intergenic splicing with *DISC1* (Millar et al. 2000). This genomic region has been shown to be closely related to susceptibility for BP (Curtis et al. 2003; Macgregor et al. 2004). It may thus also be influenced by translocation. Hennah et al.'s (2003) haplotype transmission analysis showed that *TSNAX* was associated with schizophrenia. Palo et al. (2007) reported that *TSNAX* was associated with female psychotic disorder. Thomson et al. (2005) also showed an association between *TSNAX* and male Scottish BP patients. These studies that have found association with *TSNAX* have used Caucasian populations for which the underlying linkage disequilibrium (LD) spans *TSNAX* into the first portion of the *DISC1*. In the Japanese population the two genes are on distinct LD regions according to HapMap database (release#23a/phase II, March 2008, www.hapmap.org, population: Japanese Tokyo). However, Zhang et al. (2005) reported that *TSNAX* was not associated with schizophrenia in Japanese patients. *TSNAX* was associated with impaired spatial working memory, increased reaction time to visual targets, and reduced gray matter predominantly in the superior and middle frontal gyri (Cannon et al. 2005). There are no reported gene-based association analyses between *TSNAX* and mood disorders in the Japanese population. Therefore, we conducted a case–control study with Japanese mood disorder samples. Two recent studies reported that MDD and SSRI response in MDD have common susceptibility genes. Lekman et al. (2008) reported that *FKBP5* was associated with MDD and the citalopram therapeutic response in the White non-Hispanic population. Tsai et al. (2008) also reported significant associations between plasminogen activator inhibitor type 1 gene (*SERPINE1*) and Chinese MDD patients and the SSRI therapeutic response. We therefore performed an association analysis between *TSNAX* and the efficacy of fluvoxamine treatment in Japanese patients with MDD.

Materials and Methods

Subjects

The subjects in the association analysis were 314 MDD patients (155 males and 159 females; mean age ± standard deviation 47.3 ± 14.9 years), 158 BP patients (81 males and 77 females; 99 patients with bipolar I disorder and 59 patients with bipolar II disorder; 47.9 ± 14.2 years), and 811 healthy controls (352 males and 459 females; 37.2 ± 15.9 years). Of the 314 MDD patients, 120 (59 males and 61 females; 42.0 ± 17.2 years) were treated with fluvoxamine and diagnosed according to DSM-IV criteria with the consensus of at least two experienced psychiatrists on the basis of a review of medical records and assessments with the Structured Interview Guide for Hamilton Rating Scale for Depression (SIGH-D). The remaining MDD patients were diagnosed according to DSM-IV criteria with the consensus of at least two experienced psychiatrists on the basis of unstructured interviews and a review of medical records. Fluvoxamine was taken two or three times a day for 8 weeks. The initial total dose was 50–100 mg per day, and the dosage was then increased gradually to a maximum of 150 mg, depending on the patients' condition. Patients with insomnia and severe anxiety were prescribed benzodiazepine drugs, but no other psychotropic drugs were permitted during the study. All subjects were unrelated to each other, ethnically Japanese, and lived in the central area of Japan.

All healthy controls were also psychiatrically screened based on unstructured interviews. None had severe medical complications such as cirrhosis, renal failure, heart failure, or other Axis-I disorders according to DSM-IV. The study was described to subjects and written informed consent was obtained from each. This study was approved by the Ethics Committees at Fujita Health University and Nagoya University School of Medicine.

Data Collection

The 120 MDD patients in this study had scores of 12 or higher on the 17 items of the SIGH-D (Peveler and Kendrick 2005). We defined a clinical response as a decrease of more than 50% in baseline SIGH-D within 8 weeks, and clinical remission as a SIGH-D score of less than 7 at 8 weeks. Detailed information on data collection was described in a previous paper (Saito et al. 2006). The clinical characteristics of patients in this study, classified according to these definitions, can be seen in Table 1.

SNP Selection and LD Evaluation

We first consulted the HapMap database (release#23a/phase II, March 2008, www.hapmap.org, population: Japanese Tokyo, minor allele frequencies (MAFs) of more than 0.05) and included 30 SNPs covering *TSNAX* (5′-flanking regions including about 55 kb from the initial exon and about 10 kb bp downstream (3′) from the last exon: HapMap database contig number chr 1q42.1: 229673505.229774037). Three 'tagging SNPs' in *TSNAX* were then selected with the criteria of r^2 threshold greater than 0.8 in 'pair-wise tagging only' mode using the 'Tagger' program (Paul de Bakker, http://www/broad.mit.edu/mpg/tagger) in Haploview for the following association analysis (Barrett et al. 2005).

Table 1 Clinical characteristics of the patients in both definition groups

	N			Age (mean ± SD)	Baseline SIGH-D (avg ± SD)	Fluvoxamine dose at 8 weeks (mg/day) (avg ± SD)	Number of previous episode (avg ± SD)
	Total	Male	Female				
Overall	120	59	61	42.0 ± 17.2	20.3 ± 5.88	122 ± 3.84	1.39 ± 0.658
Clinical response group[a]							
Responders	61	31	30	42.2 ± 16.2	21.4 ± 6.14	119 ± 40.8	1.36 ± 0.570
Nonresponders	59	28	31	41.7 ± 18.5	19.1 ± 5.39	125 ± 40.7	1.44 ± 0.783
P value	0.712			0.895	0.0274	0.433	0.849
Clinical remission group[b]							
Remitters	47	22	25	40.1 ± 15.1	19.5 ± 5.01	115 ± 43.6	1.36 ± 0.110
Nonremitters	73	37	36	43.0 ± 18.3	20.7 ± 6.36	127 ± 38.2	1.42 ± 0.107
P value	0.678			0.510	0.271	0.114	0.697

[a] Clinical response was defined as a 50% or greater decrease in the baseline SIGH-D score

[b] Clinical remission was defined as a final SIGH-D score of less than 7

SNP Genotyping

We used TaqMan assays (ABI: Applied Biosystems, Inc., Foster City, CA,) for all SNPs. One allelic probe was labeled with FAM dye and the other with fluorescent VIC dye. The plates were heated at 50°C for 2 min and 95°C for 10 min, followed by 45 cycles of 95°C for 15 s and 58°C for 1 min. Please refer to ABI for the primer sequence. Detailed information is available on request.

Statistical Analysis

Genotype deviation from the Hardy–Weinberg equilibrium (HWE) was evaluated by Chi-square test (SAS/Genetics, release 8.2, SAS Japan Inc, Tokyo, Japan). Marker-trait association analysis was used to evaluate allele- and genotype association with the Chi-square test (SAS/Genetics, release 8.2, SAS Japan Inc, Tokyo, Japan), and haplotype association analysis was evaluated with a likelihood ratio test using the COCAPHASE2.403 program (Dudbridge 2003). In the haplotype analysis, we determined that the cutoff for testing haplotype frequency was 0.05. We used the permutation test option as provided in the haplotype analysis to avoid spurious results and correct for multiple testing. Permutation test correction was performed using 1000 iterations (random permutations). In addition, Bonferroni's correction was used to control inflation of the type I error rate in the single marker association analysis and in the explorative analysis. For Bonferroni correction, we employed the following numbers of multiple tests: 3 for each sample set in allele- and genotype analysis (3 tagging SNPs in *TSNAX*); and 6 for the explorative analysis by sex (2 × 3 tagging SNPs). We had already performed a permutation test in the haplotype analysis. Power calculation was performed using a genetic power calculator (Purcell et al. 2003). The significance level for statistical tests was 0.05.

Results

The LD structure as determined from the HapMap database can be seen in Fig. 1. Genotype frequencies of all SNPs were in HWE. We did not detect any associations between *TSNAX* and mood disorders in the allele/genotype or haplotype analyses (Tables 2 and 3). It is known that there are sex differences in the pathophysiology of mood disorders (Currier et al. 2006; Faraone et al. 1987). Therefore, we performed an explorative analysis of subjects divided by sex. We found an association between rs766288 in *TSNAX* and female MDD in the allele/genotype analysis (Table 4). This significance remained after Bonferroni's correction. However, we did not find any association between *TSNAX* and BP in the allele/genotype analysis or haplotype analysis (Tables 4 and 5).

With regard to the clinical characteristics of patients, only one difference was detected between responders and nonresponders in baseline SIGH-D scores (P value = 0.0274) (Table 1). In addition to fluvoxamine treatment in this cohort, one patient each was prescribed alprazolam, loflazepate, and etizolam. Two patients each were prescribed lorazepam, brotizolm, flunitrazepam, and zopiclone. We did not find any association between *TSNAX* and the fluvoxamine therapeutic response in MDD patients in allele/genotype (Table 6) or haplotype analysis (Response: P value = 0.797 and Remission: P value = 0.773).

In the power analysis, we obtained more than 80% power for the detection of association when we set the genotype relative risk at 1.26–1.30 and 1.41–1.48 in MDD and BP, respectively, for *TSNAX* under a multiplicative model of inheritance.

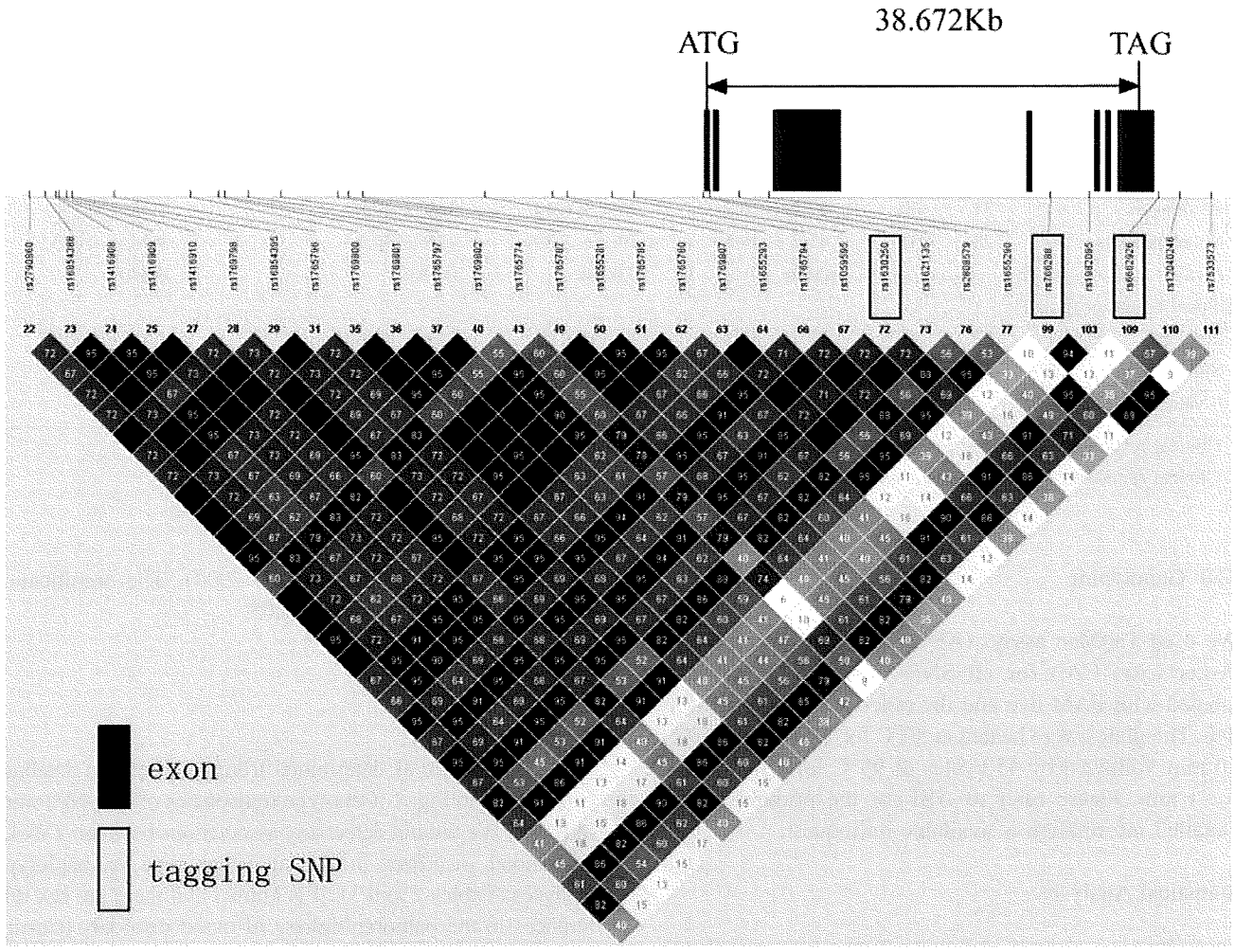

Fig. 1 LD evaluation and tagging SNPs in *TSNAX* ATG is the start codon and TAG is the stop codon. *Vertical bars* represent exons. Tagging SNPs selected from the HapMap database are represented by *black boxes*. Color scheme is based on r^2 value. Other information can be seen at the Haploview website

Table 2 Tagging SNPs and association analysis of *TSNAX*

SNP ID[a]	Phenotype[b]	MAF	*N*	Genotype distribution			*P* value[c]		
				M/M	M/m	m/m	HWE	Genotype	Allele
rs1630250	Controls	0.442	811	245	415	151	0.287		
5′ flanking region	MDD	0.475	314	85	160	69	0.700	0.356	0.165
C>G	BP	0.446	158	43	89	26	0.0789	0.493	0.892
rs766288	Controls	0.362	811	340	350	116	0.137		
Intron 4	MDD	0.322	314	141	144	29	0.367	0.0742	0.0727
C>T	BP	0.370	158	57	85	16	0.0535	0.0572	0.778
rs6662926	Controls	0.497	811	207	402	202	0.807		
3′ flanking region	MDD	0.463	314	87	163	64	0.438	0.270	0.153
C>G	BP	0.468	158	44	80	34	0.833	0.628	0.353

[a] Major allele > minor allele

[b] *MDD* Major depressive disorder, *BP* bipolar disorder, *MAF* minor allele frequency, *M* major allele, *m* minor allele

[c] Hardy–Weinberg equilibrium

Table 3 Haplotype analysis of tagging SNPs in *TSNAX*

TSNAX common haplotypes rs1630250-rs766288-rs6662926	Phenotype[a]	Individual haplotype frequency	Individual *P* value[b]	Phenotype[a]	Global *P* value
C–C–G	Control	0.263			
	MDD	0.236	0.280		
	BP	0.252	0.712		
C–T–G	Control	0.296		MDD	0.143
	MDD	0.266	0.258	BP	0.724
	BP	0.321	0.421		
G–C–C	Control	0.441			
	MDD	0.498	**0.0481**		
	BP	0.427	0.677		

[a] *MDD* Major depressive disorder, *BP* bipolar disorder

[b] Bold numbers represent significant *P* value

Table 4 Tagging SNPs and association analysis of *TSNAX* by sex

SNP ID[a]	Phenotype[b]	MAF	*N*	Genotype distribution			*P* value[c,d]			Corrected *P* value[d,e]	
				M/M	M/m	m/m	HWE	Genotype	Allele	Genotype	Allele
rs1630250	Male controls	0.440	352	107	180	65	0.482				
5′ flanking region	Male MDD	0.455	155	46	77	32	0.983	0.848	0.669		
C>G	Male BP	0.469	81	20	46	15	0.207	0.567	0.506		
	Female controls	0.443	459	138	235	86	0.425				
	Female MDD	0.494	159	39	83	37	0.577	0.286	0.120		
	Female BP	0.422	77	23	43	11	0.204	0.607	0.623		
rs766288	Male controls	0.362	352	148	153	51	0.266				
Intron 4	Male MDD	0.368	155	62	72	21	0.989	0.822	0.866		
C>T	Male BP	0.364	81	29	45	7	0.0726	0.110	0.962		
	Female controls	0.362	459	192	202	65	0.315				
	Female MDD	0.277	159	79	72	8	0.0980	**0.00661**	**0.00586**	**0.0397**	**0.0352**
	Female BP	0.377	77	28	40	9	0.351	0.429	0.721		
rs6662926	Male controls	0.492	352	92	174	86	0.835				
3′ flanking region	Male MDD	0.471	155	41	82	32	0.442	0.630	0.547		
C>G	Male BP	0.444	81	25	40	16	1.00	0.561	0.280		
	Female controls	0.501	459	115	228	116	0.889				
	Female MDD	0.456	189	46	81	32	0.735	0.363	0.166		
	Female BP	0.494	77	19	40	18	0.731	0.920	0.862		

[a] Major allele > minor allele

[b] *MDD* Major depressive disorder, *BP* bipolar disorder, *MAF* minor allele frequency, *M* major allele, *m* minor allele

[c] Hardy–Weinberg equilibrium

[d] Bold represents significant *P* value

[e] Calculated by Bonferroni's correction

Discussion

We first performed a gene-based association analysis between *TSNAX* and mood disorders including BP and MDD in the Japanese population. We found almost no association between *TSNAX* and mood disorders. However, we detected a significant association between *TSNAX* and Japanese female MDD in the Japanese population. This significant association remained after Bonferroni's correction was used to control inflation of the type I error rate due to multiple testing. *TSNAX* was associated with impaired spatial working memory, increased reaction time to visual targets, and reduced gray matter predominantly in the superior and middle frontal gyri (Cannon et al. 2005). This evidence may be involved in the pathophysiology of female MDD.

Table 5 Haplotype analysis of tagging SNPs in *TSNAX* by sex

TSNAX common haplotypes rs163 0250–rs766288–rs6662926	Phenotype[a]	Individual haplotype frequency	Individual *P* value[b]	Phenotype[a]	Global *P* value
C–C–G	Male controls	0.282			
	Male MDD	0.227	0.134		
	Male BP	0.258	0.596		
	Female controls	0.249		Male MDD	0.751
	Female MDD	0.245	0.903	Male BP	0.751
	Female BP	0.246	0.937		
C–T–G	Male controls	0.284		Female MDD	0.0744
	Male MDD	0.304	0.508	Female BP	0.885
	Male BP	0.317	0.477		
	Female controls	0.304			
	Female MDD	0.231	**0.0400**		
	Female BP	0.325	0.628		
G–C–C	Male controls	0.424			
	Male MDD	0.469	0.393		
	Male BP	0.425	0.864		
	Female controls	0.447			
	Female MDD	0.524	**0.0488**		
	Female BP	0.429	0.703		

[a] *MDD* Major depressive disorder, *BP* bipolar disorder

[b] Bold numbers represent significant *P* value

Table 6 Genotype and allele distributions of *TSNAX* in both definition groups

SNP ID[a]	Phenotype[b]	MAF	*N*	Genotype distribution			*P* value[c]		
				M/M	M/m	m/m	HWE	Genotype	Allele
rs1630250	Responders	0.500	61	15	31	15	0.898		
5′ flanking region	Nonresponders	0.551	59	14	25	20	0.270	0.507	0.430
C>G	Remission	0.510	47	12	22	13	0.664		
	Nonremission	0.534	73	17	34	22	0.584	0.942	0.721
rs766288	Responders	0.311	61	26	32	3	0.0815		
Intron 4	Nonresponders	0.271	59	31	24	4	0.823	0.429	0.492
C>T	Remission	0.266	47	23	23	1	0.0824		
	Nonremission	0.308	73	34	33	6	0.608	0.380	0.482
rs6662926	Responders	0.426	61	20	30	11	0.966		
3′ flanking region	Nonresponders	0.339	59	18	29	12	0.959	0.936	0.720
C>G	Remission	0.404	47	16	24	7	0.680		
	Nonremission	0.459	73	22	35	16	0.768	0.628	0.405

[a] Major allele > minor allele

[b] *MDD* Major depressive disorder, *BP* bipolar disorder, *M* major allele, *m* minor allele, *MAF* minor allele frequency

[c] Hardy–Weinberg equilibrium

It is known that there are sex differences in the pathophysiology of mood disorders (Currier et al. 2006; Faraone et al. 1987). We detected an association between rs766288 in intron 4 in *TSNAX* and female Japanese MDD patients. Several other investigations have also reported sex differences in associations between *TSNAX* and psychiatric disorders. Thomson et al. (2005) showed an association between several SNPs, including rs766288, in *TSNAX* and male Scottish BP patients in a haplotype analysis. Palo et al. (2007) reported that *TSNAX* (single marker association analysis: rs1655285 and haplotype analysis, including rs1655285) was associated with female psychotic disorder. However, because SNP composites for these haplotypes were "MAFs = 0" or raw data were not presented in the

HapMap database, we did not perform an association analysis for this SNP in the current study. Other genes have demonstrated gender differences in association to mood disorders. Szczepankiewicz et al. reported an association between a diagnosis of BP II in females and the glycogen synthase kinase-3 β gene (*GSK3B*) (Szczepankiewicz et al. 2006). Our previous study reported an association between prokineticin 2 receptor gene (*PROKR2*) and female BP and MDD (Kishi et al. 2009c). Several sex differences are observed in mood disorders, with the prevalence of MDD being higher in females. Hormonal variations in females, such as during the menstrual cycle, pregnancy and menopause, affect the onset and course of mood disorders, and it is possible that the etiology of mood disorders differs somewhat in females and males. Since our findings show significant associations between *TSNAX* and MDD in female Japanese patients, our results may support the supposition that the etiology of mood disorders differs somewhat in females and males.

According to HapMap database, rs1630250's MAFs in the Japanese population appear to be smaller than in Caucasians. On the other hand, rs6662926's MAFs in Caucasians were smaller than in Japanese. Also, rs766288 was the almost same MAFs in both Japanese and Caucasians. Schosser et al. (2009) reported that rs766288 was not associated with BP or MDD in the UK population. Although they selected only one SNP (rs766288) in *TSNAX*, their study was a case–control study using larger samples than our study (Schosser et al. 2009). Hennah et al. haplotype transmission analysis showed that SNPs in intron 4 in *TSNAX* (rs1615344, rs1615409, and rs766288) was associated with schizophrenia. Zhang et al. reported that *TSNAX* was not associated with Japanese schizophrenic patients. In this study, they selected rs1630250, rs1621135, and rs1655284 in *TSNAX*. We used rs1630250, and considered other SNPs that were in LD with tagging SNPs according to the HapMap database (release#23a/phase II, March 2008, www.hapmap.org, population: Japanese Tokyo). For MAF of rs1630250, our findings were almost same as those of Zhag and et al.' study (2005). In the Finnish population, Kilpinen et al. (2008) did not detect an association between *TSNAX* and autism or Asperger syndrome.

Because testing for HWE is commonly used for quality control in large-scale genotyping and is one of the few ways to identify systematic genotyping errors in unrelated individuals (Wittke-Thompson et al. 2005), we estimated HWE and confirmed the genotyping quality in this study. Genotype frequencies were in HWE for the SNPs in this study.

A few points of caution should be mentioned with respect to our results. Firstly, an association of *TSNAX* with female MDD patients may be due to biased samples, such as small sample sizes or unmatched age. In the power analysis, we obtained power of more than 80% for the detection of association when we set the genotype relative risk at 1.26–1.30 and 1.41–1.48 in MDD and BP, respectively, under a multiplicative model of inheritance. Because our samples were small, the statistical errors are possible in the results of these statistical association analyses. On average, the controls were much younger than the patients. This means that a number of young controls may go on to develop one of these disorders, most likely MDD, since the incidence of major depression is as high as 5% or more. Our subjects did not undergo structured interviews, and it is reported that MDD patients who are not diagnosed by structured interview may develop bipolar disorder in the future (Bowden 2001; Kishi et al. 2008b, 2009a; Stensland et al. 2008). However, in this study patients were carefully diagnosed according to DSM-IV criteria with consensus of at least two experienced psychiatrists on the basis of a review of medical records. In addition, when we found a patient who had been misdiagnosed, we promptly excluded the misdiagnosed case to maintain the precision of our sample (Kishi et al. 2008a, b, 2009b). Secondly, we did not perform a mutation scan of *TSNAX*. Because we consider it to be difficult to evaluate the association of extremely rare variants from the viewpoint of statistical power, a replication study using a larger sample will be required for conclusive results. Thirdly, HapMap data has been updated to release #27 to date.

In conclusion, our results suggest that *TSNAX* probably plays a role in female MDD in the Japanese population. However, because our samples are small, it will be important to replicate and confirm these findings in other independent studies using larger samples.

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Serotonin 1A receptor gene is associated with Japanese methamphetamine-induced psychosis patients

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ABSTRACT

Background: Several investigations have reported associations the serotonin 1A (5-HT1A) receptor to schizophrenia and psychotic disorders, making 5-HT1A receptor gene (*HTR1A*) an adequate candidate gene for the pathophysiology of schizophrenia and methamphetamine (METH)-induced psychosis. Huang and colleagues reported that rs6295 in *HTR1A* was associated with schizophrenia. The symptoms of methamphetamine (METH)-induced psychosis are similar to those of paranoid type schizophrenia. It may indicate that METH-induced psychosis and schizophrenia have common susceptibility genes. In support of this hypothesis, we reported that the V-act murine thymoma viral oncogene homologue 1 (AKT1) gene was associated with METH-induced psychosis and schizophrenia in the Japanese population. Furthermore, we conducted an analysis of the association of *HTR1A* with METH-induced psychosis.

Method: Using one functional SNP (rs6295) and one tagging SNP (rs878567), we conducted a genetic association analysis of case-control samples (197 METH-induced psychosis patients and 337 controls) in the Japanese population. The age and sex of the control subjects did not differ from those of the methamphetamine dependence patients.

Results: Rs878567 was associated with METH-induced psychosis patients in the allele/genotype-wise analysis. Moreover, this significance remained after Bonferroni correction. In addition, we detected an association between rs6295 and rs878567 in *HTR1A* and METH-induced psychosis patients in the haplotype-wise analysis. Although we detected an association between rs6295 and METH-induced psychosis patients, this significance disappeared after Bonferroni correction.

Conclusion: *HTR1A* may play an important role in the pathophysiology of METH-induced psychosis in the Japanese population. However, because we did not perform a mutation scan of *HTR1A*, a replication study using a larger sample may be required for conclusive results.

1. Introduction

Altered serotonergic neural transmission is hypothesized to be a susceptibility factor for schizophrenia (Geyer and Vollenweider, 2008; Meltzer et al., 2003). Several postmortem studies reported increased serotonin 1A (5-HT1A) receptor in the prefrontal cortex

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of schizophrenic patients (Burnet et al., 1996; Hashimoto et al., 1993, 1991; Simpson et al., 1996; Sumiyoshi et al., 1996). Huang and colleagues reported that rs6295 in an SNP (C-1019G: rs6295) in the promoter region of the 5-HT1A receptor gene (*HTR1A*), which regulate HTR1A transcription (Le Francois et al., 2008; Lemonde et al., 2003), was associated with schizophrenia (Huang et al., 2004). These facts suggest a crucial relationship between the 5-HT1A receptor and schizophrenia, and that *HTR1A* is an adequate candidate for the etiology of schizophrenia. *HTR1A* (OMIM*109 760, 1 exon in this genomic region spanning 2.069 kb) is located on 5q11.

The symptoms of methamphetamine (METH)-induced psychosis are similar to those of paranoid type schizophrenia (Sato et al., 1992). It may indicate that METH-induced psychosis and schizophrenia have common susceptibility genes (Bousman et al., 2009). In support of this hypothesis, we reported that the V-act murine thymoma viral oncogene homologue 1 (AKT1) gene was associated with METH-induced psychosis (Ikeda et al., 2006) and schizophrenia (Ikeda et al., 2004) in the Japanese population. Furthermore, we conducted an analysis of the association of these genes with METH-induced psychosis, using the recently recommended strategy of 'gene-based' association analysis (Neale and Sham, 2004).

2. Materials and methods

2.1. Subjects

The subjects in the association analysis were 197 METH-induced psychosis patients (164 males: 83.2% and 33 females; mean age ± standard deviation (SD) 37.6 ± 12.2 years) and 337 healthy controls (271 males: 80.4% and 66 females; 37.6 ± 14.3 years). The age and sex of the control subjects did not differ from those of the methamphetamine dependence patients. All subjects were unrelated to each other, ethnically Japanese, and lived in the central area of Japan. The patients were diagnosed according to DSM-IV criteria with consensus of at least two experienced psychiatrists on the basis of unstructured interviews and a review of medical records. METH-induced psychosis patients were divided into two categories of psychosis prognosis, the transient type and the prolonged type, which showed remission of psychotic symptoms within 1 month and after more than 1 month, respectively, after the discontinuance of methamphetamine consumption and beginning of treatment with neuroleptics; 112 patients (56.9%) were the transient type, and 85 patients (43.1%) were the prolonged type. One hundred thirty-seven subjects with METH-induced psychosis also had dependence on drugs other than METH. Cannabinoids were the most frequency abused drugs (31.4%), followed by cocaine (9.09%), LSD (9.09%), opioids (7.69%), and hypnotics (7.69%). Subjects with METH-induced psychosis were excluded if they had a clinical diagnosis of psychotic disorder, mood disorder, anxiety disorder or eating disorder. More detailed characterizations of these subjects have been published elsewhere (Kishi et al., 2008b). All healthy controls were also psychiatrically screened based on unstructured interviews. None had severe medical complications such as liver cirrhosis, renal failure, heart failure or other Axis-I disorders according to DSM-IV.

The study was described to subjects and written informed consent was obtained from each. This study was approved by the Ethics Committee at Fujita Health University, Nagoya University School of Medicine and each participating member of the Institute of the Japanese Genetics Initiative for Drug Abuse (JGIDA).

2.2. SNPs selection and linkage disequilibrium (LD) evaluation

We first consulted the HapMap database (release#23.a.phase2, Mar 2008, www.hapmap.org, population: Japanese Tokyo: minor allele frequencies (MAFs) of more than 0.05) and included 3 SNPs (rs6449693, rs878567 and rs1423691) covering *HTR1A* (5′-flanking regions including about 1 kb from the initial exon and about 2 kb downstream (3′) from the last exon: HapMap database contig number chr5: 63287418...63291774). Then one tagging SNP was selected with the criteria of an r^2 threshold greater than 0.8 in 'pair-wise tagging only' mode using the 'Tagger' program (Paul de Bakker, http://www/broad.mit.edu/mpg/tagger) of the HAPLOVIEW software (Barrett et al., 2005).

HTR1A has also been reported to have one biologically functional SNP (C-1019G: rs6295) (Albert et al., 1996; Albert and Lemonde, 2004; Lemonde et al., 2003). Rs6295 (C-1019G) in the promoter region regulate *HTR1A* transcription (Le Francois et al., 2008; Lemonde et al., 2003). The C allele is a part of a 26 palindrome that connect transcription factors (Deaf-1, Hes1 and Hes5) by NUDR (nuclear deformed epidermal autoregulatory factor), whereas the G allele abolishes repression by NUDR (Le Francois et al., 2008; Lemonde et al., 2003). This would lead to elevated levels of 5-HT1A receptor in the presynaptic raphe nucleus in GG genotypes, compared with CC genotype (Le Francois et al., 2008; Lemonde et al., 2003). Since no information about rs6295 was shown in the HapMap database, we included this SNP. These two SNPs were then used for the following association analysis.

2.3. SNPs genotyping

We used TaqMan assays (Applied Biosystems, Inc., Foster City, CA) for both SNPs. Detailed information, including primer sequences and reaction conditions, is available on request.

2.4. Statistical analysis

Genotype deviation from the Hardy–Weinberg equilibrium (HWE) was evaluated by chi-square test (SAS/Genetics, release 8.2, SAS Japan Inc., Tokyo, Japan).

Marker-trait association analysis was used to evaluate allele- and genotype-wise association with the chi-square test (SAS/Genetics, release 8.2, SAS Japan Inc., Tokyo, Japan), and haplotype-wise association analysis was conducted with a likelihood ratio test using the COCAPHASE2.403 program (Dudbridge, 2003). We used the permutation test option as provided in the haplotype-wise analysis to avoid spurious results and correct for multiple testing. Permutation test correction was performed using 1000 iterations (random permutations). In addition, Bonferroni's correction was used to control inflation of the type I error rate in the single marker association analysis and in the explorative analysis. For Bonferroni correction, we employed the following numbers for multiple testing: 2 for each sample set in allele- and genotype-wise analysis (2 examined SNPs). We had already performed a permutation test in the haplotype-wise analysis. Power calculation was performed using a genetic power calculator (Purcell et al., 2003).

The significance level for all statistical tests was 0.05.

3. Results

The LD from rs6449693, rs878567 and rs1423691 was tight in from the HapMap database samples ($r^2 = 1.00$). However, the LD structure of rs6295 (functional SNP) and rs878567 (tagging SNP) in our control samples was not tight ($r^2 = 0.160$). Genotype frequencies of all SNPs were in HWE (Table 1). Rs878567 was associated with METH-induced psychosis patients in the allele/genotype-wise analysis (P allele = 0.000122 and P genotype = 0.00103) (Table 1). Moreover, these significances remained after Bonferroni correction (P allele = 0.000244 and P genotype = 0.00203) (Table 1). In addition, we detected an association between rs6295 and rs878567 in *HTR1A* and METH-induced psychosis patients in the haplotype-wise analysis ($P = 0.0000643$) (Table 2). Although we detected an association between rs6295 and METH-induced psychosis patients (P allele = 0.0271), this significance disappeared after Bonferroni correction (P allele = 0.0542) (Table 1).

4. Discussion

We found associations between *HTR1A* and Japanese METH-induced psychosis patients. Therefore, we reasoned that *HTR1A* may play an important role in the pathophysiology of METH-induced psychosis in the Japanese population. However, our samples are small. Although Bonferroni's correction was used to control inflation of the type I error rate, we considered that there is a possibility of type I error in these results.

The 5-HT1A receptor is present in various regions of the brain, including the cortex, hippocampus, amygdala, hypothalamus and septum (Aznar et al., 2003; Barnes and Sharp, 1999; Le Francois et al., 2008; Varnas et al., 2004). Presynaptic 5-HT1A autoreceptors play an important role in the autoregulation of serotonergic neurons (Le Francois et al., 2008; Lemonde et al., 2003; Riad et al., 2000; Sotelo et al., 1990). The 5-HT1A receptor activation by serotonin induces the hyperpolarization of serotonergic neurons, decreasing their firing rate and consequently the release of serotonin in the brain (Le Francois et al., 2008; Lemonde et al., 2003; Riad et al., 2000; Sotelo et al., 1990). Also, the 5-HT1A receptor was associated hippocampal neurogenesis. The hippocampus is a part of the limbic system involved in cognitive function such as memory. Stimulation of 5-HT1A receptors has been known to reduce the

Table 1
Association analysis of *HTR1A* with methamphetamine-induced psychosis.

SNP[a]	Phenotype[b]	MAFs[c]	N	Genotype distribution[d]			*P*-value[f]			Corrected *P*-value[f,g]	
				M/M	M/m	m/m	HWE[e]	Genotype	Allele	Genotype	Allele
rs6295	Controls	0.254	336	192	117	27	0.132				
C > G	METH-induced psychosis	0.317	197	92	85	20	0.955	0.0657	**0.0271**		0.0542
rs878567	Controls	0.126	336	258	71	7	0.423				
C > T	METH-induced psychosis	0.216	197	124	61	12	0.233	**0.00103**	**0.000122**	**0.00203**	**0.000244**

[a] Major allele > minor allele.
[b] METH-induced psychosis: methamphetamine-induced psychosis.
[c] MAFs: minor allele frequencies.
[d] M: major allele, m: minor allele.
[e] Hardy–Weinberg equilibrium.
[f] Bold represents significant *P*-value.
[g] Calculated using Bonferroni's correction.

negative symptoms and cognitive dysfunction of schizophrenia (Meltzer et al., 2003; Meltzer and Sumiyoshi, 2008; Sumiyoshi et al., 2001, 2007). Mason and Reynolds (1992) reported that one of the major pharmacological therapeutic targets of clozapine is 5-HT1A receptors on cortical glutamatergic neurons. Several post-mortem studies reported increased 5-HT1A receptor in the prefrontal cortex of schizophrenic patients (Burnet et al., 1996; Hashimoto et al., 1993, 1991; Simpson et al., 1996; Sumiyoshi et al., 1996). NAN-190 (5-HT1A receptor antagonist) produced an inhibitory action on methamphetamine-induced hyperactivity (Ginawi et al., 2004; Millan and Colpaert, 1991). These facts suggest that altered serotonergic neural transmission caused by abnormalities in 5-HT1A receptor may be involved in the development of psychotic disorders such as schizophrenia and METH-induced psychosis (Geyer and Vollenweider, 2008; Meltzer et al., 2003).

Serretti et al. (2007) reported that rs878567 in *HTR1A* was associated with German and Italian suicidal attempters. Also, previous study have reported that rs878567 in *HTR1A* was found the interaction with childhood physical abuse in mood disorders (Brezo et al., 2009). These authors suggested rs878567 might influence hippocampus-mediated memory deficits in mood disorders (Brezo et al., 2009). The LD from rs6449693, rs878567 and rs1423691 was tight in from the HapMap database samples (r^2 = 1.00). As these results show, rs878567 covers a wide and important region including the exon and the promoter region in *HTR1A*. Because it is possible that rs878567 influences biological function in the brain, we suggest that functional analysis for rs878567 should be performed in future studies.

Rs6295 (C-1019G) in the promoter region regulate *HTR1A* transcription (Le Francois et al., 2008; Lemonde et al., 2003). The C allele is a part of a 26 palindrome that connect transcription factors (Deaf-1, Hes1 and Hes5) by NUDR (nuclear deformed epidermal autoregulatory factor), whereas the G allele abolishes repression by NUDR (Le Francois et al., 2008; Lemonde et al., 2003). This would lead to elevated levels of 5-HT1A receptor in the presynaptic raphe nucleus in GG genotyps, compared with CC genotype (Le Francois et al., 2008; Lemonde et al., 2003). This variant was associated with several studies, including major depressive disorder (Anttila et al., 2007; Kraus et al., 2007; Lemonde et al., 2003; Neff et al., 2009; Parsey et al., 2006) and panic disorder (Strobel et al., 2003) and antidepressant response in MDD (Arias et al., 2005; Hong et al., 2006; Lemonde et al., 2004; Parsey et al., 2006; Serretti et al., 2004; Yu et al., 2006). Huang et al. (2004) reported that rs6295 was associated with schizophrenia. Recent studies reported that rs6295 was associated with the improvement in negative symptoms from antipsychotics such as risperidone (Mossner et al., 2009; Reynolds et al., 2006; Wang et al., 2008) and that 5-HT1A receptor agonists such as tandospirone produced improvements in the cognitive impairment in schizophrenia (Meltzer and Sumiyoshi, 2008; Sumiyoshi et al., 2001, 2007).

A few points of caution should be mentioned with respect to our results. Firstly, the positive association may be due to small sample size. Ideal samples for this study are METH use disorder samples with and without psychosis. Because we had only a few METH use disorder samples without psychosis, and we wanted to avoid statistical error, we did not perform an association analysis with these samples. Secondly, we did not include a mutation scan to detect rare variants. We designed the study based on the common disease-common variants hypothesis (Chakravarti, 1999). However, Weickert et al. (2008) have shown associations between a common disease such as schizophrenia and rare variants. If the genetic background of METH-induced psychosis is described by the common disease-rare variants hypothesis, further investigation will be required, such as medical resequencing using larger samples. However, statistical power is needed to evaluate the association of rare variants. Lastly, our subjects did not undergo structured interviews. However, in this study patients were carefully diagnosed according to DSM-IV criteria with consensus of at least two experienced psychiatrists on the basis of a review of medical records (Kishi et al., 2008a,c, 2009). In addition, when we found misdiagnosis in a patient, we promptly excluded the misdiagnosed case to maintain the precision of our sample. To overcome these limitations, a replication study using larger samples or samples of other populations will be required for conclusive results.

In conclusion, our results suggest that *HTR1A* may play a major role in the pathophysiology of METH-induced psychosis in the Japanese population. However, because we did not perform a mutation scan of *HTR1A*, a replication study using a larger sample may be required for conclusive results.

Table 2
Haplotype-wise analysis of *HTR1A*.

Haplotype rs6295-rs878567	Phenotype[a]	Individual haplotype frequency	Individual *P*-value[b]	Phenotype[a]	Global *P*-value[b]
C–C	Control	0.811			
	METH-induced psychosis	0.694	**0.0000364**	METH-induced psychosis	**0.0000643**
G–C	Control	0.189			
	METH-induced psychosis	0.306	**0.0000364**		

[a] METH-induced psychosis: methamphetamine-induced psychosis.
[b] Bold numbers represent significant *P*-value.

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Identification of Novel Candidate Genes for Treatment Response to Risperidone and Susceptibility for Schizophrenia: Integrated Analysis Among Pharmacogenomics, Mouse Expression, and Genetic Case-Control Association Approaches

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Background: Pharmacogenomic approaches based on genomewide sets of single nucleotide polymorphisms (SNPs) are now feasible and offer the potential to uncover variants that influence drug response.

Methods: To detect potential predictor gene variants for risperidone response in schizophrenic subjects, we performed a convergent analysis based on 1) a genomewide (100K SNP) SNP pharmacogenetic study of risperidone response and 2) a global transcriptome study of genes with mRNA levels influenced by risperidone exposure in mouse prefrontal cortex.

Results: Fourteen genes were highlighted as of potential relevance to risperidone activity in both studies: *ATP2B2, HS3ST2, UNC5C, BAG3, PDE7B, PAICS, PTGFRN, NR3C2, ZBTB20, ST6GAL2, PIP5K1B, EPHA6, KCNH5,* and *AJAP1*. The SNPs related to these genes that were associated in the pharmacogenetic study were further assessed for evidence for association with schizophrenia in up to three case-control series comprising 1564 cases and 3862 controls in total (Japanese [JPN] 1st and 2nd samples and UK sample). Of 14 SNPs tested, one (rs9389370) in *PDE7B* showed significant evidence for association with schizophrenia in a discovery sample ($p_{allele} = .026$ in JPN_1st, two-tailed). This finding replicated in a joint analysis of two independent case-control samples ($p_{JPN\_2nd+UK} = .008$, one-tailed, uncorrected) and in all combined data sets ($p_{all} = .0014$, two-tailed, uncorrected and $p_{all} = .018$, two-tailed, Bonferroni correction).

Conclusions: We identified novel candidate genes for treatment response to risperidone and provide evidence that one of these additionally may confer susceptibility to schizophrenia. Specifically, *PDE7B* is an attractive candidate gene, although evidence from integrated methodology, including pharmacogenomics, pharmacotranscriptomic, and case-control association approaches.

Key Words: Expression: PDE7B, pharmacogenomics, risperidone, schizophrenia

Schizophrenia is a severe psychiatric disorder with a lifetime risk of approximately 1%. With its early onset, typically in late teens to early 20s, frequent relapse and chronic course, schizophrenia imposes a considerable burden on sufferers, their families, and society. Worldwide, it is a major source of morbidity, but it is often overlooked that it is also associated with a considerable truncation in life span, the mortality rate in individuals with schizophrenia being more than twice that of the age- and sex-matched population (1). A large number of antipsychotics have been developed as treatment agents. However, individual response to these drugs is highly variable, and identifying the optimal treatment for any patient is often a trial and error process that can span many years and even then, response is often poor. There is a pressing need both to identify new treatments and to attempt to improve the information based on which response to treatment can be predicted.

Genetic factors are generally assumed to contribute to variable treatment response (2), and on this basis, a number of pharmacogenetic studies have been performed. Here, the aim was to detect DNA sequence predictors for treatment response. Most studies have focused on genes encoding neurotransmitter receptors, such as dopamine or serotonin receptors, the logic being that antipsychotics usually have high affinities with members of these classes of receptor. Although a number of variants have been correlated with treatment response in several stud-

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ies—for example, dopamine D2 and D3 receptor variants (2)—there are no definitive predictors of response.

Pharmacogenetics has been driven by a candidate gene approach. This approach has the disadvantage that targets for study are limited by our current understanding of the mechanisms of drugs, and therefore, this method cannot identify unsuspected predictor genes. Approaches that are independent of prior functional hypotheses of gene action based on genome-wide surveys of SNPs are, however, now feasible. The genome-wide approach has its disadvantages, but one of the most important is that, with effectively random sets of SNPs, the low prior probability that any is truly associated with disease requires a stringent type I error rate to control the enormous potential for reporting false positives. One way to address this issue is to use very large (and therefore highly powered) studies in which such stringent statistical support might realistically be achieved. Another approach that is more economical in genotyping costs is to undertake multistage analysis in which candidate variants from a screening sample are validated by replication in other data sets (3). However, because the samples for pharmacogenomics require a large amount of clinical data and are preferably prospective, large samples are difficult to collect.

Another approach is to try to enhance the prior probability for a given gene by integrating pharmacogenomic data with other sources of data—for example, from studies of gene expression (4). Under the hypothesis that genes related to drug response may be regulated by exposure to that drug, genes in which expression is altered in animals exposed to that drug have a higher probability of being genuinely associated in a pharmacogenetic study than any random gene. If this is correct, genes in which expression is altered by drug exposure require less stringent statistical support.

We aimed to detect predictor genes for risperidone response in schizophrenic patients using this convergent approach (4). Specifically, we compared data from a pharmacogenetic study based on first-episode, previously drug-naive subjects with schizophrenia who were treated with risperidone with data from a pharmacotranscriptomic study based on mice exposed to the same drug. Moreover, candidate variants from genes implicated by convergent data were also tested for evidence for association to schizophrenia per se because variants that are related to drug response may also be related to disease risk (5). Evidence that this occurs can be considered an additional independent line of circumstantial support that the convergence between the pharmacogenetic and transcriptomics does not merely reflect chance.

Methods and Materials

Subjects and Collection of Clinical Data

We performed an open-labeled pharmacogenetic study involving 108 first-episode, previously antipsychotic-naive schizophrenic patients. All received risperidone monotherapy after enrollment. Details are described elsewhere (6,7). Briefly, patients were entered into the study if they 1) met DSM-IV-TR criteria for schizophrenia (and then remained in follow-up to at least 6 months), 2) were physically healthy and had all laboratory parameters within normal limits, and 3) had neither a current nor a past DSM-IV-TR diagnosis of mood disorders or substance abuse. Consensus diagnoses were made by at least two experienced psychiatrists on the basis of unstructured interviews with patients and families and review of medical records. Duration of untreated psychosis (DUP) was defined as the period from the onset of psychotic symptoms to that of first antipsychotic exposure. Sixty subjects were recruited from outpatient clinics, and 48 subjects were treated as inpatients.

Subjects received risperidone monotherapy (starting dosage: .5–4 mg/day, mean starting dosage: 2.5 mg), and dosage was adjusted in accordance with symptomatic response by trained psychiatrists (1–8 mg/day, mean dosage: 3.4 mg at 8 weeks) for 8 weeks. Patients with insomnia were prescribed brotizolam, .25 mg or .5 mg, at bedtime. No other psychotropic drugs were permitted.

Clinical symptoms were evaluated at the first visit and after 8 weeks of treatment by the use of the Positive and Negative Syndrome Scale (PANSS). Evaluations were carried out by qualified psychiatrists and psychologists (the interrater reliability was measured by intraclass correlation coefficient was .90, unpublished data).

The clinical characteristics of subjects that we used as potential covariates were selected from another report (8): sex (57 male, 51 female), age (mean 30.2 ± 9.5 years), DUP (1.5–32 months, mean 7.6 ± 7.1 months), and baseline PANSS total score (mean 83.0 ± 22.9).

Samples used in the schizophrenia case-control association analysis consisted of three sets: (1) JPN_1st: this was used for identifying genes of potential interest and comprised 540 patients with schizophrenia (275 male and 265 female; aged 43.3 ± 15.0 years) and 425 healthy controls (236 male and 189 female; aged 36.3 ± 13.9 years) from the Japanese population; 2) JPN_2nd sample (used to follow up genes of interest) comprised 545 patients with schizophrenia (282 male and 263 female; aged 50.7 ± 14.9 years) and 500 controls (279 male and 221 female; aged 40.8 ± 15.4 years) from the Japanese population; 3) Additional follow-up data for SNPs of interest were extracted from a UK genomewide association study (GWAS) of schizophrenia comprising 479 patients with schizophrenia and 2937 controls from the UK population (9).

Controls in the Japanese population were screened for past history of mental disorders. All individuals were unrelated. 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, University of Occupational and Environmental Health, Nagoya University Graduate School of Medicine, Osaka University Graduate School of Medicine and by multiple ethics committees across the UK where sample recruitment was performed.

Microarray Experiments

See also Methods in Supplement 1.

SNP Chip. Genomewide genotyping was carried out using Illumina Sentrix human 1 Genotyping BeadChip (109,363 SNPs randomly distributed throughout the genome) according to the manufacturer's instructions (Illumina, San Diego, California). Details are given in the Supplement 1.

Mouse Expression Chip. We compared mRNA levels of the prefrontal cortex (PFC) between control ($n = 3$) and risperidone-exposed mice (2.4 mg/kg given orally, once a day for 21 days, $n = 3$). Affymetrix Mouse Gene 1.0 St. Array, which profiles the expression of 28,853 genes (Affymetrix, Santa Clara, California), was used to measure the amount of mRNA.

The procedures involving animals and their care were conducted in conformity with the international guidelines, Principles of Laboratory Animal Care (National Institutes of Health Publication 85-23, revised 1985).

Experimental Procedures and Statistical Analysis

Study 1: Pharmacogenomics. Quality control (QC) regarding population stratification (Figure S1 in the Supplement 1),

Hardy-Weinberg equilibrium (HWE), genotyping rate, and minor allele frequency was conducted by PLINK (10). Details are described in Supplement 1.

After QC, 99 samples (51 males and 48 females) and 62,935 autosomal SNPs (a mean call rate of 99.2%, indicating a high rate of successful genotyping) were analyzed to evaluate the effect of each SNP on antipsychotic response to risperidone.

To evaluate the effect of each SNP on antipsychotic response to risperidone, multiple regression analysis was carried out with a dependent variable [% PANSS change = 100 × ((PANSS at week 0) − (PANSS at Week 8))/PANSS at Week 0] and independent variables that included sex, age, duration of illness, initial PANSS score, and the genotype of each polymorphism. Each genotype was assessed using dominant, recessive, and multiplicative genetic models, respectively.

To calculate the best empiric p values based on the most significant result in each genetic model, we generated 1 million simulated data sets by randomizing the PANSS changes (the covariates stay with the genotypes) with respect to the GWAS data. This approach retains the linkage disequilibrium (LD) relationships between SNPs, and therefore allows for the appropriate degree of nonindependence in the data sets. The same multiple regression analysis model as applied to the real data were applied to each SNP in each permuted data set, and the empiric significance for a SNP was the proportion of the simulated data sets in which the test statistic was equal to, or greater than, that observed in the true data set (11–15).

SNPs were annotated to the closest genes with an up- and downstream span of 20 kb by WGAViewer (16).

Study 2: Mouse Expression Assay. In the mouse expression assay, data sets passing QC were normalized using GeneChip Operating Software (Affymetrix) and the raw intensity values exported for further analysis. Only genes called present based on Affymetrix detection p value for the presence of each gene on either chip were included. A t test was performed to assess the statistical significance of genes in which expression differed between control and risperidone-exposed mice. Power analysis was carried out using PowerAtlas (17). Our data set provides expected discovery rate (corresponding to power) of .37, an expected proportion of true positives (PTPs) of .72, and an expected proportion of true negatives of .80 at alpha set at .05. A major aim of this study was to prioritize genes showing convergent evidence in the pharmacogenomic study, thus we consider a high PTP optimal.

These data were submitted to CIBEX (http://cibex.nig.ac.jp/index.jsp, accession number: CBX77).

Study 3: Checking Overlap Results Between Pharmacogenomics (Study 1) and Mouse Expression Assay (Study 2). We checked candidate SNPs from the genes that showed convergent evidence for relevance to risperidone action from Study 1 and Study 2. Candidate genes were defined as follows: 1) genes for which there was at least one SNP with p values less than 5.0×10^{-4} in the pharmacogenomic study and in which expression significantly differed between groups at $\alpha < .05$ and 2) genes with much stronger evidence for $p < 1.0 \times 10^{-4}$ but that had weaker evidence for association in the pharmacogenomic study ($p < .05$).

Study 4: Case-Control Association Analysis of Strong Candidate Genes from Pharmacogenomics and Mouse Expression Assay. The candidate SNPs from Study 3 were further assessed for evidence for association with schizophrenia. These SNPs were genotyped by TaqMan assay (Applied Biosystems, California) in the Japanese case-control samples. Genotypes for the SNP in *PDE7B* in the UK samples were extracted from the Affymetrix GWAS data (9) after confirmation of good-quality cluster plots.

Genotype deviation from HWE was evaluated by a goodness of fit chi-square test. Marker-trait association was evaluated for allele/genotype-wise using standard contingency tables (SPSS 15.0, SPSS, Tokyo, Japan).

For SNPs analyzed in multiple samples, we conducted a meta-analysis using a random-effects model. Heterogeneity was measured using a Q statistic test in the combined studies. Odds ratios (ORs) were pooled using DerSimonian and Laird methods. The significance of the pooled OR was determined using a Z test. All data were analyzed using an R package, meta (http://www.r-project.org/index.html).

Results

Possible Predictor SNPs for Risperidone Treatment: From Pharmacogenomic Result (Study 1)

Among the 62,935 SNPs we examined in the pharmacogenomics study, 51,550 SNPs were annotated to 14,655 genes (annotation span: 5′ or 3′ ± 20 kb). For a number of genes, we had multiple SNPs with p values less than 5.0×10^{-4} because of the high LD among genotyped markers. Where this occurred, we list only the strongest associated SNP from that gene (the top 10 hits and SNPs with p value less than 5.0×10^{-4} in Table 1 and Table S1 in Supplement 1, respectively).

Table 1. Predictor Genes in the Pharmacogenomics (Top 10)

Ranking	SNP	Chr	Position[a]	Closest Gene[b]	p Value (Pharmacogenomics)
1	rs2289273	3	10,388,601	*ATP2B2*	1.60×10^{-5}
2	rs234091	1	183,186,172	*FAM129A*	2.00×10^{-5}
3	rs241202	8	28,689,604	*INTS9*	3.20×10^{-5}
4	rs4340422	19	48,604,802	*TEX101*	5.00×10^{-5}
5	rs6682786	1	23,615,883	*TCEA3*	7.30×10^{-5}
6	rs1001220	7	72,748,539	*WBSCR22*	7.70×10^{-5}
7	rs3829241	11	68,611,939	*TPCN2*	8.90×10^{-5}
8	rs460473	16	22,740,528	*HS3ST2*	1.03×10^{-4}
9	rs9792264	8	135,640,117	*ZFAT*	1.10×10^{-4}
10	rs6443999	3	186,056,249	*VPS8*	1.17×10^{-4}

Chr, chromosome; SNP, single nucleotide polymorphism.
[a]Based on Ensemble *Homo sapiens* Version 54.36p (NCBI36).
[b]SNPs are annotated to the closest genes with ± 20-kb span.

Table 2. Overlap Genes Based on the Pharmacogenomcis ($p < 5.0 \times 10^{-4}$) with Mouse Expression Assay ($p < .05$)

Ranking	SNP	Chr	Position[a]	Closest Gene[b]	*p* Value (Pharmacogenomics)	*p* Value[c] (Mouse Expression)	Fold Change
1	rs2289273	3	10,388,601	*ATP2B2*	1.60×10^{-5}	.000710	.504
8	rs460473	16	22,740,528	*HS3ST2*	1.03×10^{-4}	.00600	.259
28	rs3775003	4	96,390,234	*UNC5C*	2.20×10^{-4}	.0132	1.85
32	rs196290	10	121,398,061	*BAG3*	2.81×10^{-4}	.0283	1.33
35	rs9389370	6	136,472,958	*PDE7B*	2.88×10^{-4}	.00806	.710
53	rs1356787	4	57,012,104	*PAICS*	4.26×10^{-4}	.0368	.660
54	rs4641299	1	117,284,884	*PTGFRN*	4.27×10^{-4}	.00160	.283

Chr, chromosome; SNP, single nucleotide polymorphism.
[a]Based on Ensemble *Homo sapiens* Version 54.36p (NCBI36).
[b]SNPs are annotated to the closest genes with ± 20-kb span.
[c]Comparison between risperidone-treated mice ($n = 3$) and saline-treated mice ($n = 3$).

We also looked specifically in our data for support for genes recently suggested as associated with iloperidone based on the only other available antipsychotic GWAS data set (18) and candidate genes implicated in earlier studies (2) including *DRD2*, *DRD3*, *HTR2A*, and others (Tables S2 and S3 in Supplement 1). No strong evidence for association to any of these was found in our pharmacogenomics data set.

Genes Influenced by Risperidone Exposure in Mouse PFC (Study 2)

We examined 22,556 probes in 12,706 genes in RNA extracted from the PFC of mice treated with either risperidone or with vehicle. Of these, 754 (5.9%) and 2227 (17.5%) genes had at least one probe that showed nominally significant differences at $p <$ .01 and .05, respectively, a rate much higher than chance. The top genes with *p* value less than 5.0×10^{-4} are presented in Table S4 in Supplement 1.

Overlapping Genes Between Pharmacogenomic and Mouse Expression Assays (Study 3)

We looked to see whether the pharmacogenetic data (excluding 14 SNPs that could not be annotated to the closest gene) and expression overlapped. Seven genes containing nominally significant alteration in expression in mice also contained SNPs with *p* value less than 5×10^{-4} (Table 2). The relation between PANSS changes and physical locations of each SNP and the genotype effects to risperidone response can be seen in Figures S2 and S3 and Table S5 in Supplement 1. In addition, we found seven genes that met the more stringent threshold for expression change in the mouse and that had at least one significant SNP ($p < .05$) in the pharmacogenomic data (Table 3). It should be stressed these SNPs were not strongly associated with treatment response ($p = .0047$-$.0472$).

Consequently 14 SNPs were further assessed for case-control association analysis in Study 4.

Examining Candidate SNPs as Susceptibility Factor for Schizophrenia (Study 4)

The 14 candidate SNPs in genes showing convergent evidence from Study 3 were further tested for association with schizophrenia (Table 4). For rs242056, a proxy for rs2071999 in *AJAP1*, the genotypes significantly deviated from HWE in controls ($p = .0016$). This SNP was therefore excluded.

Of the remaining 13 SNPs, a single SNP (rs9389370) in *PDE7B* showed a nominally significant association in the JPN_1st case-control sample ($p_{allele} = .026$, two-tailed). In an attempt to extend this putative association, we used two other samples. In the second Japanese sample, we obtained significant evidence for association (second set, $P_{allele} = .02$, one-tailed) and a nonsignificant trend in the UK sample ($p_{allele} = .07$, one-tailed) (Table 4). Meta-analysis of the two replication data sets showed significant evidence for association ($p_{JPN2nd+UK} = .008$, one-tailed). As expected, in all data sets combined, the evidence was stronger than observed in the screening sample alone ($p_{all} = .0014$, two-tailed, uncorrected; $p = .018$, 13 times Bonferroni correction for number of SNPs tested in Study 4) with no evidence for heterogeneity ($p = .56$; Table 5).

Discussion

Combined Analysis as a Tool for Prioritizing Candidate Genes for Pharmacogenomics and Susceptibility

Genomewide approaches to pharmacogenomics have the capacity to provide novel insights into mechanisms and predictors of drug response. However, a major concern of this approach, which is not specific to pharmacogenomics, relates to balancing the need to set a stringent threshold for the type I error rate against the desire to achieve power to detect findings at that threshold. Unless the genetic effect sizes in pharmacogenetics are substantially greater than is typical for complex diseases (19), the sorts of sample sizes currently available for studies of

Table 3. Overlap Genes Based on the Mouse Expression Assay ($p < 1.0 \times 10^{-4}$) with the Pharmacogenomics ($p < .05$)

Ranking	Gene	Probe ID	Fold Change	*p* Value (Mouse Expression)	*p* Value (Pharmacogenomics)	SNP ID
2	*Nr3c2*	1435991-at	5.86	2.23×10^{-6}	.0297	rs2070951
3	*Zbtb20*	1439278-at	4.94	5.04×10^{-6}	.0230	rs9883949
4	*St6gal2*	1434819-at	.23	6.52×10^{-6}	.0102	rs1448110
7	*Pip5k1b*	1450389-s-at	2.46	1.02×10^{-5}	.0472	rs1414944
8	*Epha6*	1421527-at	3.18	1.46×10^{-5}	.0047	rs727229
9	*Kcnh5*	1441742-at	.44	2.72×10^{-5}	.0305	rs10141458
24	*Ajap1*	1438662-at	.66	9.21×10^{-5}	.0208	rs2071999

SNP, single nucleotide polymorphism.

Table 4. Case-Control Analysis of the Candidate SNPs from the Pharmacogenomics and Mouse Expression Data

SNP	Sample	Proxy SNPs	Phenotype	N	Genotype M/M	Genotype M/m	Genotype m/m	*p* Value Allele	*p* Value Genotype	*p* Value HWE	MAF
ATP2B2	JPN_1st		Case	536	275	208	53	.676	.184	.14	29.3
rs2289273			Control	417	209	179	29			.26	28.4
HS3ST2	JPN_1st		Case	538	163	263	112	.408	.682	.76	45.3
rs460473			Control	407	117	196	94			.50	47.2
UNC5C	JPN_1st		Case	540	336	178	26	.249	.136	.70	21.3
rs3775003			Control	406	231	159	16			.07	23.5
BAG3	JPN_1st		Case	539	234	243	62	.609	.877	.93	34.0
rs196290			Control	407	183	180	44			.98	32.9
PDE7B	JPN_1st		Case	535	259	222	54	**.0255**	.0738	.53	30.8
rs9389370			Control	422	229	165	28			.81	26.2
	JPN_2nd		Case	536	278	200	58	**.0214**[a]	.0966	.018	29.5
			Control	500	281	183	36			.41	25.5
	UK		Case	478	181	226	71	.0672[a]	.327	.97	38.5
			Control	2,932	1203	1348	381			.91	36.0
PAICS	JPN_1st		Case	540	181	274	85	.662	.808	.27	41.1
rs1356787			Control	424	134	223	67			.10	42.1
PTGFRN	JPN_1st		Case	535	319	180	36	.222	.219	.13	23.6
rs4641299			Control	413	255	141	17			.65	21.2
NR3C2	JPN_1st		Case	534	295	206	33	.284	.509	.71	25.5
rs2070951			Control	414	213	173	28			.37	27.7
ZBTB20	JPN_1st		Case	537	234	245	58	.494	.420	.61	33.6
rs9883949			Control	423	169	211	43			.05	35.1
ST6GAL2	JPN_1st	rs2241991	Case	533	224	246	63	.936	.814	.72	34.9
rs1448110		$r2 = 1$	Control	409	169	196	44			.25	34.7
PIP5K1B	JPN_1st		Case	536	173	275	88	.550	.764	.22	42.1
rs1414944			Control	420	145	208	67			.6	40.7
EPHA6	JPN_1st		Case	539	146	274	119	.877	.949	.65	47.5
rs727229			Control	419	110	217	92			.44	47.9
KCNH5	JPN_1st		Case	539	169	265	105	.224	.476	.95	44.1
rs10141458			Control	413	142	201	70			.94	41.3
AJAP1	JPN_1st	rs242056	Case	—	—	—	—	—	—	—	—
rs2071999		$r2 = .46$	Control	418	191	161	66			.0016	35.0

Bold numbers represent significant *p* value.

JPN_1st, first Japanese sample; JPN_2nd, second Japanese sample; MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium; M, major allele, m, minor allele; SNP, single nucleotide polymorphism; UK, United Kingdom sample.

[a]Based on one-tailed analysis.

antipsychotics have no realistic prospect of attaining the sorts of levels of significance suggested for genomewide significance (7.2×10^{-8} or 1×10^{-7}) (20,21). Although it is at least possible that the typical effects on gene expression of drugs may be much more substantial than that of SNPs on disease risk, broadly similar balances of power and type I error also apply to our genomewide expression study. Therefore, with the aim of prioritizing our findings, we attempted to cross-validate the top findings from our study using independent approaches as suggested (4). Our methods of prioritizing our findings were based on two hypotheses; one that the most highly significant sets of SNPs from our pharmacogenetic study of risperidone are likely to be enriched among genes whose expression is altered by that drug (and vice versa), the other that SNPs related to drug response may also be enriched among SNPs associated with disease. To what extent these hypotheses are correct is currently unknown.

From our data, we found 14 markers in genes that showed some degree of overlapping support in the pharmacogenomics

Table 5. Meta-Analysis of rs9389370 in PDE7B

Analysis	Sample	OR	95% CIs Lower Limit	95% CIs Upper Limit	*p* Value
	JPN_1st	1.26	1.03	1.54	**.0255**
	JPN_2nd	1.22	1.01	1.48	**.0214**[a]
	UK	1.11	.967	1.28	.0672[a]
Meta (Replication)	JPN_2nd+UK	1.15	1.03	1.29	**.0082**[a]
Meta (All)	JPN1st+JPN2nd+UK	1.17	1.06	1.30	**.0014**

Bold number represent significant *p* value.

CI, confidence interval; JPN_1st, first Japanese sample; JPN_2nd, second Japanese sample; OR, odds ratio; UK, United Kingdom Sample.

[a]Based on one-tailed analysis.

and mouse expression experiments. These top convergent candidate genes have no previous support for association with schizophrenia or risperidone response and are thus novel candidates for antipsychotic response. However, at present, they have no clinical utility in terms of predicting treatment response, and independent replication using other samples will be required. Moreover, even if replicated, the potential clinical utility for pharmacogenetics is questionable because the effect sizes in each case are small, although it is conceivable given the limited coverage of each gene that the true functional variants have much stronger effects.

Another method for prioritizing genes from genomewide data are to apply a gene ontology (GO) based approach to investigate whether sets of findings tend to converge on particular biological pathways or functions. Our previous experience of GO category analysis suggests that with respect to genetic data, these require large data sets (22). Nevertheless, in response to an anonymous reviewer's comments, for interested readers, we provide the results of our GO category analyses based on ALIGATOR (22) and David Bioinformatics Resources 2008 (http://david.abcc.ncifcrf.gov/) in Supplement 1. Although a number of categories were observed to be significant in each analysis (Tables S6 and S7 in Supplement 1), there is no overlap between the results of the two analytic approaches. Moreover, our favored approach based on ALIGATOR did not reveal any categories that were significant after correction for multiple testing, so it is likely that all of those findings represent chance positives.

Possible Predictor SNPs for Response to Risperidone

In this study, several genes were detected as possible novel predictors for treatment response to risperidone: *ATP2B2, HS3ST2, UNC5C, BAG3, PDE7B, PAICS, PTGFRN, NR3C2, ZBTB20, ST6GAL2, PIP5K1B, EPHA6, KCNH5,* and *AJAP1*. Because the multiple testing burden in SNPs is more severe, our primary analysis included selecting genes based on the more stringent thresholds in the pharmacogenomics data (Table 2) and were additionally shown to have altered expression in the mouse expression study. However, in response to review, we additionally provide data for much more weakly associated SNPs that have highly significant expression changes in the mouse brain (Table 3). Given the weak evidence for most of the latter group of SNPs, we think those are most likely to be chance positives but report the findings for others to test.

Among genes of particular interest in Table 2 is *ATP2B2*, which encodes one of four isoforms of the plasma membrane Ca^{2+} pumps of mammalian cells, showed both the strongest statistical association with treatment response ($p = 1.60 \times 10^{-5}$) and was among those genes that had the strongest association with differential expression because of exposure to risperidone ($p = .00071$). The product of this gene is thought to be involved in neurodevelopment (23) because of its influence on Ca^{2+} homeostasis and Ca^{2+} signaling. This in turn regulates multiple neuronal functions, including synaptic transmission, plasticity, and cell survival (24). Interestingly, several of the other genes with convergent evidence for a role in risperidone response might also be related to neurodevelopment via association with netrin (*UNC5C*) (25,26), interaction with heat shock proteins (*BAG3*) (27,28), cyclic adenosine monophosphate (cAMP) systems (*PDE7B*; details discussed later), glucocorticoids (*NR3C2*) (29), and ephirin (*EPHA6*) (30). Given the neurodevelopmental hypothesis of schizophrenia (31) and evidence that second-generation antipsychotics, including risperidone, have neurogenic actions in hippocampus and PFC (32), our findings suggest that genes involved in the regulation of neurodevelopment or neurogenesis are candidate genes for treatment response in schizophrenics, as well as for schizophrenia per se.

PDE7B Is Candidate Gene Either for Treatment Response and Susceptibility for Schizophrenia

We pursued the top findings from Study 3 to see whether the findings with best convergent evidence (human and mouse) for relevance to risperidone response might also influence susceptibility to schizophrenia. After correction for multiple testing, we found evidence for association between disease status and *PDE7B*, which was therefore the only gene supported across all study designs.

Phosphodiesterases (PDEs) are central in regulating degradation of cAMP and cyclic guanosine monophosphate (cGMP), which are important second messengers for many cellular functions (33). There are 21 known genes encoding PDEs in human, spread across 11 distinct PDE families (*PDE1* to *PDE11*). Among these, *PDE4B* has been reported as a candidate susceptibility gene for schizophrenia. This was on the basis of a translocation found in two affected members of a single pedigree and the observation that the protein interacts with Disrupted in Schizophrenia 1 (*DISC1*), itself another strong candidate gene for schizophrenia and affective disorders (34). Elevation of cellular cAMP leads to dissociation of *PDE4B* from DISC1 and an increase in *PDE4B* activity (34).

PDE7B degrades cAMP, but not cyclic guanosine monophosphate (cGMP), and is predominantly expressed in brain (33). To date, no direct evidence for association of *PDE7B* with schizophrenia has been reported; however, several findings provide some functional plausibility to our results. First, mRNA for *PDE7B* and dopamine D1, D2, and D3 receptors show a similar pattern of distribution, and it is thought that the dopamine D1 receptor activates *PDE7B* through the cAMP pathway (35). Second, *PDE7B* maps to 6q23-24, one of the most significant linkage regions for schizophrenia (OMIM %600511; SCZD3) (36). Lastly, association has recently been reported to the *Abelson Helper Integration Site 1* (AHI1) and *Family with sequence similarity 54 A* (*FAM54A*), which are respectively in the 5′ and 3′ regions of *PDE7B*. However, it should be noted that in those studies, SNPs in *PDE7B* were not associated with disease (37,38).

PDE inhibitors have recently emerged as being of interest as therapeutic agents for neuropsychiatric disorders, such as schizophrenia, depression, and dementia (33). Our results indicate that among these, drugs acting on *PDE7B* may be of particular value in schizophrenia, although particularly for clinical applications, our results should be treated with caution until independent replications have been reported.

Limitations and Conclusion

The major limitation in this study is that the sample sizes we used for the genomewide pharmacogenetics and gene expression studies are small. In particular, the phramacogenetics study is only highly powered to detect effects that are much larger than typical of common susceptibility alleles for diseases to date. This is less of a limitation with respect to one major goal of pharmacogenetics, namely, the identification of common markers with sufficiently large effects to be of value in guiding therapeutics. Our study suggests that in such large common effects may not exist, although being based on one of the earliest chips, the coverage of genes is incomplete, and it would be desirable to repeat this experiment with a denser set of SNPs. The extent to which clinical heterogeneity is likely to have an impact on treatment response, and therefore power to detect association to that response, is also currently

unknown. We presume it is likely to play some role, as is the possibility of imperfect adherence to treatment. More subtle effects are of potential value in informing about drug mechanisms relevant to therapeutic response, and here, power is limited. Given that limitation, we tried to minimize false negatives through the use of relaxed significance criteria but tried to control false positives by combining expression and genetic data. Nevertheless, replication of our findings are required. Our follow-up observation of association between *PDE7B*, a novel candidate gene, and schizophrenia does, however, suggest that the use of convergent data may have successfully enriched for findings of true relevance to schizophrenia and its response to treatment.

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Supplementary material cited in this article is available online.

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ORIGINAL ARTICLE

Serotonin 1A receptor gene and major depressive disorder: an association study and meta-analysis

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Several genetic studies have shown an association between the 5-HT1A receptor gene (*HTR1A*) and major depressive disorder (MDD); however, results have been rather inconsistent. Moreover, to our knowledge, no association study on *HTR1A* and MDD in the Japanese population has been reported. Therefore, to evaluate the association between *HTR1A* and MDD, we conducted a case–control study of Japanese population samples with two single-nucleotide polymorphisms (SNPs), including rs6295 (C-1019G) in *HTR1A*. In addition, we conducted a meta-analysis of rs6295, which has been examined in other papers. Using one functional SNP (rs6295) and one tagging SNP (rs878567) selected with the HapMap database, we conducted a genetic association analysis of case–control samples (331 patients with MDD and 804 controls) in the Japanese population. Seven population-based association studies, including this study, met our criteria for the meta-analysis of rs6295. We found an association between rs878567 and Japanese MDD patients in the allele-wise analysis, but the significance of this association did not remain after Bonferroni's correction. We also did not detect any association between *HTR1A* and MDD in the allele/genotype-wise or haplotype-wise analysis. On the other hand, we detected an association between rs6295 and MDD in the meta-analysis ($P(Z)$=0.0327). In an explorative analysis, rs6295 was associated with Asian MDD patients after correction for multiple testing ($P(Z)$=0.0176), but not with Caucasian MDD patients ($P(Z)$=0.138). Our results suggest that *HTR1A* may not have a role in the pathophysiology of Japanese MDD patients. On the other hand, according to the meta-analysis, *HTR1A* was associated with MDD patients, especially in the Asian population.
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Keywords: case–control study; functional SNP; major depressive disorder (MDD); meta-analysis; serotonin 1A receptor gene (*HTR1A*); tagging SNP

INTRODUCTION

Altered serotonergic neural transmission is hypothesized to be a susceptibility factor for major depressive disorder (MDD). The evidence for such an association is discussed in more detail in reviews.[1,2]

Several genetic studies have shown an association between the serotonin 1A (5-HT1A) receptor gene (*HTR1A*) and MDD; however, results have been rather inconsistent. A recent meta-analysis showed no association between *HTR1A* and MDD.[3] However, two very recent studies reported that rs6295 (C-1019G) in the promoter region of *HTR1A*, which regulates *HTR1A* transcription,[4,5] was associated with MDD in the Chinese population.[6,7] Moreover, to our knowledge, no association study of *HTR1A* and MDD in the Japanese population has been reported.

Therefore, we examined the association between *HTR1A* and MDD in the Japanese, using the recently recommended strategy of 'gene-based' association analysis.[8] Moreover, we conducted an updated meta-analysis of rs6295, which has been intensively investigated in other studies.

MATERIALS AND METHODS

Subjects

The subjects in the association analysis were 331 patients with MDD (162 men and 169 women; mean age ± s.d. 44.3 ± 14.2 years) and 804 healthy controls (352 men and 452 women; mean age ± s.d. 38.6 ± 12.9 years). The patients were diagnosed according to the DSM-IV criteria with the consensus of at least two experienced psychiatrists on the basis of unstructured interviews and a review of medical records. In addition, at least 125 of the 331 MDD patients had been diagnosed according to the DSM-IV criteria with the consensus of at least two experienced psychiatrists on the basis of a review of medical records and assessment with the SIGH-D (Structured Interview Guide for Hamilton Rating Scale for Depression). All subjects were unrelated to each other, ethnically Japanese and lived in the central area of Japan. All healthy controls were also psychiatrically screened on the basis of unstructured interviews. None of them had severe medical complications, such as cirrhosis, renal failure, heart failure

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