matsuhama Hospital, Minamihama Hospital, Niigata Prefectural Psychiatric Center, Niigata University Hospital, Niitsu-Shin-ai Hospital, Ohjima Hospital, Sado General Hospital, Sagata Hospital, Seki Hospital, Shirone-Kensei Hospital, Shirone-Midorigaoka Hospital and Suehirobashi Hospital. The diagnosis of schizophrenia had been assigned based on all available sources of information, including unstructured interviews, clinical observations and medical records, and was subsequently reassessed by an experienced psychiatrist (T.M. or N.K.). The mean age of the patients at onset was 23.1 (SD 7.2) years. Regarding the subtypes of

schizophrenia, 22 patients suffered from paranoid schizophrenia, 142 from disorganized schizophrenia, 10 from catatonic schizophrenia, 172 from undifferentiated schizophrenia and 3 from residual schizophrenia, according to the defined criteria. The control subjects were mainly recruited from the staff of the participating hospitals. Although these subjects were not assessed by a structured psychiatric interview, they all showed good social and occupational skills and reported that they had no history of psychiatric disorders. The Ethics Committee on Genetics of Niigata University School of Medicine approved the present study, and written informed consent was obtained from all participants.

2.2. Genotyping

Genomic DNA was extracted from peripheral blood using the standard phenol/chloroform method. We genotyped three single nucleotide polymorphisms (SNPs), namely rs988748 (GenBank accession no. **AF411339**; at position 50611), C132T (GenBank accession no. **AF411339**; at position 53620) and rs6265 (GenBank accession no. **AF411339**; at position 95422), of the *BDNF* gene using TaqMan

5'-exonuclease assays. The primer and probe sets were designed and synthesized by Applied Biosystems (Foster City, CA). We carried out polymerase chain reaction amplification using TaqMan 2× Universal Master Mix, No AmpErase UNG (Applied Biosystems), 5-10 ng of DNA, 0.9 μM of each primer and 200 nM of each probe in a total volume of 5 μl. Each 96-well plate contained 94 test samples and 2 control samples with no DNA template. The thermal cycling conditions were 95°C for 10 min, followed by 40 cycles of 92°C for 15 s and 60°C for 1 min. Fluorescence and allelic discrimination were measured using an ABI PRISM 7900HT Sequence Detection System and SDS 2.0 software (Applied Biosystems).

2.3. Statistical analysis

Deviation from Hardy-Weinberg equilibrium (HWE) was tested by using the χ^2 test for goodness-of-fit. Allele and genotype frequencies between patients and control subjects were compared using Fisher's exact test. Haplotype frequencies were estimated using the expectation maximization algorithm with SNPAlyse (DYNACOM, Yokohama, Japan). Pair-wise linkage disequilibrium (LD) indices, D' and r^2, were

calculated in the control subjects. Case-control haplotype analyses were performed by the permutation test. A probability level of $p < 0.05$ was considered to indicate statistical significance.

3. Results

Table 1 shows the genotype and allele frequencies of the three SNPs in the *BDNF* gene among the patients and control subjects. The genotype frequencies of all three SNPs in both groups did not differ significantly from the values expected from HWE ($p > 0.05$). The genotype and allele frequencies of rs98874 and rs6265 in the patients did not differ from those in the control subjects ($p > 0.05$). Regarding the C132T polymorphism, there was a trend toward a higher frequency of the C/T genotype in patients than in control subjects (7.4% vs. 4.5%; $p = 0.090$). A similar tendency was also found for the T allele frequency (3.7% in patients vs. 2.2% in control subjects; $p = 0.090$). The values of absolute D' and r^2 for the control subjects are presented in Table 2. There was statistically significant evidence of LD for each pair of SNPs ($p < 0.05$). Next, we performed haplotype analyses (Table 3), in which rare haplotypes with

frequencies of less than 1% were not assessed. There was no significant overall difference in the haplotype frequencies between the patients and control subjects (global permutation $p = 0.113$). One individual haplotype, C-T-G, was significantly more frequent in patients than in control subjects (3.5% vs. 1.8%; $p = 0.044$), although the p value for the difference did not exceed the level of significance after Bonferroni correction for multiple testing ($p = 0.132$).

4. Discussion

In the present study, we found no significant association between three *BDNF* gene polymorphisms (rs988748, C132T and rs6265) and schizophrenia in our Japanese subjects. Neves-Pereira et al. (2005) found that the G allele frequency for rs6265 was significantly higher in schizophrenia patients than in control subjects, while Rosa et al. (2006) reported that the G allele was preferentially transmitted from parents to affected offspring. However, other studies, including the present study, failed to find this association (Egan et al., 2003; Hong et al., 2003; Anttila et al., 2005; de Krom et al., 2005; Dempster et al., 2005; Gourion et al., 2005; Szeszko et al., 2005; Tan et al., 2005;

Chen et al., 2006). The G allele frequency in our Japanese control subjects was 58.0%, which is lower than the frequencies for the Scottish control subjects (78.2%) in Neves-Pereira et al. (2005) and the Spanish control subjects (76.0%) in Rosa et al. (2006). Therefore, this discrepancy may stem from an ethnic heterogeneity in this polymorphism. Using the Genetic Power Calculation (Purcell et al., 2003), our sample has a power of 0.88 for detecting a significant association between the G allele and schizophrenia with an α value of 0.05, assuming a disease prevalence of 0.01, risk allele frequency of 0.580 and genotypic relative risks for G/G of 2.0 and G/A of 1.5. Accordingly, the likelihood of a type II error with our sample size appears to be very low. Recently, Schumacher et al. (2005) demonstrated that the haplotype constructed from rs988748-$(GT)_n$-rs6265 was associated with schizophrenia. However, a single-marker analysis did not provide evidence for an association, and our results therefore do not contradict those of Schumacher et al. (2005).

Szekeres et al. (2003) reported that the 132T allele frequency was significantly higher in Caucasian schizophrenia patients than in control subjects (13.9% vs. 2.9%). Nanko et al. (2003) detected a weak, but significant, association between the T allele and