Neuromol Med (2009) 11:114–122
DOI 10.1007/s12017-009-8067-0

ORIGINAL PAPER

Possible Association of Prokineticin 2 Receptor Gene (*PROKR2*) with Mood Disorders in the Japanese Population

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Received: 23 March 2009 / Accepted: 21 May 2009 / Published online: 20 June 2009

Abstract Several investigations have suggested that disruption of circadian rhythms may provide the foundation for the development of mood disorders such as bipolar disorder (BP) and major depressive disorder (MDD). Recent animal studies reported that prokineticin 2 or prokineticin 2 receptor gene deficient mice showed disruptions in circadian and homeostatic regulation of sleep. This evidence indicates that prokineticin 2 gene (*PROK2*) and prokineticin 2 receptor gene (*PROKR2*) are good candidate genes for the pathogenesis of mood disorders. To evaluate the association between *PROK2*, *PROKR2*, and mood disorders, we conducted a case-control study of Japanese samples (151 bipolar patients, 319 major depressive disorder patients, and 340 controls) with four and five tagging SNPs in *PROK2* or *PROKR2*, respectively, selected by HapMap database. We detected a significant association between *PROKR2* and major depressive disorder and bipolar disorder in the Japanese population. In conclusion, our findings suggest that *PROKR2* may play a role in the pathophysiology of mood disorders in the Japanese population. However, because our samples were small, it will be important to replicate and confirm these findings in other independent studies using larger samples.

Keywords Major depressive disorder · Bipolar disorder · Prokineticin 2 gene · Prokineticin 2 receptor gene · Linkage disequilibrium · Tagging SNP · Clock genes · Circadian rhythm

Introduction

Sleep-wake disturbance is frequently observed in mood disorders such as bipolar disorder (BP) and major depressive disorder (MDD), and negatively affects the clinical status of patients. Abnormalities in circadian rhythms are believed to occur in mood disorders (Barnard and Nolan 2008; Mansour et al. 2005; McClung 2007a, b; Wirz-Justice 2006). Severe sleep-wake rhythm disturbance is also often observed in mood disorder patients (Boivin 2000). Circadian sleep disorders such as delayed sleep phase syndrome are often associated with mood symptoms (Dagan et al. 1998). These facts suggest a close relationship between circadian rhythms and mood disorders, and genes associated with the molecular clock mechanism are good candidates for involvement in mood disorders. The evidence for such an association is discussed in more detail in our previous paper and a review by Barnard and Nolan (Barnard and Nolan 2008; Kishi et al. 2008b; Kishi et al. 2009a, b).

Cheng and colleagues reported that prokineticin 2 (PK2), a secreted molecule expressed in gut and brain including suprachiasmatic nucleus (SCN), plays a major role as an output molecule for the SCN clock (Cheng et al. 2002; Li et al. 2006; Zhou and Cheng 2005). PK2 gene deficit mice showed reduced physiological and behavioral parameters,

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including circadian locomotor activity, circulating glucocorticoid, glucose levels, and the expression of peripheral clock genes such as *Bmal1* and *Dbp* compared with WT mice (Li et al. 2006; Li et al. 2009). The expression of PK2 gene in SCN was activated by the Clock/Bmal1 complex, and suppressed by Per/Cry in vitro studies (Cheng et al. 2002). A recent animal study reported that PK2 gene deficit mice showed disruptions in the homeostatic regulation of sleep, such as reduced non-rapid eye movement sleep time and increased rapid eye movement (REM) sleep time in both light and dark periods (Hu et al. 2007). Mice lacking the PK2 gene also showed significantly reduced anxiety and depression-like behaviors in the forced swimming test (Li et al. 2009). In addition, PK2 gene deficit mice showed deceased responses to new environments in terms of locomotor activity, arousal, body temperature, and food intake (Li et al. 2009). On the other hand, PK2 receptor, a G-coupled receptor for PK2 expressed in SCN and its targets (Cheng et al. 2002), has also been shown to be essential for circadian behavior control; PK2 receptor gene mutant mice showed disrupted circadian coordination of the activity cycle and lost precision in the timing of the onset of nocturnal locomotor activity (Prosser et al. 2007). Li and colleagues suggested that PK2 signaling plays a major role in stress-related traits in mice, and indicated a possible molecular connection between circadian rhythms and mood regulation (Li et al. 2009).

Considering the above, the prokineticin 2 gene (*PROK2*) and prokineticin 2 receptor gene (*PROKR2*) seem to be good candidate genes for the pathogenesis of mood disorders. *PROK2* (OMIM * 607002, 4 exons in this genomic region spanning 14.206 kb) is located on chromosome 3p13. Also, *PROKR2* (OMIM * 607123, 2 exons in this genomic region spanning 13.130 kb) is located on chromosome 20p12.3, which was shown to be a susceptibility region for BP, according to three linkage analysis studies (Detera-Wadleigh et al. 1997; Fanous et al. 2008; Ross et al. 2008). To evaluate the association between *PROK2* and *PROKR2* and mood disorders, we conducted a case-control study of Japanese samples.

Materials and Methods

Subjects

The subjects in the association analysis were 151 BP patients (80 males and 71 females; 97 patients with bipolar I disorder and 54 patients with bipolar II disorder; mean age ± standard deviation (SD) 46.1 ± 12.1 years), 319 MDD patients (157 males and 162 females; mean age ± standard deviation (SD) 46.1 ± 13.8 years), and 340 healthy controls (187 males and 153 females; 38.9 ± 14.7 years). 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. In addition, at least 117 of the 319 MDD patients, who were treated with fluvoxamine, had been diagnosed according to DSM-IV criteria with consensus of at least two experienced psychiatrists on the basis of a review of medical records and assessment with the Structured Interview Guide for Hamilton Rating Scale for Depression (SIGH-D). Subjects were in-patients or outpatients of hospitals, unrelated to each other, ethnically Japanese, and living in the central area of Japan; they were collected between January 2000 and December 2008. Almost patients were treated in the Department of Psychiatry, Fujita health University Hospital, Toyoake, Japan. All healthy controls were also psychiatrically screened based on unstructured interviews. None had severe medical complications such as cirrhosis, renal failure, and heart failure or other Axis-I disorders according to DSM-IV. No structured methods were used to assess psychiatric symptoms in the controls, which included hospital staff and medical students.

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 and Nagoya University School of Medicine.

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 9 SNPs covering *PROK2* (5′-flanking regions including about 3100 bp from the initial exon and about 9800 bp downstream (3′) from the last exon: HapMap database contig number chr3: 71894788.. 71919925). We also 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 38 SNPs covering *PROKR2* (5′-flanking regions including about 2100 Kbp from the initial exon and about 1360 bp downstream (3′) from the last exon: HapMap database contig number chr20: 5229253.. 5245172). Four and five 'tagging SNPs' in *PROK2* and *PROKR2*, respectively, 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).

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 the fluorescent VIC dye. The plates were heated for 2 min at 50 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.

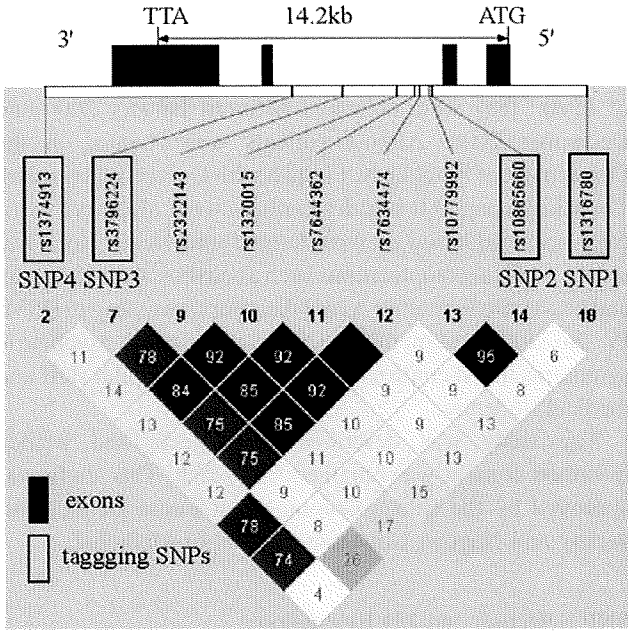

Fig. 1 LD evaluation and tagging SNPs in *PROK2* ATG is the start codon and TAA is the stop codon. *Vertical bars* represent exons. Tagging SNPs selected from the HapMap database are represented by *black boxes*. Color scheme is based on r^2 value. Other information can be seen at the Haploview website

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 evaluated with a likelihood ratio test using the COCAPHASE2.403 program (Dudbridge 2003). In the haplotype-wise analysis, we determined cutoff for testing a haplotype frequency was 0.05. 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 numbers of multiple testing as follows; 9 for each sample set in allele- and genotype-wise analysis in *PROKR2* (9 tagging SNPs in *PROK2* and *PROKR2*); 18 for the explorative analysis either by sex (2 × 9 tagging SNPs). Also, we already have performed permutation test in the haplotype-wise analysis. Therefore, for Bonferroni correction, we performed the numbers of multiple testing as follows; 2 for the explorative analysis either by sex in the haplotype-wise analysis. Power calculation was performed using a genetic power calculator (Purcell et al. 2003). The significance level for statistical tests was 0.05.

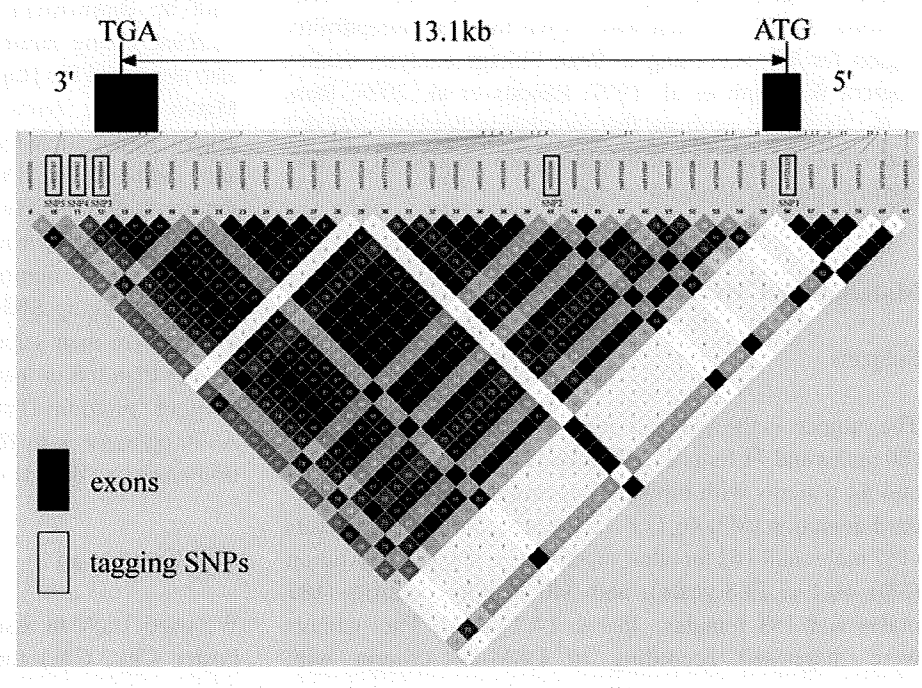

Fig. 2 LD evaluation and tagging SNPs in *PROKR2* ATG is the start codon and TGA is the stop codon. *Vertical bars* represent exons. Tagging SNPs selected from the HapMap database are represented by *black boxes*. Color scheme is based on r^2 value. Other information can be seen at the Haploview website

Results

The LD structure as determined from the HapMap database can be seen in Figs. 1 and 2. Genotype frequencies of all SNPs were in HWE. We detected significant associations between SNP1 (rs17721321) and SNP4 (rs3746682) in *PROKR2* with BP in the allele- and/or genotype-wise analyses after Bonferroni correction (Table 1). We also found an association between SNP5 (rs4815787) in *PROKR2* and MDD in the allele/genotype-wise analysis after Bonferroni correction (Table 1). Moreover, we detected an association between *PROKR2* and not only BP but also MDD in the haplotype-wise analysis (Table 2). It is known that there are sex differences not only in the pathophysiology of mood disorders (Currier et al. 2006; Faraone et al. 1987) but also in circadian rhythms (Lehnkering and Siegmund 2007). Therefore, we performed an explorative analysis of subjects divided by sex. We detected significant associations between SNP4 (rs3746682) in *PROKR2* with female BP in the allele- and/or genotype-wise analyses and the haplotype-wise analysis after Bonferroni correction (Tables 4 and 5). In addition, we showed an association between *PROKR2* and female MDD in the haplotype-wise analysis after Bonferroni correction

Table 1 Tagging SNPs and association analysis of *PROK2* and *PROKR2*

Gene		SNP ID[a]	Phenotype[b]	MAF[c]	*N*	Genotype distribution[d]			*P* value[e,f]			Corrected *P* value[f,g]	
						M/M	M/m	m/m	HWE	Genotype	Allele	Genotype	Allele
PROK2	SNP1	rs1316780	Controls	0.385	340	128	162	50	0.914				
	T > A	5′ Flanking region	MDD	0.384	319	114	165	40	0.0956	0.527	0.962		
			BP	0.401	151	56	69	26	0.551	0.771	0.649		
	SNP2	rs10865660	Controls	0.338	340	148	154	38	0.828				
	A > G	Intron2	MDD	0.326	319	145	140	34	0.981	0.882	0.638		
			BP	0.311	151	70	68	13	0.536	0.653	0.407		
	SNP3	rs3796224	Controls	0.200	340	223	98	19	0.0672				
	G > A	Intron2	MDD	0.213	319	202	98	19	0.133	0.832	0.556		
			BP	0.185	151	101	44	6	0.663	0.754	0.595		
	SNP4	rs1374913	Controls	0.376	340	137	150	53	0.266				
	T > G	3′ Flanking region	MDD	0.382	319	129	136	54	0.0818	0.875	0.823		
			BP	0.387	151	59	67	25	0.423	0.950	0.744		
PROKR2	SNP1	rs17721321	Controls	0.0706	340	294	44	2	0.800				
	G > A	5′ Flanking region	MDD	0.0987	319	258	59	2	0.485	0.145	0.0658		
			BP	0.129	151	117	29	5	0.0725	**0.0100**	**0.00289**	0.0900	**0.0261**
	SNP2	rs6085086	Controls	0.275	340	185	123	32	0.0725				
	G > A	Intron1	MDD	0.313	319	155	128	36	0.226	0.314	0.125		
			BP	0.341	151	66	67	18	0.875	0.0905	**0.0363**		0.327
	SNP3	rs3746684	Controls	0.449	340	110	155	75	0.148				
	G > A	Exon2	MDD	0.434	319	106	149	64	0.378	0.821	0.600		
		Synonymous	BP	0.470	151	40	80	31	0.436	0.289	0.529		
	SNP4	rs3746682	Controls	0.408	340	123	156	61	0.349				
	G > C	Exon2	MDD	0.357	319	137	136	46	0.200	0.169	0.549		
		Synonymous	BP	0.298	151	76	60	15	0.536	**0.00552**	**0.000931**	**0.0497**	**0.00838**
	SNP5	rs4815787	Controls	0.321	340	160	142	38	0.447				
	G > A	3′ Flanking region	MDD	0.411	319	117	142	60	0.151	**0.00418**	**0.000684**	**0.0376**	**0.00616**
			BP	0.401	151	55	71	25	0.797	0.0571	**0.0149**		0.134

[a] Major allele > minor allele

[b] MDD: major depressive disorder, BP: bipolar disorder

[c] MAF: minor allele frequency

[d] M: major allele, m: minor allele

[e] Hardy-Weinberg equilibrium

[f] Bold numbers represent significant *P* value

[g] Calculated by Bonferroni's correction

Table 2 Haplotype-wise analysis of tagging SNPs in *PROK2* and *PROKR2*

	Phenotype	Individual haplotype frequency	Individual *P* value	Phenotype	Global *P* value
PROK2 Common haplotypes					
SNP1-SNP2-SNP3-SNP4					
T-A-G-T	Control	0.249			
	MDD	0.257	0.795		
	BP	0.288	0.323		
T-A-G-G	Control	0.237		MDD	0.838
	MDD	0.240	0.933	BP	0.455
	BP	0.201	0.337		
T-G-G-G	Control	0.168			
	MDD	0.192	0.395		
	BP	0.212	0.206		
A-A-G-T	Control	0.168			
	MDD	0.144	0.387		
	BP	0.152	0.645		
A-A-A-T	Control	0.179			
	MDD	0.167	0.669		
	BP	0.147	0.345		
PROKR2 Common haplotypes					
SNP1-SNP2-SNP3-SNP4-SNP5					
G-G-G-G-G	Control	0.0577			
	MDD	0.0688	0.466		
	BP	0.0779	0.350		
G-G-G-C-G	Control	0.433			
	MDD	0.344	**0.00385**	MDD	**0.000685**
	BP	0.320	**0.000697**	BP	**0.00204**
G-G-A-G-G	Control	0.148			
	MDD	0.107	0.0522		
	BP	0.131	0.349		
G-A-A-G-A	Control	0.252			
	MDD	0.300	0.0893		
	BP	0.369	**0.00543**		

Bold numbers represent significant *P* value

MDD major depressive disorder, *BP* bipolar disorder

(Table 5). No association was detected between other SNPs in *PROKR2* and any subgroup or either sex (Tables 3 and 5). We did not detect any significant association of *PROK2* with MDD or BP in allele/genotype-wise analysis or haplotype-wise analysis (Tables 1, 2, and 3).

Moreover, to evaluate the interactions with each SNP in *PROK2* and *PROKR2*, we analyzed gene–gene interactions with the Multifactor Dimensionality Reduction (MDR) method (Hahn et al. 2003). In this analysis, however, no interactions were seen with MDD and BP (data not shown).

In the power analysis, we obtained more than 80% power for the detection of association when we set the genotype relative risk at 1.41–1.48 and 1.67–1.76 in MDD and BP, respectively, for *PROK2,* and at 1.45–1.88 and 1.66–2.21 in MDD and BP, respectively, for *PROKR2* under a multiplicative model of inheritance.

Discussion

We first preformed a genetic association analysis between *PROK2*, *PROKR2*, and mood disorders including BP and MDD in the Japanese population. We detected significant associations between *PROKR2* and both of mood disorders in the Japanese population. These significant associations remained after Bonferroni's correction was used to control inflation of the type I error rate due to multiple testing. Since we detected significant associations between

Table 3 Tagging SNPs and association analysis of *PROK2* and *PROKR2* in males

Gene		SNP ID[a]	Phenotype[b]	MAF[c]	*N*	Genotype distribution[d]			*P* value[e,f]			Corrected *P* value[f,g]	
						M/M	M/m	m/m	HWE	Genotype	Allele	Genotype	Allele
PROK2	SNP1	rs1316780	Controls	0.380	187	72	88	27	0.989				
	T > A	5′ Flanking region	MDD	0.382	157	57	80	20	0.322	0.757	0.947		
			BP	0.387	80	32	34	14	0.349	0.730	0.865		
	SNP2	rs10865660	Controls	0.356	187	77	87	23	0.836				
	A > G	Intron2	MDD	0.354	157	64	75	18	0.572	0.960	0.954		
			BP	0.338	80	34	38	8	0.578	0.865	0.688		
	SNP3	rs3796224	Controls	0.187	187	126	52	9	0.239				
	G > A	Intron2	MDD	0.207	157	100	49	8	0.536	0.768	0.514		
			BP	0.169	80	55	23	2	0.825	0.684	0.613		
	SNP4	rs1374913	Controls	0.401	187	67	90	30	0.981				
	T > G	3′ Flanking region	MDD	0.389	157	64	67	26	0.232	0.572	0.554		
			BP	0.406	80	28	39	13	0.925	0.992	0.911		
PROKR2	SNP1	rs17721321	Controls	0.0588	187	166	20	1	0.641				
	G > A	5′ Flanking region	MDD	0.0701	157	136	20	1	0.779	0.833	0.548		
			BP	0.125	80	62	16	2	0.443	**0.0416**	**0.00926**	0.74	0.167
	SNP2	rs6085086	Controls	0.259	187	107	63	17	0.0924				
	G > A	Intron1	MDD	0.283	157	82	61	14	0.586	0.600	0.479		
			BP	0.306	80	38	35	7	0.791	0.283	0.265		
	SNP3	rs3746684	Controls	0.468	187	57	85	45	0.233				
	G > A	Exon2	MDD	0.411	157	55	75	27	0.869	0.277	0.133		
		Synonymous	BP	0.456	80	24	39	17	0.876	0.849	0.804		
	SNP4	rs3746682	Controls	0.385	187	74	82	31	0.311				
	G > C	Exon2	MDD	0.360	157	65	71	21	0.817	0.710	0.500		
		Synonymous	BP	0.325	80	38	32	10	0.430	0.440	0.187		
	SNP5	rs4815787	Controls	0.329	187	85	81	21	0.798				
	G > A	3′ Flanking region	MDD	0.401	157	58	72	72	0.568	0.150	**0.0490**		0.882
			BP	0.356	80	33	37	10	0.940	0.814	0.540		

[a] Major allele > minor allele

[b] MDD: major depressive disorder, BP: bipolar disorder

[c] MAF: minor allele frequency

[d] M: major allele, m: minor allele

[e] Hardy-Weinberg equilibrium

[f] Bold numbers represent significant *P* value

[g] Calculated by Bonferroni's correction

PROKR2, which has been shown to be an essential molecule in circadian rhythms in previous studies, and not only MDD but also BP, our results may support the hypothesis that abnormalities in circadian rhythms may be a common pathophysiology in mood disorders.

In the explorative analysis we also found an association between *PROKR2* and female BP and MDD. It is known that there are sex differences in not only the pathophysiology of mood disorders (Currier et al. 2006; Faraone et al. 1987) but also in circadian rhythms (Lehnkering and Siegmund 2007). Szczepankiewicz et al. (2006) reported an association between a diagnosis of BP II in females and the glycogen synthase kinase-3 β gene (*GSK3B*). 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 *PROKR2* and Japanese bipolar disorder patients in female, our results may support the supposition that the etiology of mood disorders differs somewhat in females and males.

Table 4 Tagging SNPs and association analysis of *PROK2* and *PROKR2* in females

Gene		SNP ID[a]	Phenotype[b]	MAF[c]	*N*	Genotype distribution[d]			*P* value[e,f]			Corrected *P* value[f,g]	
						M/M	M/m	m/m	HWE	Genotype	Allele	Genotype	Allele
PROK2	SNP1	rs1316780	Controls	0.392	153	56	74	23	0.858				
	T > A	5′ Flanking region	MDD	0.386	162	57	85	20	0.173	0.697	0.870		
			BP	0.415	71	24	35	12	0.900	0.894	0.639		
	SNP2	rs10865660	Controls	0.317	153	71	67	15	0.889				
	A > G	Intron2	MDD	0.299	162	81	65	16	0.579	0.793	0.632		
			BP	0.282	71	36	30	5	0.710	0.730	0.450		
	SNP3	rs3796224	Controls	0.216	153	97	46	10	0.168				
	G > A	Intron2	MDD	0.219	162	102	49	11	0.139	0.995	0.916		
			BP	0.204	71	46	21	4	0.448	0.960	0.782		
	SNP4	rs1374913	Controls	0.346	153	70	60	23	0.0975				
	T > G	3′ Flanking region	MDD	0.386	162	65	69	28	0.197	0.592	0.305		
			BP	0.366	71	31	28	12	0.205	0.925	0.683		
PROKR2	SNP1	rs17721321	Controls	0.0850	153	128	24	1	0.913				
	G > A	5′ Flanking region	MDD	0.127	162	122	39	1	0.257	0.177	0.0907		
			BP	0.134	71	55	13	3	0.0767	0.143	0.110		
	SNP2	rs6085086	Controls	0.294	153	78	60	15	0.492				
	G > A	Intron1	MDD	0.343	162	73	67	22	0.298	0.445	0.192		
			BP	0.380	71	28	32	11	0.712	0.210	0.0692		
	SNP3	rs3746684	Controls	0.425	153	53	70	30	0.430				
	G > A	Exon2	MDD	0.457	162	51	74	37	0.311	0.732	0.420		
		Synonymous	BP	0.486	71	16	41	14	0.189	0.156	0.226		
	SNP4	rs3746682	Controls	0.442	153	49	74	31	0.750				
	G > C	Exon2	MDD	0.355	162	72	65	25	0.115	0.0673	**0.0261**		0.470
		Synonymous	BP	0.268	71	38	28	5	0.959	**0.00258**	**0.000429**	**0.0464**	**0.00772**
	SNP5	rs4815787	Controls	0.310	153	75	61	17	0.395				
	G > A	3′ Flanking region	MDD	0.420	162	59	70	33	0.151	**0.0248**	**0.00444**	0.446	0.0799
			BP	0.451	71	22	34	15	0.782	**0.0204**	**0.00389**	0.367	0.0702

[a] Major allele > minor allele

[b] MDD: major depressive disorder, BP: bipolar disorder

[c] MAF: minor allele frequency

[d] M: major allele, m: minor allele

[e] Hardy-Weinberg equilibrium

[f] Bold numbers represent significant *P* value

[g] Calculated by Bonferroni's correction

O'Donovan et al. (2008) suggested a method of genetic study in the future: First, several candidate genes would be detected with Genome wide association study (GWAS). Second, these candidate genes would be tested with a gene-based case control study using large and differential ethic samples, and then be included in a meta-analysis (O'Donovan et al. 2008). We also consider the simple case-control approach is now transitive.

A few points of caution should be mentioned with respect to our results. Firstly, our sample sizes were small, especially BP. In the power analysis, we obtained power of more than 80% for the detection of association when we set the genotype relative risk at 1.67–1.76 in BP for *PROKR2*, and at 1.66–2.21 in BP for *PROKR2*, under a multiplicative model of inheritance. Secondly, we did not perform a mutation scan of *PROK2* and *PROKR2*. Because we consider it to be difficult to evaluate the association of such extremely rare variants from the viewpoint of statistical power, a replication study using a larger sample is required for conclusive results. Lastly, our subjects did not undergo structured interviews. MDD patients who are not diagnosed by structured interview may develop bipolar disorder in the future (Bowden 2001). However, in this study, patients were carefully diagnosed according to DSM-IV criteria

Table 5 Explorative haplotype-wise analysis of subjects divided by sex in *PROKR2*

Common haplotypes SNP1-SNP2-SNP3-SNP4-SNP5 in male	Phenotype	Individual haplotype frequency	Individual *P* value	Phenotype	Global *P* value
G-G-G-C-G	Control	0.490			
	MDD	0.487	0.956		
	BP	0.421	0.279		
G-G-A-G-G	Control	0.204		MDD	0.441
	MDD	0.158	0.270	BP	0.242
	BP	0.171	0.507		
G-A-A-G-A	Control	0.306			
	MDD	0.355	0.333		
	BP	0.409	0.0898		

Common haplotypes SNP1-SNP2-SNP3-SNP4-SNP5 in female	Phenotype	Individual haplotype frequency	Individual *P* value	Phenotype	Global *P* value	Corrected *P* value[a]
G-G-G-C-G	Control	0.645				
	MDD	0.500	**0.0224**	MDD	**0.0224**	**0.0448**
	BP	0.406	**0.00175**	BP	**0.00175**	**0.00350**
G-A-A-G-A	Control	0.355				
	MDD	0.500	**0.0224**			
	BP	0.594	**0.00175**			

Bold numbers represent significant *P* value

MDD major depressive disorder, *BP* bipolar disorder

[a] Calculated by Bonferroni's correction

with consensus of at least two experienced psychiatrists on the basis of a review of medical records. In addition, when we found a misdiagnosis in a patient, we promptly excluded the misdiagnosed case in consideration of the precision of our sample. Detailed information on our samples was provided in previous papers (Kishi et al. 2008a, b; Kishi et al. 2009a).

In conclusion, our results suggest that *PROKR2* probably plays a role in mood disorders 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.

Acknowledgments We thank Ms M. Miyata and Ms S. Ishihara for their technical support. This work was supported in part by research grants from the Ministry of Education, Culture, Sports, Science and Technology, the Ministry of Health, Labor and Welfare, and the Japan Health Sciences Foundation (Research on Health Sciences focusing on Drug Innovation).

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Neuromol Med (2009) 11:123–127
DOI 10.1007/s12017-009-8068-z

ORIGINAL PAPER

No Association Between Polymorphisms of Neuronal Oxide Synthase 1 Gene (*NOS1*) and Schizophrenia in a Japanese Population

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Received: 8 April 2009 / Accepted: 21 May 2009 / Published online: 10 June 2009

Abstract The neuronal nitric oxide synthase gene (*NOS1*) is located on 12q24, in a susceptibility region for schizophrenia, and produces nitric oxide (NO) in the brain. NO plays a role in neurotransmitter release and is the second messenger of the *N*-methyl-D-aspartate (NMDA) receptor. Furthermore, it is connected to the dopaminergic and serotonergic neural transmission systems. Therefore, abnormalities in the NO pathway are thought to be involved in the pathophysiology of schizophrenia. Several genetic studies showed an association of *NOS1* with schizophrenia. However, results of replication studies have been inconsistent. Therefore, we conducted a replication study of *NOS1* with schizophrenia in a Japanese sample. We selected seven SNPs (rs41279104, rs3782221, rs3782219, rs561712, rs3782206, rs2682826, and rs6490121) in *NOS1* that were positively associated with schizophrenia in previous studies. Two SNPs showed an association with Japanese schizophrenic patients (542 cases and 519 controls, rs3782219: P allele = 0.0291 and rs3782206: P allele = 0.0124, P genotype = 0.0490), and almost these significances remained with an increased sample size (1154 cases and 1260 controls, rs3782219: P allele = 0.0197 and rs3782206: P allele = 0.0480). However, these associations also might have resulted from type I error on account of multiple testing (rs3782219: P allele = 0.133 and rs3782206: P allele = 0.168). In conclusion, we could not replicate the association between seven SNPs in *NOS1* and schizophrenia found in several earlier studies, using larger Japanese schizophrenia and control samples.

Keywords Schizophrenia · Neuronal nitric oxide syntase 1 gene (*NOS1*) · Case-control association study

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Introduction

Schizophrenia is a common psychiatric disease, seen in approximately 1% of the world population. It is characterized by delusions, hallucinations, and cognitive dysfunction. Genetic factors play an important role in susceptibility to schizophrenia (Cardno and Gottesman 2000), and several genetic studies have identified susceptibility genes (Ross et al. 2006).

The nitric oxide synthase 1 gene (*NOS1*) is located on 12q24, and consists of 12 alternative untranslated first exons, termed exon 1a_1l, and 28 exons in a genomic region spanning 149.404 Kb. *NOS1* is considered to be a likely candidate gene for schizophrenia owing to its

chromosomal location, 12q24, which has been reported to be a susceptibility locus from several linkage studies (Bailer et al. 2000, 2002; DeLisi et al. 2002), and to produce nitric oxide (NO). NO is synthesized from L-arginine by a family of isoformic enzymes known as nitric oxide synthases (NOSs). Three isoforms of NOS have been identified: neuronal (nNOS), endothelial (eNOS), and inducible (iNOS) (McLeod et al. 2001). NO is involved in a variety of mechanisms, such as neurotransmitter release, *N*-methyl-D-aspartate (NMDA) receptor activation (Joca et al. 2007; Snyder and Ferris 2000), and oxidative stress in the brain (Yao and Reddy 2005). Abnormalities in these mechanisms are thought to be involved in the pathophysiology of psychotic disorders (Bennett 2008). Moreover, evidence from pharmacological studies in animal and postmortem studies supports an association between NO and psychotic disorders (Wass et al. 2009; Yao et al. 2004).

A number of genetic association studies showed that single nucleotide polymorphisms (SNP) in *NOS1* were associated with schizophrenia. Shinkai et al. (2002) examined the association between a synonymous SNP (rs2682826) in exon 29 and schizophrenia in a Japanese population, and showed that it was significant. Fallin et al. (2005) identified a haplotype (rs3782221–rs3782219–rs561712–rs3782206) and reported it to be associated with schizophrenia and schizoaffective disorder. *NOS1* has a complex promoter–exon1 region. Expression of the different mRNA from distinct promoters in *NOS1* is controlled by the 5′ flanking region (Bros et al. 2006). Reif et al. (2006) reported a polymorphism (rs41279104) in the promoter region of exon 1c associated with schizophrenia and prefrontal brain function. Recently, a whole genome association study reported an association between rs6490121 in intron 2 of *NOS1* and schizophrenia (Moskvina et al. 2009).

In this study, we conducted a replication study of association between significant seven SNPs in *NOS1* and schizophrenia in a Japanese samples.

Materials and Methods

Subjects

A total of 542 patients with schizophrenia (276 males and 266 females; mean age ± standard deviation; 43.8 ± 14.8 years) and 519 healthy controls (264 males and 255 females; 36.5 ± 14.1 years) were recruited. For rs3782219 and rs3782206, which showed a significant association in the allele and/or genotype-wise analysis, additional samples were included for this association analysis, bringing the totals to 1154 schizophrenics (additional 612 cases) and 1260 controls (additional 741 controls). All subjects were unrelated to each other and ethnically Japanese. The 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, and who were outpatients or inpatients of psychiatric hospitals. The patients were grouped according to the following DSM-IV subtypes of schizophrenia: Paranoid Type ($n = 429$), Disorganized Type ($n = 441$), Catatonic Type ($n = 39$), Residual Type ($n = 138$), and Undifferentiated Type ($n = 107$). All healthy control subjects 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. No structured methods were used to assess psychiatric symptoms in the controls (hospital staffs and medical students). None of the subjects were known to be related to each other, and all were ethnically Japanese. Written informed consent was obtained from each subject. This study was approved by the ethics committees at Fujita Health University, Okayama University, and Nagoya University Graduate School of Medicine.

SNP Selection and Genotyping

We selected seven SNPs (rs41279104, rs3782221, rs3782219, rs561712, rs3782206, rs2682826, and rs6490121) in *NOS1* shown by previous studies to have a positive association with schizophrenia (Fallin et al. 2005; Moskvina et al. 2009; Reif et al. 2006; Shinkai et al. 2002). We used TaqMan assays (Applied Biosystems, Foster City, CA, USA) for all SNPs. Detailed information is available on request.

Statistical Analysis

Genotype deviation from the Hardy–Weinberg equilibrium (HWE) was evaluated by the chi-square test (SAS/Genetics, release 8.2, SAS Japan Inc., Tokyo, Japan). Marker–trait association was also evaluated by the chi-square test in allele- and genotype-wise analyses. Haplotype frequencies were estimated in a two- to four-marker sliding window fashion and log likelihood ratio tests were performed for global P values with COCAPHASE program version 3.0.6 (Dudbridge 2003). In these haplotype-wise analyses, rare haplotypes (less than 0.05) in either of cases and controls were excluded from the association analysis. Power calculation was performed using a statistical program prepared by Genetic Power Calculator (http://pngu.mgh.harvard.edu/~purcell//gpc/). To correct for problems of multiple comparisons, we applied the Benjamini–Hochberg (BH) method, which is a procedure to control for false discovery

rate (FDR) (Dudbridge 2003). The level of significance for all statistical tests was 0.05.

Results

Genotype frequencies of subjects and controls did not deviate significantly from HWE. In the first-set of analysis, two SNPs (rs3782219 and rs3782206) showed a significant association with schizophrenia in allele and/or genotype-wise analysis (rs3782219: *P* allele = 0.0291 and rs3782206: *P* allele = 0.0124, *P* genotype = 0.0490). Five other SNPs did not show evidence of association with schizophrenia (Table 1). There was no evidence of association with schizophrenia in haplotype-wise analysis (Table 2).

To validate the significant association of rs3782219 and rs3782206 found in the first-set samples, and even some significances remained with an increased sample size (1154 cases and 1260 controls, rs3782219: *P* allele = 0.0197 and rs3782206: *P* allele = 0.0480); however, these associations also might have resulted from type I error on account of multiple testing (rs3782219: *P* allele = 0.133 and rs3782206: *P* allele = 0.168) (Table 1).

We obtained power of more than 80% for the detection of association when we set the genotype relative risk at 1.27–1.36 under a multiplicative model of inheritance in the first-set samples.

Discussion

We found marginal associations between two SNPs (rs3782219 and rs3782206) and schizophrenia in allele and/or genotype-wise analysis, and almost these significances remained with an increased sample size. However, we suggested that it might have resulted from type I error due to multiple testing. Fallin et al. (2005) reported that significant associations of haplotypes were identified with four SNPs in intron 2 (rs3782221, rs3782219, rs561712, and rs3782206). However, no association was found in our study. Shinkai et al. (2002) showed a strongly positive association between rs2682826 and schizophrenia in a Japanese population. However, although we examined more large Japanese samples than original study (Shinkai et al. 2002), we found no significant association with schizophrenia. The result of this study was in concordance with replication studies in other ethnic population samples (Liou et al. 2003; Tang et al. 2008). Recently, a whole genome association study reported a possible association between rs6490121 in *NOS1* and schizophrenia (O'Donovan et al. 2008). To avoid multiple testing problems, it is important to conduct replication study. Our samples were provided for replication study and showed a significant association and odds ratio that were opposite to UK samples (O'Donovan et al. 2008). However, although we performed a replication study using larger different samples

Table 1 Association study between *NOS1* and schizophrenia

SNP ID	Position	Phenotype[a]	MAF[b]	*N*	Genotype distribution[c]			*P* value[e]			Corrected *P* value[f]
					M/M	M/m	m/m	HWE[d]	Genotype	Allele	Allele
rs41279104	114886493	SCZ	0.197	542	355	162	25	0.243	0.436	0.230	
	Promoter region of exon 1c	CON	0.175	519	354	148	17	0.751			
rs3782221	114805000	SCZ	0.448	542	173	252	117	0.161	0.488	0.275	
	Intron 1	CON	0.424	519	175	247	97	0.550			
rs3782219	114797355	SCZ	0.411	1154	409	540	205	0.248	0.0655	0.0197	0.133
	Intron 1	CON	0.444	1260	394	611	255	0.518			
rs561712	114761232	SCZ	0.176	542	374	145	23	0.0677	0.128	0.856	
	Intron 2	CON	0.179	519	346	160	13	0.274			
rs3782206	114754240	SCZ	0.279	1154	610	443	101	0.111	0.133	0.0480	0.168
	Intron 3	CON	0.254	1260	706	467	87	0.415			
rs6490121	114717324	SCZ	0.390	542	203	255	84	0.790	0.244	0.0952	
	Intron 10	CON	0.425	519	175	246	98	0.484			
rs2682826	114662005	SCZ	0.353	542	223	255	64	0.491	0.469	0.228	
	Exon 29	CON	0.328	519	230	237	52	0.424			

[a] *SCZ* schizophrenia, *CON* control

[b] *MAF* minor allele frequency

[c] *M* major allele, *m* minor allele

[d] Hardy–Weinberg equilibrium

[e] Bold numbers represent significant *P* value

[f] Calculated using Benjamini–Hochberg (BH) method

Table 2 Haplotype-wise analysis between *NOS1* and schizophrenia

SNP ID	Global *P* value		
	2 Window	3 Window	4 Window
rs41279104			
	0.228		
rs3782221		0.187	
	0.051		0.106
rs3782219		0.223	
	0.180		0.203[a]
rs561712		0.0770	
	0.0620		0.112
rs3782206		0.0780	
	0.0600		0.223
rs2682826		0.211	
	0.131		
rs6490121			

[a] Fallin et al. reported

than original study, we could not replicate. In other recent study, Tang et al. (2008) reported a significant association with schizophrenia of rs3782206 in a Chinese population. This discordance of results may reflect problems in the replication study, such as population difference, in each sample. Therefore, it is necessary to evaluate a polymorphism for this association with schizophrenia in various ethnic populations.

A few points of caution should be noted in interpreting our results. First, we did not apply a LD-based approach and a mutation scan to detect rare variants with functional effects. Moreover, we did not examine a VNTR in exon 1f within the promoter region in *NOS1*. Reif et al. (2006) reported a significant association of the haplotype constructed by rs41279106 and this VNTR with schizophrenia. These problems are future topics for study. Second, our sample was not matched in terms of age. Moreover, our samples were not assessed by a standard structured interview, and thus there is a chance of false negatives due to misdiagnosis or sampling bias (Kishi et al. 2009).

In conclusion, we suggest that these seven SNPs in *NOS1* may not play a role in the susceptibility to schizophrenia in the Japanese population. However, other functional polymorphisms in *NOS1* may show important roles in the pathophysiology of schizophrenia, and further investigations will be necessary.

Acknowledgments We thank Ms S. Ishihara and Ms M. Miyata for their technical support. This study was supported in part by research grants from the Ministry of Education, Culture, Sports, Science and Technology, the Ministry of Health, Labor and Welfare, the Japan Health Sciences Foundation (Research on Health Sciences Focusing on Drug Innovation), MEXT, Academic Frontier, and the Smoking Research Foundation.

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Humana Press

Neuroscience Research 64 (2009) 231–234

Contents lists available at ScienceDirect

Neuroscience Research

journal homepage: www.elsevier.com/locate/neures

Genetic association analysis of serotonin 2A receptor gene (*HTR2A*) with bipolar disorder and major depressive disorder in the Japanese population

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ARTICLE INFO

Article history:
Received 19 November 2008
Received in revised form 3 March 2009
Accepted 5 March 2009
Available online 20 March 2009

Keywords:
Serotonin 2A receptor gene (*HTR2A*)
SNPs
Major depressive disorder
Bipolar disorder

ABSTRACT

Because several investigations, including genetic studies, have reported associations between serotonin (5-HT) 2A receptor gene and mood disorders, 5-HT 2A receptor gene (*HTR2A*) is a good candidate gene for the pathophysiology of mood disorders such as major depressive disorder (MDD) and bipolar disorder (BP). Using two functional SNPs (T102C and -A1438G) and two SNPs (rs7997012 and rs1928040) in *HTR2A*, which reported an association with therapeutic response to the SSRI, we conducted a genetic association analysis of case-control samples (325 MDD patients, 155 BP patients and 802 controls) in the Japanese population. We did not detect significant an association of *HTR2A* with MDD and BP in allele/genotype-wise or haplotype-wise analysis. In this study, we could detect no evidence of genetic association between 4 markers near *HTR2A* and mood disorders in the Japanese population, but sample sizes, especially BP, were probably too small to allow a meaningful test.

1. Introduction

Altered serotonergic neural transmission is hypothesized to be a susceptibility factor for mood disorders, such as major depressive disorder (MDD) and bipolar disorder (BP). The evidence for such an association is discussed in more detail in reviews (Levinson, 2006; Murphy et al., 2004; Serretti and Mandelli, 2008).

Selective serotonin reuptake inhibitors (SSRIs), which are major therapeutic agents for MDD, block serotonin transport on the presynaptic neuron, increasing extracellular serotonin level and stimulating serotonin 2A (5-HT2A) receptors on the postsynaptic neuron. This mechanism is believed to relieve depressive symptoms. Imipramine, an antidepressant, is an antagonist for 5-HT2A receptors. Also, valproic acid, one of the most well-known mood stabilizers in the pharmacotherapy of BP, is reported to effect a signaling cascade on 5-HT2A receptors (Sullivan et al., 2004; Yatham et al., 2005). Moreover, atypical antipsychotics such as risperidone and olanzapine augment the clinical response in treatment-resistant mood disorder patients (Philip et al., 2008). One of the major pharmacological therapeutic targets of atypical antipsychotics is 5-HT2A receptors. Thus, 5-HT2A receptors are suggested to be involved in the pathophysiology of mood disorders (Serretti and Artioli, 2004a,b; Serretti et al., 2007).

Several genetic studies showed an association between the 5-HT2A receptor gene (*HTR2A*) and MDD and BP; however, results have been rather inconsistent. The evidence for such an association is discussed in more detail in reviews (Kato, 2007; Serretti et al., 2007). Moreover, to our knowledge, no association study of *HTR2A* and MDD or BP in the Japanese population has been reported. Also, the results of pharmacogenetic studies of *HTR2A* and the SSRI response in MDD have also been rather inconsistent. Recently, McMahon and colleagues reported an association between rs7997012 and rs1928040 in *HTR2A* and outcome of citalopram treatment in a very large sample of outpatients with MDD (McMahon et al., 2006). Recent two studies reported that MDD and SSRI response in MDD have common susceptibility genes. Lekman and colleagues reported that the *FKBP5* was associated with MDD and citalopram therapeutic response in the White non-Hispanic population (Lekman et al., 2008). Also, Tsai and colleagues reported the significant associations between plasminogen activator inhibitor type 1 gene (*SERPINE1*) and Chinese MDD patients and SSRI therapeutic response (Tsai et al., 2008).

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0168-0102/$ – see front matter.
doi:10.1016/j.neures.2009.03.003

Table 1
HTR2A and mood disorders.

SNPs[a]	Phenotype[b]	MAF[c]	N	Genotype distribution[d]			*P*-value[e]		
				M/M	M/m	m/m	HWE	Genotype	Allele
rs6313	Controls	0.485	802	220	386	196	0.301		
(102T/C)	Mood disorders	0.508	480	125	222	133	0.102	0.430	0.254
Exon1	MDD	0.495	325	87	154	84	0.346	0.883	0.656
	BP	0.535	155	38	68	49	0.141	0.172	0.104
rs2070040	Controls	0.440	802	262	374	166	0.128		
(-1438A/G)	Mood disorders	0.423	480	164	226	90	0.438	0.675	0.394
Intron1	MDD	0.431	325	108	154	63	0.542	0.884	0.684
	BP	0.406	155	56	72	27	0.643	0.559	0.273
rs1928040	Controls	0.300	802	400	322	80	0.203		
T>C	Mood disorders	0.303	480	235	199	46	0.682	0.894	0.888
Intron2	MDD	0.286	325	163	138	24	0.478	0.370	0.499
	BP	0.339	155	72	61	22	0.130	0.287	0.182
rs7997012	Controls	0.181	802	535	243	24	0.568		
G>A	Mood disorders	0.192	480	311	154	15	0.438	0.781	0.518
Intron2	MDD	0.208	325	202	111	12	0.496	0.336	0.149
	BP	0.158	155	109	43	3	0.598	0.591	0.325

[a] Major allele > minor allele, SNP position.
[b] Mood disorders: mood disorders group, MDD: major depressive disorder, BP: bipolar disorder.
[c] MAF: minor allele frequency.
[d] M: major allele m: minor allele.
[e] Hardy-Weinberg equilibrium.

Therefore, using two functional SNPs (T102C and -A1438G) and two SNPs (rs7997012 and rs1928040) in *HTR2A*, which reported an association with therapeutic response to the SSRI, we conducted a genetic association analysis of case-control samples (325 MDD patients, 155 BP patients and 802 controls) in the Japanese population.

2. Materials and methods

2.1. Subjects

The subjects in the association analysis were 325 MDD patients (159 males and 166 females; mean age ± standard deviation 47.3 ± 14.9 years), 155 BP patients (80 males and 75 females: 96 patients with bipolar I disorder and 59 patients with bipolar II disorder; 47.9 ± 14.2 years) and 802 healthy controls (351 males and 451 females; 37.6 ± 14.3 years). All subjects were unrelated to one another, 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. 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. This study was approved by the Ethics Committee at Fujita Health University and Nagoya University School of Medicine.

2.2. SNP selection and linkage disequilibrium (LD) evaluation

HTR2A has been reported to have two biologically functional SNPs (T102C: rs6313 and -A1438G: rs2070040) (Myers et al., 2007; Spurlock et al., 1998). According to the HapMap database, LD among these two SNPs in *HTR2A* was $r^2 = 0.765$. Therefore, we performed an association study for these two SNPs. Moreover, because McMahon and colleagues reported an association between rs7997012 and rs1928040 in *HTR2A* and outcome of citalopram treatment in a very large sample of outpatients with MDD (McMahon et al., 2006), we included these two SNPs. These four SNPs were selected for the following association analysis.

2.3. SNP genotyping

We used TaqMan assays (Applied Biosystems, Inc., Foster City, CA,) for all 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 evaluated with a likelihood ratio test using the COCAPHASE2.403 program (Dudbridge, 2003). Power calculation was performed using the Genetic Power Calculator (Purcell et al., 2003).

The significance level for all statistical tests was 0.05.

3. Results

Genotype frequencies of all SNPs were in HWE. We did not detect any significant association of *HTR2A* with MDD or BP in allele/genotype-wise analysis (Table 1) or haplotype-wise analysis (BP: $P = 0.344$ and MDD: $P = 0.198$). It is known that there are sex differences in the pathophysiology of mood disorders (Baron, 1981; Currier et al., 2006; Faraone et al., 1987). Therefore, we performed an explorative analysis of subjects divided by sex. However, no association was detected between four SNPs in *HTR2A* and either sex (Tables 2 and 3). Moreover, because the sample size is not large enough to detect the possible small effect of this gene, especially in bipolar disorder, we performed to combine the MDD and BP to one "mood disorders group" as a case group for the association analysis. However, we did not detect any significant association of *HTR2A* with "mood disorders group" in allele/genotype-wise analysis (Tables 1–3) or haplotype-wise analysis ($P = 0.0810$).

4. Discussion

We analyzed the genetic association between *HTR2A* and mood disorders in the Japanese population. However, no association was found between 4 markers near *HTR2A* and mood disorders by allele/genotype-wise or haplotype-wise analysis. In addition, no association was detected between four SNPs in *HTR2A* and either sex.

McMahon and colleagues detected a significant association of rs7997012 and rs1928040 in *HTR2A* with the outcome of citalopram treatment in a very large sample of outpatients with MDD (McMahon et al., 2006). Also, Kato et al. (2006) reported an association between -A1438G and the fluvoxamine therapeutic response in Japanese MDD, but Sato et al. (2002) reported no such association. Although there are two reported association analyses of *HTR2A* with SSRI response in Japanese MDD patients, the results were rather inconsistent and the problem of these studies was

Table 2
HTR2A and mood disorders in male.

SNPs[a]	Phenotype[b]	MAF[c]	N	Genotype distribution[d]			*P*-value[e]		
				M/M	M/m	m/m	HWE	Genotype	Allele
rs6313	Controls	0.494	351	96	163	92	0.183		
(102T/C)	Mood disorders	0.529	239	54	117	68	0.786	0.424	0.240
Exon1	MDD	0.525	159	37	77	45	0.715	0.616	0.361
	BP	0.538	80	17	40	23	0.960	0.533	0.324
rs2070040	Controls	0.447	351	112	164	75	0.303		
(-1438A/G)	Mood disorders	0.406	239	85	114	40	0.865	0.339	0.158
Intron1	MDD	0.406	159	57	75	27	0.784	0.456	0.214
	BP	0.406	80	28	39	13	0.925	0.582	0.345
rs1928040	Controls	0.296	351	179	136	36	0.184		
T>C	Mood disorders	0.297	239	120	96	23	0.555	0.929	0.977
Intron2	MDD	0.277	159	83	64	12	0.944	0.623	0.524
	BP	0.338	80	37	32	11	0.345	0.592	0.307
rs7997012	Controls	0.174	351	241	98	12	0.603		
G>A	Mood disorders	0.192	239	153	80	6	0.235	0.317	0.414
Intron2	MDD	0.201	159	99	56	4	0.229	0.236	0.293
	BP	0.174	80	54	24	2	0.603	0.869	0.971

[a] Major allele > minor allele, SNP position.
[b] Mood disorders: mood disorders group, MDD: major depressive disorder, BP: bipolar disorder.
[c] MAF: minor allele frequency.
[d] M: major allele m: minor allele.
[e] Hardy-Weinberg equilibrium.

small samples (Sato et al., 2002: 54 MDD patients, Kato et al., 2006: 49 MDD patients treated with fluvoxamine only). To overcome these problems, replication study using larger samples will be required for conclusive results. More recently, although Wilkie and colleagues reported an association between rs6314 (C1354T) in *HTR2A* and both response and remission to the paroxetine in MDD (Wilkie et al., 2008), this SNP was shown to have "minor allele frequencies: 0%" in the HapMap database (Japanese population).

HTR2A has been reported to have biologically functional SNPs (T102C: rs6313 and -A1438G: rs2070040) (Myers et al., 2007; Spurlock et al., 1998). In genetic analysis of *HTR2A*, so far either SNPs have been selected till now. However, according to the HapMap database, LD among these two SNPs in *HTR2A* was $r^2 = 0.765$, so we performed an association study for these SNPs. In this study, we detected r^2 less than 0.85 for all phenotypes (r^2 = Control 0.719, BP 0.840 and MDD 0.709). This result suggests that association analyses for both SNPs should be performed in future studies. Also, although we confirmed LD between the two functional SNPs selected in this study and rs7997012 and rs1928040 according to the HapMap database, this LD was not found to be tight.

A few points of caution should be noted in interpreting our results. Firstly, our sample sizes were small, especially BP. We obtained power of more than 80% for the detection of association when we set the genotype relative risk at 1.58–1.68 in BP and 1.32–1.39 in MDD under a multiplicative model of inheritance (Purcell et al., 2003). The lack of association may be due to biased samples, such as small sample sizes, especially BP. Because our BP samples are small, there are possibilities of type II errors in these results of an association analysis for these phenotypes statistically. Secondly,

Table 3
HTR2A and mood disorders in female.

SNPs[a]	Phenotype[b]	MAF[c]	N	Genotype distribution[d]			*P*-value[e]		
				M/M	M/m	m/m	HWE	Genotype	Allele
rs6313	Controls	0.478	451	124	223	104	0.846		
(102T/C)	Mood disorders	0.488	241	71	105	65	0.0629	0.310	0.731
Exon1	MDD	0.467	166	50	77	39	0.380	0.763	0.732
	BP	0.533	75	21	28	26	0.0804	0.0643	0.208
rs2070040	Controls	0.435	451	150	210	91	0.265		
(-1438A/G)	Mood disorders	0.440	241	79	112	50	0.377	0.982	0.851
Intron1	MDD	0.455	166	51	79	36	0.603	0.818	0.526
	BP	0.407	75	28	33	14	0.445	0.786	0.522
rs1928040	Controls	0.304	451	221	186	44	0.596		
T>C	Mood disorders	0.309	241	115	103	23	0.993	0.930	0.837
Intron2	MDD	0.295	166	80	74	12	0.358	0.551	0.771
	BP	0.340	75	35	29	11	0.231	0.436	0.374
rs7997012	Controls	0.187	451	294	145	12	0.236		
G>A	Mood disorders	0.191	241	158	74	9	0.927	0.703	0.874
Intron2	MDD	0.214	166	103	55	8	0.851	0.374	0.297
	BP	0.140	75	55	19	1	0.651	0.357	0.163

[a] Major allele > minor allele, SNP position.
[b] Mood disorders: mood disorders group, MDD: major depressive disorder, BP: bipolar disorder.
[c] MAF: minor allele frequency.
[d] M: major allele m: minor allele.
[e] Hardy-Weinberg equilibrium.

because we did not perform association analysis based on LD and a mutation scan of *HTR2A*, a replication study using a larger sample and based on LD may be required for conclusive results. Lastly, our subjects did not undergo structured interviews. MDD patients who are not diagnosed by structured interview may develop bipolar disorder in the future (Bowden, 2001; Kishi et al., 2008; 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 misdiagnosis of a patient, we promptly excluded the misdiagnosed case in consideration of the precision of our sample.

In this study, we could detect no evidence of genetic association between 4 markers near *HTR2A* and MDD and BP, but sample sizes (especially BP) were probably too small to allow a meaningful test.

Acknowledgements

We thank Ms. M. Miyata and Ms. S. Ishihara for their technical support. This work was supported in part by research grants from the Ministry of Education, Culture, Sports, Science and Technology, the Ministry of Health, Labor and Welfare, and the Japan Health Sciences Foundation (Research on Health Sciences focusing on Drug Innovation).

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Association analysis of functional polymorphism in estrogen receptor alpha gene with schizophrenia and mood disorders in the Japanese population

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Psychiatric Genetics 2009, 19:217–218

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Received 2 July 2008 Revised 2 March 2009
Accepted 28 March 2009

Postmortem studies reported alternations of estrogen receptor alpha (ERalpha) mRNA in the dorsolateral prefrontal cortex, amygdala, and hippocampus in patients with schizophrenia, major depressive disorder (MDD), and bipolar disorder (BP) compared with control participants (Osterlund *et al.*, 2000; Perlman *et al.*, 2005). A recent study reported an association between rs2234693, which influenced enhancer activity levels of ESR1 in ERalpha gene (*ESR1*), and schizophrenia (Weickert *et al.*, 2008). This study reported that the schizophrenia patients with CC genotype have significantly lower ESR1 mRNA levels in the prefrontal cortex than the patients who are with other genotypes (Weickert *et al.*, 2008). We conducted a replication analysis rs2234693 with Japanese schizophrenia and mood disorders patients.

The study participants were 738 schizophrenia patients (395 male, 343 female; mean age ± standard deviation 36.3 ± 18.4 years) (subtypes: paranoid 216, disorganized 221, catatonic 29, residual 142, undifferentiated 130), 155 BP patients (80 male, 75 female; 45.1 ± 13.6: BPI 98, BPII 57), 325 MDD patients (159 male, 166 female; 47.5 ± 16.1), and 802 healthy controls (351 male, 451 female; 37.6 ± 14.3). All participants were unrelated, ethnic Japanese. The patients were diagnosed according to *The Diagnostic and Statistical Manual of Mental Disorders*, fourth edition criteria with consensus of at least two experienced psychiatrists, based on unstructured interviews and a review of medical records. When we found a misdiagnosis of a patient, we excluded the misdiagnosed case in consideration of the precision of our sample. Detailed information on our samples was described in our previous paper (Kishi *et al.*, 2008). Written informed consent was obtained from each participant. This study was approved by the Ethics Committees at Fujita Health University and Nagoya University Graduate School of Medicine. Genotyping was carried out with TaqMan assays (Applied Biosystems, California, USA). Detailed information is available on request. Genotype deviation from the Hardy–Weinberg equilibrium and marker-trait association analysis used to evaluate allele/genotype-wise association was evaluated by χ^2 test (SAS/Genetics, release 8.2, SAS Japan Inc., Tokyo, Japan). Power calculation was performed using a statistical program prepared by Ohashi *et al.* (2001). The significance level for all statistical tests was 0.05.

Genotype frequencies were in Hardy–Weinberg equilibrium for this single nucleotide polymorphism. We detected no association between rs2234693 (genotype counts: TT/TC/CC) and each disorder in the allele/genotype-wise analysis (241/375/122 for schizophrenia, 51/77/27 for BP, 103/154/68 for MDD, and 273/392/137 for controls). In the power analysis, we obtained more than 80% power for the detection of association when we set the genotype relative risk at 1.22, 1.42, and 1.29 in schizophrenia, BP, and MDD, respectively, for *ESR1* under a multiplicative model of inheritance.

Our results suggest that *ESR1* may not play a major role in the pathophysiology of these disorders. However, Weickert *et al.* (2008) reported associations of several ERalpha mRNA splicing variants and a rare variant in *ESR1* with schizophrenia, and further study such as resequencing in *ESR1* will be necessary. The lack of association may be because of biased samples such as unmatched age or sex samples. However, although we included an explorative analysis of participants divided by clinical diagnosis (except MDD) or sex, no association was detected in any subgroup or in either sex (data not shown).

Acknowledgements

The authors thank Ms M. Miyata and Ms S. Ishihara for their technical support. This work was supported in part

DOI: 10.1097/YPG.0b013e32832cee98

by research grants from the Japan Ministry of Education, Culture, Sports, Science and Technology; the Ministry of Health, Labor and Welfare; and the Health Sciences Foundation (Research on Health Sciences focusing on Drug Innovation).

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