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Prepulse inhibition of the startle response with chronic schizophrenia: A replication study

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ABSTRACT

Prepulse inhibition (PPI) deficit, the acoustic startle reflex (ASR) and habituation (HAB) impairment are considered to be endophenotypes for schizophrenia. The recent two studies have reported that a PPI deficit was detected in Japanese schizophrenic patients. We replicated that study using larger samples (115 schizophrenic patients and 111 normal controls) than the original study and a method same as original study. A startle response monitoring system was used to deliver acoustic startle stimuli, and to record and score the electromyographic activity of the orbicularis oculi muscle. We evaluated the startle measures of mean magnitude of ASR, HAB, and PPI at prepulse sound pressure intensities of 82 dB (PPI82), 86 dB (PPI86), and 90 dB (PPI90). ASR was significantly different between schizophrenic patients and controls. HAB and all PPI session data from schizophrenic patients were significantly lower than in controls. In addition, we detected significant differences for ASR, HAB and each PPI (82, 86 and 90 dB) between schizophrenic patients and controls with the use of multiple regression analysis. The gender and smoking state were not correlated with ASR, HAB or any PPI in multiple regression analysis. In conclusion, we were able to replicate the finding of HAB impairment and PPI deficit in chronic Japanese schizophrenic patients.

1. Introduction

Disruption of sensorimotor gating is considered to lead to abnormalities in cognitive dysfunction, such as attention-related information processing (Braff et al., 1992; McGhie and Chapman, 1961). Several investigations suggested that disruption of sensorimotor gating was measured mainly by prepulse inhibition (PPI) and the acoustic startle reflex (ASR) (Braff et al., 1992; Kunugi et al., 2007; Takahashi et al., 2008). PPI deficit, ASR and habituation (HAB) impairment are considered endophenotypes for schizophrenia (Braff et al., 1992; Kunugi et al., 2007; Takahashi et al., 2008; Walters and Owen, 2007).

Recently, Kunugi et al. (2007) have reported that a PPI deficit was detected in Japanese schizophrenic patients. More recently, Takahashi et al. (2008) evaluated the startle measures of mean magnitude of ASR, HAB, and PPI at prepulse sound pressure intensities of 82, 86, and 90 dB, respectively. Although they found that ASR was not significantly different between the patients and controls, they reported that patients showed significantly reduced HAB and PPI for all prepulse intensities compared to controls (Takahashi et al., 2008). In this study, we performed a replication using larger samples (115 schizophrenic patients and 111 normal controls) than the original study and a method same as original study (Takahashi et al., 2008).

2. Materials and methods

2.1. Subjects

One hundred and fifteen schizophrenic patients (71 males and 40 females: mean age ± standard deviation (SD) 52.0 ± 1.46 years)

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and 111 normal controls (72 males and 38 females: mean age ± standard deviation (SD) 32.7 ± 0.674 years) were recruited. 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 the consensus of at least two experienced psychiatrists on the basis of a structured interview using the Structured Clinical Interview for DSM-IV disorders (SCID-1) and a review of medical records. All the patients met the following inclusion criteria: (1) age 25–70 years, (2) no systemic or neurologic disease, (3) no electroconvulsive therapy, (4) no history of head trauma, (5) no lifetime history of substance dependence or history of substance abuse within 3 months and (6) maintenance of main antipsychotic drug therapy for 2 months. All healthy control subjects were also psychiatrically screened based on structured interviews. None had severe medical complications or other Axis-I disorders according to DSM-IV using SCID-1. No structured methods were used to assess psychiatric symptoms in the controls. 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 School of Medicine and Okehazama Hospital.

2.2. Startle response measurement

2.2.1. Apparatus and stimuli

We measured startle measure using a commercial computerized human startle response monitoring system (Startle Eyeblink Reflex Analysis System Map1155SYS, Nihonsanteku Co., Osaka, Japan). Startle eyeblink electromyographic responses were recorded from the left orbicularis oculi muscle with 2 Ag/AgCl disposable electrodes (sensor area 15 mm^2) filled with wet gel. The first electrode (Blue Sensor N-00-S, Ambu, Ballerup, Denmark) was positioned approximately 1 cm directly below the pupil of the left eye and low enough to not touch the lower eyelid, while the second electrode (Blue Sensor M-00-S, Ambu, Ballerup, Denmark) was placed laterally and slightly superior to the first one, with the centers of the electrodes separated by approximately 2 cm. The impedance between the two electrodes was measured and deemed acceptable if below 5 kΩ. The impedance was measured with an electrode impedance meter (MaP811, Nihonsanteku Co., Osaka, Japan) at a measurement frequency = 30 Hz. The ground electrode (Blue Sensor M-00-S) was placed on the left angle of the mandible. We used a method same as Takahashi et al.'s (2008) study. Detailed information can be seen in Takahashi et al.'s (2008) paper.

2.2.2. The stimulus sequence, procedure and response scoring and data reduction

Measurements were made with startle paradigm constructed of 3 blocks with continuous 70 dB SPL background white noise. Pulse stimuli consisted of broadband white noises with an instantaneous rise/fall time lasting for 40 ms and presented at 115 dB SPL. Prepulse stimuli were also broadband white noises with an instantaneous rise/fall time lasting for 20 ms and presented at three different intensities (82, 86, and 90 dB SPL). The lead interval (LI) (from prepulse onset to pulse onset) in our study was set at 120 ms. In block 1, the startle response for pulse alone trials (PA trial) was recorded 6 times. Block 2 consisted of PA trials or trials of pulse with prepulse at three intensities (PP trials), performed eight times for each condition. Block 3 was the same as block 1 to measure the habituation phenomenon. All trials were presented in a fixed pseudorandom order, separated by inter-trial intervals of 15–25 s (20 s on average). The startle paradigm consisted of a total of 44 trials. Together with 5 min acclimation to the background noise, the session lasted approximately 20 min. We used a method same as Takahashi et al.'s (2008) study. Detailed information can be seen in Takahashi et al.'s (2008) paper.

2.3. Statistical analysis

Individual t test and chi-square tests (Fisher's exact test when appropriate) were used to compare means and categorical proportions, respectively. All PPI measures were examined with analysis of variance (ANOVA) with repeated measures of prepulse intensities. In addition, because several investigators have reported significant differences in startle response with gender difference, between smokers and nonsmokers, and with the effect of benzodiazepine in not only schizophrenic patients but also normal controls (Kumari et al., 2004, 1996, 1997; Swerdlow et al., 1999), we performed exploratory analysis of the possible correlations between all startle measures (PPI, ASR and HAB, respectively) and diagnosis (schizophrenia or controls), gender, and smoking history (smoker or nonsmoker) by multiple regression analysis. In these analyses, all startle measures were set as the dependent variable, and diagnosis (schizophrenia or controls), gender and current smoking status (smoker or nonsmoker) were set as the independent variables. We performed another multiple regression analysis on the effect of several clinical variables on all startle measures with schizophrenic patients. In these analyses, all startle measures were set as the dependent variable, and age, duration of illness, gender, smoking status, and medication dosage (antipsychotics and anxiolytics/hypnotics) were set as the independent variables. The significance level for all statistical tests was 0.05.

All statistical analyses were performed using JMP (JMP 5.0. 1J, SAS Japan Inc., Tokyo, Japan).

3. Results

3.1. Patient and control demographics and disposition

Among the subjects (schizophrenia or controls) in this study, only one difference with recruited age was detected (P value ≤ 0.0001) (Table 1). Also, there were 15 nonresponders in the patient group and 4 in the control group. Therefore, we performed the analysis of startle measure with 100 patients and 107 controls. Clinical demographics and disposition of analysed schizophrenic patients are shown in Table 1.

3.2. Schizophrenic patients vs. controls

We detected significant differences for all startle measures between schizophrenic patients and controls (Fig. 1). In addition, we detected significant differences for ASR, HAB and each PPI (82, 86 and 90 dB) between schizophrenic patients and controls with the use of multiple regression analysis (Table 2). The gender and smoking state were not correlated with ASR, HAB or any PPI in multiple regression analysis (Table 2).

3.3. Startle response with schizophrenia reflected clinical variables

There was no significant correlation of ASR, HAB or any PPI for either side with age, duration of illness, gender, smoking status, and medication dosage (antipsychotics and anxiolytics/hypnotics) (data not shown).

4. Discussion

We investigated startle response with schizophrenic patients and controls using larger samples than the original study. We were able to replicate HAB impairment and PPI deficit in chronic Japanese schizophrenic patients using a multiple regression model, controlling for other parameters known to influence the startle measure. To our knowledge, this type of analysis has not been previously used for startle response study. Controlling for

Table 1
Patients and controls demographics and disposition.

	Participated control subjects	Participated schizophrenia subjects	*P* value[a]
N	111	115	
Current smoker/nonsmoker	42 (37.4%)	32 (27.8%)	0.0791
Sex (males/females)	72/38	71/40	0.513
Age, years (mean ± SD)	32.7 ± 0.674	52.0 ± 1.46	**<0.0001**
	Analysed control subjects	**Analysed schizophrenia subjects**	***P* value[a]**
N	107	100	
Current smoker/nonsmoker, *n* (%)	40/67 (62.6)	26/74 (26.0)	0.0781
Sex (males/females)	71/36	62/38	0.514
Age, years (mean ± SD)	32.7 ± 7.16	51.6 ± 15.7	**<0.0001**
Duration of illness (day, mean ± SD)		9750 ± 524	
Clinical diagnosis, *n* (%)			
Paranoid type		30 (30)	
Disorganeized type		9 (9)	
Residual type		61 (61)	
PANSS total score		77.1 ± 23.5	
Medication dosage			
Antipsychotics (mg/day)[b]		693 ± 48.3	
Antiparkinsonian drugs, *n* (%)		0 (0)	
Mood stabilizers, *n* (%)		21 (21)	
Anxiolytics/hypnotics, *n* (%) (mg/day)[c]		69 (69) (8.46 ± 9.51)	

[a] Bold represents significant *P* value.
[b] Chlorpromazine-equivalent.
[c] Diazepam-equivalent.

important covariates, we found that startle response changes did not differ with smoking status or gender. We showed that the only factor influencing startle measure was diagnosis (schizophrenia or controls). None of the startle measures in schizophrenic patients was correlated with age, duration of illness, gender, smoking status, or medication dosage (antipsychotics and anxiolytics/hypnotics). Therefore, we considered that startle response might have diagnosis specificity associated with sensorimotor gating deficit.

Kunugi et al. (2007) reported reduced ASR and PPI despite of no reduced HAB in schizophrenia. On the other hand, Takahashi et al. (2008) reported reduced PPI and HAB despite of no reduction of

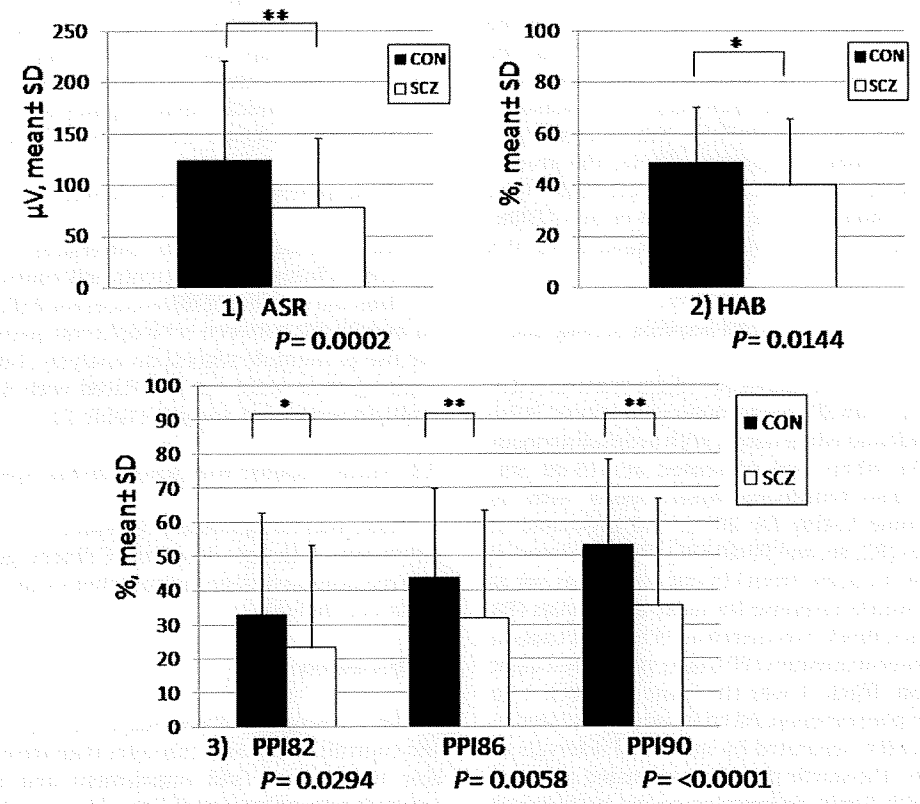

Fig. 1. ANOVA of startle measures with schizophrenia and controls. $^{*}P < 0.05$, $^{**}P < 0.01$. (1) Acoustic startle reflex (ASR) (μV, mean ± SD); schizophrenia: 78.0 ± 67.6; controls: 124 ± 96.5. (2) Habituation (HAB) (%, mean ± SD); schizophrenia: 39.8 ± 26.0; controls: 48.6 ± 21.7. (3) Prepulse inhibition (PPI); PPI 82 (%, mean ± SD); schizophrenia: 23.3 ± 30.2; controls: 33.0 ± 29.9. PPI 86 (%, mean ± SD); schizophrenia: 32.0 ± 31.7; controls: 43.8 ± 26.2. PPI 90 (%, mean ± SD); schizophrenia: 35.5 ± 31.5; controls: 53.1 ± 25.3.

Table 2
Multiple regression analysis of startle measure with clinical variables. All startle measures were set as the dependent variable, and diagnosis (schizophrenia or controls), gender and current smoking status (smoker or nonsmoker) were set as the independent variables.

Startle measure[a]	Clinical variables[b]	*P* value[c]	SRC[d]
ASR	Diagnosis	**<0.0001**	0.402
	Current smoker/nonsmoker	0.189	0.0480
	Sex	0.591	0.110
Habituation	Diagnosis	**0.0194**	0.177
	Current smoker/nonsmoker	0.264	−0.0879
	Sex	0.294	0.0820
PPI 82db	Diagnosis	**0.0249**	0.163
	Current smoker/nonsmoker	0.107	0.124
	Sex	0.0756	−0.136
PPI 86db	Diagnosis	**0.00350**	0.212
	Current smoker/nonsmoker	0.174	0.181
	Sex	0.0818	−0.132
PPI 90db	Diagnosis	**<0.0001**	0.310
	Current smoker/nonsmoker	0.0652	0.139
	Sex	0.428	−0.0592

[a] ASR: acoustic startle reflex; PPI: prepulse inhibition.
[b] Diagnosis: schizophrenia or controls.
[c] Bold represents significant *P* value.
[d] SRC: standardized regression coefficient.

ASR in schizophrenia. In this study, we detected significant differences for all of ASR, HAB and each PPI (82, 86 and 90 dB) between schizophrenic patients and controls with the use of multiple regression analysis. We considered that the difference of some results in these studies might be the following reasons: (1) Taking different antipsychotic in patient group (Kumari and Sharma, 2002; Kumari et al., 2002; Wynn et al., 2007). Several studies showed the difference of the kind of the antipsychotics influenced startle response (Kumari and Sharma, 2002; Kumari et al., 2002; Wynn et al., 2007). (2) A difference of the numbers of the samples in each studies. Schizophrenic patients and healthy controls of Kunugi's study and Takahashi's study were reported to be 20 patients and 16 controls, and 51 patients and 55 controls, respectively (Kunugi et al., 2007; Takahashi et al., 2008). We replicated this study using larger samples (115 schizophrenic patients and 111 normal controls) than the two original studies (Kunugi et al., 2007; Takahashi et al., 2008).

A few points of caution should be noted in interpreting our results. First, the significant association may be due to biased samples, such as unmatched age samples. We were apprehensive that the age might influence ASR, HAB and all PPI. Therefore, we performed another exploratory analysis of the possible correlations between all startle measures (PPI, ASR and HAB, respectively) and diagnosis (schizophrenia or controls), age, gender, and smoking history (smoker or nonsmoker) by multiple regression analysis for confirmation. However, the gender, age and smoking state were not correlated with ASR, HAB or any PPI in multiple regression analysis (data not shown). Second, 21 patients needed to take mood stabilizers such as valproic acid and carbamazepine. Since a recent animal study using DBA/2NCrl mice reported that mood stabilizers increased PPI (Flood et al., 2009), our results for these 21 schizophrenic patients may have been influenced by the mood stabilizers.

In conclusion, we were able to replicate the HAB impairment and PPI deficit in chronic Japanese schizophrenic patients. However, because our samples are small, it will be necessary to conduct a longer term replication study using a larger patient group in the future.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neures.2009.07.009.

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Orphan Nuclear Receptor Rev-erb Alpha Gene *(NR1D1)* and Fluvoxamine Response in Major Depressive Disorder in the Japanese Population

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Key Words

Major depressive disorder · Orphan nuclear receptor Rev-erbα gene, *NR1D1* · Fluvoxamine · Linkage disequilibrium · Tagging SNP · Clock genes · Circadian rhythm

Abstract

Background: Sleep-wake disturbance, frequently observed in major depressive disorder (MDD), negatively influences clinical status. Treatment with antidepressants also reportedly affects circadian rhythms. In a recent in vitro study, the nuclear receptor Rev-erbα was reported to be related to circadian rhythms, and was shown to be involved in the biological action of lithium therapy. Therefore, we examined the association between the orphan nuclear receptor Rev-erbα gene *(NR1D1)* and the efficacy of fluvoxamine treatment in 118 Japanese patients with major depressive disorder. ***Methods:*** The scores of the MDD patients in this study on the 17 items of the Structured Interview Guide for the Hamilton Rating Scale for Depression (SIGH-D) were 12 or higher. 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. We selected 3 'tagging SNPs' in *NR1D1* for the following association analysis. ***Results:*** We did not detect a significant association between *NR1D1* and the fluvoxamine therapeutic response in MDD in allele/genotype-wise analysis or haplotype-wise analysis. ***Conclusion:*** Our results suggest that *NR1D1* does not play a major role in the therapeutic response to fluvoxamine in Japanese MDD patients. However, because our sample was small, a replication study using another population and a larger sample will be required for conclusive results.

Introduction

Sleep-wake disturbance, frequently observed in major depressive disorder (MDD), negatively affects the clinical status of patients. It has been suggested that abnormalities in circadian rhythms are related to the pathophysiology of MDD [1, 2]. The evidence for this relation has been discussed in more detail in our previous paper [3] and a review by Barnard and Nolan [4].

Selective serotonin reuptake inhibitors (SSRIs), which are major therapeutic agents for MDD, act on the presynaptic neurons to increase the extracellular serotonin level, and this mechanism is believed to relieve depressive

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symptoms. However, serotonin affects circadian rhythms [5], and SSRIs have also been reported to have circadian properties: SSRIs have a phase-shifting effect in rat suprachiasmatic nucleus neural firing [6] and change the expression of clock genes in the striatum and hippocampus of mice [7], suggesting that the SSRIs' antidepressive action also may be attributable to circadian mechanisms. In addition, the clock gene was reported to be associated with greater severity of insomnia during antidepressant treatment, a higher recurrence rate and reduced need for sleep in bipolar disorder patients [8–10]. Therefore, we considered that clock genes might be therapeutic targets of SSRIs.

In the mammalian circadian feedback loop, it is known that the CLOCK/Bmal1 heterodimer drives the transcription of multiple clock genes including *Cry*, *Per* and Rev-erbα gene *(NR1D1)* through E-box elements (detailed evidence for the molecular clock mechanism in mammals has been discussed in several reviews [4, 11–13]). Recently, orphan nuclear receptor Rev-erbα and glycogen synthase kinase-3β (GSK-3β) were shown to be important circadian components [14]. Orphan nuclear receptor Rev-erbα, which belongs to the rev-erb family of transcription factors called 'orphan nuclear receptors', is a key negative-feedback regulator of the circadian clock, and is itself expressed in a circadian manner that is finely controlled both transcriptionally and post-transcriptionally [14, 15]. For example, orphan nuclear receptor Rev-erbα represses Bmal1 gene transcription [15]. Yin et al. [14] showed that orphan nuclear receptor Rev-erbα is a target of GSK-3β kinase activity, which is needed to mediate the regulation of circadian rhythms. These authors also found that in cultured mammalian cells, lithium treatment leads to rapid proteasomal degradation of Rev-erbα and subsequent activation of the Bmal1 gene, a clock gene [14]. Therefore, we thought that *NR1D1* was a good candidate gene for the pathophysiology of mood disorders, and performed an association analysis of *NR1D1* and mood disorders in the Japanese population [3]. No association was found [3], suggesting the possibility that MDD and the antidepressant treatment response in MDD do not have common susceptibility genes. Evidence in support of this hypothesis has been reported (e.g. *DTNBP1* and *NGFR*) [16, 17]. Therefore, although *NR1D1* was not found to play a major key role in the pathophysiology of mood disorders [3], we considered that *NR1D1* might be a susceptibility gene for SSRI treatment response.

In the present study, we examined the association between *NR1D1* and the efficacy of fluvoxamine treatment in Japanese MDD patients.

Materials and Methods

Subjects

The subjects were 118 MDD patients (59 males and 59 females: mean age ± SD 44.5 ± 16.5 years). 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 2 experienced psychiatrists on the basis of a review of medical records. Although these subjects were part of the MDD subject group in our previous study [3], all MDD patients in this study have specific characteristics: being treated with fluvoxamine and undergone a semi-structured interview for assessment of treatment response. Detailed information can be seen in 'Data collection'.

Fluvoxamine was taken 2 or 3 times a day for 8 weeks. The initial total dose in 1 day was 50–100 mg. Fluvoxamine was increased gradually to a maximum of 150 mg, depending on the patient's condition. Patients with insomnia and severe anxiety were prescribed benzodiazepine drugs, but no other psychotropic drugs were permitted during the study. The study was described to subjects and written informed consent was obtained from each participant. This study was approved by the Ethics Committee at Fujita Health University.

Data Collection

The scores of the MDD patients in this study on the 17 items of the Structured Interview Guide for the Hamilton Rating Scale for Depression (SIGH-D) were 12 or higher [18]. 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 [19]. The clinical characteristics of patients in this study, classified according to these definitions, can be seen in table 1.

SNP Selection and Linkage Disequilibrium Evaluation

We first consulted the HapMap database (release 20/phase II, Jan 2006, www.hapmap.org; population: Japan Tokyo, minor allele frequencies of >0.05) and included 5 SNPs covering *NR1D1* [5′-flanking regions including about 750 bp from the initial exon and about 1 kb downstream (3′) from the last exon: HapMap database contig number chr17: 35501880–35510616]. Then 3 tagging SNPs were 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, www.broad.mit.edu/mpg/tagger) in Haploview [20]. These 3 tagging SNPs (rs939347, rs2071427 and rs3744805) were used for the following association analysis. Detailed information can be seen in our previous paper [3].

SNP Genotyping

We used TaqMan assays (Applied Biosystems, Foster City, Calif., USA) for all SNPs. Detailed information can be seen in our previous paper [3].

Statistical Analysis

Genotype deviation from the Hardy-Weinberg equilibrium was evaluated by the χ^2 test (SAS/Genetics, version 8.2, SAS Japan, Tokyo, Japan).

Marker-trait association analysis was used to evaluate allele- and genotype-wise association with the χ^2 test or Fisher's exact

Table 1. Clinical characteristics of the patients in both groups

	Total	Males	Females	Age years	Baseline SIGH-D	Fluvoxamine dose at 8 weeks mg/day	Number of previous episodes
Overall	118	59	59	44.5 ± 16.5	20.2 ± 5.88	122 ± 40.9	1.39 ± 0.658
Clinical response group[1]							
Responders	59	31	28	44.4 ± 16.3	21.5 ± 6.19	118 ± 41.1	1.36 ± 0.574
Nonresponders	59	28	31	44.3 ± 17.3	18.8 ± 5.28	125 ± 40.7	1.43 ± 0.774
p value		0.644		0.801	0.0145	0.391	0.480
Clinical remission group[2]							
Remitters	45	22	23	43.7 ± 15.9	19.6 ± 5.06	113 ± 43.9	1.37 ± 0.598
Nonremitters	73	37	36	45.1 ± 17.1	20.5 ± 6.34	127 ± 38.2	1.41 ± 0.715
p value		0.757		0.750	0.750	0.101	0.856

Average values presented with SD. [1] A decrease of ≥50% in baseline SIGH-D score. [2] Final SIGH-D score <7.

test (SAS/Genetics), and haplotype-wise association analysis was conducted with a likelihood ratio test using the Cocaphase 2.403 program [21]. The power calculation was performed using the Genetic Power Calculator [22]. In addition, we included another test for the association between percentage decrease from baseline to the end of the period of observation at the SIGH-D score and each tagging SNP genotype data, with analysis of covariance (ANCOVA) using the least-squares method. Gender, age at the time of recruitment, fluvoxamine dose at 8 weeks and SIGH-D total score at the baseline were covariates used in the analysis to better model the effect of genotype on percentage decrease from baseline to the end of the period of observation at the SIGH-D score. The statistical package JMP for Windows was used for ANCOVA (JMP 5.0. 1J, SAS). Bonferroni's correction was used to control inflation of the type I error rate. The significance level for all statistical tests was 0.05.

Results

Among the clinical characteristics of patients in this study, only 1 difference with total SIGH-D score at the baseline was detected ($p = 0.0145$; table 1). Genotype frequencies of all SNPs were in Hardy-Weinberg equilibrium. We did not detect an association between *NR1D1* and the fluvoxamine therapeutic response in MDD in the allele/genotype-wise analysis (table 2) or the haplotype analysis (response: $p = 0.695$, and remission: $p = 0.384$). Also, ANCOVA was performed to test the effect of the tagging SNP genotype at percentage decrease from baseline to the end of the period of observation with the SIGH-D score when MDD patients were treated with fluvoxamine. There were no statistically significant differences in the change in percentage decrease from baseline to the end of the period of observation with the SIGH-D score in which there was a fluvoxamine response to *NR1D1* genotype (rs939347: $p = 0.434$, rs2071427: $p = 0.891$, and rs3744805: $p = 0.450$).

Discussion

We first performed an association analysis of clock genes with fluvoxamine response in MDD patients. However, we did not detect a significant association between *NR1D1* and the fluvoxamine therapeutic response in MDD in allele/genotype-wise analysis or haplotype-wise analysis. In addition, we performed another test for the differences in percentage decrease from baseline to the end of the period of observation. In this test, we used the SIGH-D score among the data for each tagging SNP genotype that was evaluated by ANCOVA after adjustment for sex, age at the time of recruitment, fluvoxamine dose at 8 weeks and SIGH-D total score at the baseline. No association was found. Therefore, our results suggest that *NR1D1* does not play a major role in the therapeutic response to fluvoxamine in Japanese MDD patients.

We recently reported that *NR1D1* does not play a major role in the pathophysiology of Japanese MDD patients [3]. We consider that the present study strongly supports our previous study. However, because 1 of the biological actions of lithium treatment has been reported to affect the expression of clock genes mediated by Rev-erbα in vitro [14], the pharmacogenomics of bipolar disorder (lithium response) and gene-gene interactions among clock genes will also need to be investigated in the future.

Table 2. Genotype and allele distributions of *NR1D1* in both groups

	Clinical groups	Minor allele frequency	n	Genotype distribution			HWE	p value (HWE)	
				M/M	M/m	m/m		genotype	allele
SNP ID (major allele→minor allele)									
rs939347	responders	0.551	59	14	25	20	0.270		
G→A	nonresponders	0.475	59	16	30	13	0.880	0.355	0.241
	remission	0.522	45	12	19	14	0.302		
	nonremission	0.507	73	18	36	19	0.908	0.740	0.818
rs2071427	responders	0.542	59	11	32	16	0.477		
A→G	nonresponders	0.483	59	15	31	13	0.689	0.625	0.362
	remission	0.500	45	12	21	12	0.655		
	nonremission	0.521	73	14	42	17	0.192	0.483	0.759
rs3744805	responders	0.424	59	20	28	11	0.828		
A→G	nonresponders	0.483	59	15	31	13	0.689	0.596	0.360
	remission	0.478	45	14	19	12	0.302		
	nonresponders	0.438	73	21	40	12	0.335	0.307	0.555

M = Major allele; m = minor allele; HWE = Hardy-Weinberg equilibrium.

It will also be important to investigate the association between other clock genes and SSRIs response in MDD using larger samples.

A few points of caution should be noted in interpreting our results. Firstly, our sample sizes were small. We obtained power of more than 80% for the detection of association when we set the genotype relative risk at 1.55–1.85 in all 118 samples, under a multiplicative model of inheritance [22]. Therefore, a replication study using a larger sample may be required for conclusive results. Secondly, we did not include a mutation scan to detect rare variants with functional effects. However, it is difficult to evaluate the association of such extremely rare variants (e.g. minor allele frequencies of less than 0.01) from the viewpoint of power. Furthermore, the analysis of copy number variation, acetylation and methylation rates in *NR1D1* were not analyzed in our study.

In conclusion, our results suggest that *NR1D1* does not play a major role in the therapeutic response to fluvoxamine in Japanese MDD patients. However, because our sample was small, a replication study using another population and larger sample will be required for conclusive results.

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Regular Article

Risk factors for obstructive sleep apnea syndrome screening in mood disorder patients

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Aims: Previous studies have reported that the incidence of obstructive sleep apnea syndrome (OSAS) in patients with depression is higher than in the general population. We examined the risk factors to predict OSAS in mood disorder patients with depressive symptoms.

Method: We conducted polysomnography for patients who satisfied the following criteria: (i) diagnosis of major depressive disorder or bipolar disorder according to the Mini-International Neuropsychiatric Interview (MINI); (ii) a score of ≥10 on the Hamilton Rating Scale for Depression (HAM-D); (iii) fulfillment of either (a) or (b) below: (a) at least one of the following: severe snoring, witnessed apnea during sleep, excessive daytime sleepiness; (b) at least one of the following plus an oxygen desaturation index of 4% ≥5 times/h by pulse oximeter: mild snoring, sleep disturbance, headache, high blood pressure. The patients with apnea hypopnea index ≥5 were diagnosed with OSAS.

Results: Of the 32 mood disorder patients who met the subject conditions, 59.4% had OSAS. The diagnosis rate with our criteria was significantly higher than the previously reported incidence of OSAS in patients with depression. There was no significant difference among diagnosis rates as to individual risk factors or the number of risk factors. A multiple regression test showed no significant association between apnea–hypopnea index and other clinical factors including depression severity.

Conclusion: The present results showed that OSAS can be detected at a remarkably higher rate by considering appropriate OSAS risk factors in mood disorder patients, and suggested that there is a high rate of undetected and therefore untreated OSAS among mood disorder patients.

Key words: depression, mood disorders, obstructive sleep apnea syndrome, risk factors, screening.

OBSTRUCTIVE SLEEP APNEA SYNDROME (OSAS) has attracted attention as a cause of various physical disorders, as well as traffic or industrial accidents and occupational difficulties due to excessive daytime sleepiness and cognitive dysfunction. Depression and OSAS have many common symptoms, including sleep disturbance, general fatigue, decreased volition and judgment ability, and other various physical manifestations. Earlier studies have reported that the incidence of OSAS complications in cases of depression is about 11–18%, higher than in the general population.[1,2] From this, one may predict that there is much untreated OSAS among depression patients, and it would be reasonable to assume that some of these cases are treated as

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treatment-resistant depression.[3] There are also a number of reports of OSAS treatment that improved comorbid depressive symptoms.[4,5] By identifying and treating OSAS in depression patients, therefore, it may be possible to alleviate not only the various symptoms that accompany OSAS and the deterioration in quality of life, but also the depressive symptoms themselves.

Screening with the use of appropriate risk factors is needed for the easy detection of OSAS in the daily clinical setting. Reported risk factors for OSAS include sex, age, obesity, snoring and/or witnessed apnea during sleep, pharyngeal abnormalities, and cephalometric features.[6–10] Some reports presented the usage of risk factors for OSAS prediction;[6,8,11,12] however, there is lack of consensus, because the subjects of these previous studies were the general population. Additionally there has been no reported study on risk factors or screening for OSAS in mood disorder patients, as far as we know.

With the aim of identifying and treating OSAS in mood disorder patients presenting with depression, we conducted OSAS screening with known risk factors in these patients. And we examined whether the screening model in this study was useful and valid in the clinical setting of psychiatry, and which or how many risk factors would contribute most to OSAS screening.

METHODS

Subjects

Among patients who were treated for mood disorder in the departments of psychiatry at Fujita Health University Hospital and Okehazama Hospital, those who had no previous diagnosis of OSAS and who met the following criteria were selected as candidates for inclusion with high risk for latent OSAS, between April 2007 and March 2008.

Selection criteria

1 Diagnosis of major depressive disorder or bipolar disorder (in a major depressive episode) through a structured Mini-International Neuropsychiatric Interview (MINI) based on the Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV); excluding untreated patients or patients in the acute phase whose drug regimen is being adjusted.
2 A score of ≥10 on the Hamilton Rating Scale for Depression (HAM-D).
3 Fulfillment of either (a) or (b) below.
   (a) At least one of the following: severe snoring, witnessed apnea during sleep, or excessive daytime sleepiness.
   (b) At least one of the following: mild snoring, sleep disturbance (arousal during sleep, unrefreshing sleep or mild daytime sleepiness), headache, or high blood pressure. In addition, oxygen desaturation index of 4% (ODI 4%) of ≥5 times per h, measured by a pulse oximeter.

Here, excessive daytime sleepiness is defined as drowsiness that interferes with social activities even though the patient feels that he or she received sufficient sleep. Pulse oximetry was carried out using Pulsox 6 (Minolta, Tokyo, Japan), a continuous, noninvasive arterial oxygen saturation monitor that was attached to one of the patient's fingers. The number of times that arterial oxygen saturation decreased by ≥4% over one night was divided by the number of hours in bed to get the ODI 4%.

Assessment

Overnight polysomnography (PSG) was conducted according to the following procedure for patients who satisfied the above conditions. Patients with an apnea–hypopnea index (AHI) of ≥5 times per h were diagnosed as having OSAS that should be considered for treatment, according to the International Classification of Sleep Disorders 2nd edition (ICSD2). Hypnotics that have a muscle relaxant effect were discontinued before PSG, with the exception of short-acting ones. Also, benzodiazepines were discontinued in principle prior to examination, however, if the patients could not discontinue benzodiazepines because of symptoms such as anxiety, only the short-acting varieties were allowed to be continued. Other psychotropic drugs, including antidepressants and mood stabilizers, were continued.

Besides the HAM-D, we also conducted the Beck Depression Inventory (BDI), Epworth Sleepiness Scale (ESS) and Pittsburgh Sleep Quality Index (PSQI) for included subjects when they underwent PSG.

The study was approved by the ethics committees of Fujita Health University and Okehazama Hospital, and conducted after written explanations were given to all patients and their consent was obtained.

PSG

PSG was carried out following the procedure below, according to the clinical electroencephalography standards of the Japanese Society of Clinical Neurophysiology. Electroencephalogram (EEG), eye movement, chin electromyogram (EMG), expiration thermistor measurements, chest and abdominal expansion, electrocardiogram (ECG), arterial oxygen saturation, snoring, and leg EMG were recorded. Recordings from EEG electrodes were made at C3, C4, O1, and O2 according to the ten-twenty electrode system. A1 and A2 were placed at the bilateral mastoid processes as reference electrodes and connected to the contralateral cranial electrodes. For EMG, electrodes were attached 1 cm lateral and 1 cm superior to the lateral orbit margin of the left eye, and 1 cm lateral and 1 cm inferior to the lateral orbit margin of the right eye, with connections from two leads of these electrodes with the mastoid process A1 (single lead). For chin EMG, two electrodes were attached to the submental muscle, and for leg EMG two electrodes were attached to the tibialis anterior muscle and bipolar leads were used. ECG employed a CM5 lead. Measurements were made from 21.00 hours to 06.00 hours the following morning in an air-conditioned, sound insulated and dark room. The obtained signals were stored as digital data into a personal computer using one of two PSG systems, Alice 3 (Respironics, Murrysville, PA, USA) or Somnostar (Viasys, Conshohocken, PA, USA). The sleep stages and parameters were based on the criteria of Rechtschaffen and Kales, revised manually by trained technicians following automatic scoring by either PSG system. Apnea events were based on complete cessation or ≥50% reduction of air flow of 10 sec or longer, and hypopnea events were the air flow reductions accompanied by SaO_2 reduction of ≥3% or an arousal response when the reductions were <50%. The apnea or hypopnea counts were also revised manually by trained technicians after automatic scoring, and the mean number of both events per h divided by the total sleep time was taken as the AHI.

Statistical analysis

The χ^2-test was conducted to verify the increase in the OSAS diagnosis rate for all cases included in this study based on the previously reported OSAS complication rate in depression. The difference in OSAS diagnosis rate in patients with each risk factor, and the difference in OSAS diagnosis rate between patients with one risk factor and those with two or more risk factors, was compared using the χ^2-test or Fisher's exact test. To test the relation between the other clinical factors and the severity of OSAS in the subject group, multiple regression analysis was conducted with AHI as the dependent variable and the clinical items (sex, age, psychiatric diagnosis, body mass index (BMI), ESS score, HAM-D score, BDI score, and PSQI score) as independent variables. Statistical calculations were conducted with JMP 5.0.1J computer software package (SAS Institute Japan, Tokyo, Japan). The significant level in the tests was set at $P < 0.05$.

RESULTS

Thirty-two subjects (24 men and eight women) met the screening criteria and were included in the study. The mean age of these patients was 48.4 ± 11.8 years. The psychiatric diagnosis was major depressive disorder in 19 and bipolar affective disorder in 13. Their mean HAM-D score was 15.69 ± 3.94 for 17 items. The risk factors matched for screening were witnessed apnea during sleep in six subjects, severe snoring in 21, excessive daytime sleepiness in 15, and mild symptoms plus ODI 4% of five or more times per h by pulse oximeter in four (multiple factors applied to some patients).

On PSG, 19 patients had OSAS of AHI ≥ 5 (AHI ≥ 10 in 15 patients, AHI ≥ 20 in 10 patients). In the group with AHI ≥ 5 (OSAS group), the risk factors matched for inclusion were witnessed apnea during sleep in five patients, severe snoring in 13, excessive daytime sleepiness in seven, and mild symptoms plus ODI 4% of ≥5 times/h by pulse oximeter in four (multiple factors applied to some patients). The OSAS group had 17 men and two women (Table 1). Setting the rate of complication with OSAS in mood disorder patients at 20%, which was higher than that from previous studies, the increase in the OSAS diagnosis rate was still statistically significant even when using the criterion of AHI ≥ 10 from the present results ($P = 0.00005$).

The OSAS diagnosis rate for each risk factor in the patients included in the present study was as follows: OSAS was diagnosed in 83.3% (5/6) of patients with witnessed apnea during sleep, 61.9% (13/21) with severe snoring, and 46.7% (7/15) with excessive daytime sleepiness. Although no BMI inclusion criterion was considered in this screening, OSAS was diag-

Table 1. Demographic data and risk factors of the subjects

			OSAS				
		All patients	AHI ≥ 5	AHI ≥ 10	AHI ≥ 20	Non-OSAS AHI < 5	*P*-value
n		32	19	15	10	13	
Sex (M/F)		24/8	17/2	13/2	8/2	7/6	0.753
Age		48.4 ± 11.8	51.3 ± 12.2	53.1 ± 12.4	53.8 ± 13.4	44.2 ± 10.2	0.186
Diagnosis (MDD/BD)		19/13	10/9	8/7	5/5	9/4	0.503
BMI (kg/m^2)		27.2 ± 5.2	26.5 ± 5.3	26.2 ± 5.7	26.9 ± 6.7	28.1 ± 5.2	0.114
ESS (score)		7.6 ± 5.2	6.7 ± 5.6	5.9 ± 5.0	6.4 ± 5.8	8.9 ± 4.4	0.255
HAM-D (score)		15.7 ± 3.9	14.8 ± 3.6	14.4 ± 5.4	15.3 ± 4.3	17.0 ± 4.16	0.978
BDI (score)		22.2 ± 10.9	21.7 ± 11.4	19.4 ± 9.7	18.6 ± 9.8	22.9 ± 10.4	0.114
PSQI (score)		11.8 ± 4.3	11.4 ± 3.8	11.5 ± 3.9	11.8 ± 4.3	12.2 ± 5.1	0.079
Risk factors	Apnea (*n*)	6	5	3	2	1	–
	Snore (*n*)	21	13	10	7	8	–
	EDS (n)	15	7	5	4	8	–
	ODI4% ≥ 5 (*n*)	4	4	3	1	0	–
	†BMI > 25 (*n*)	22	13	9	6	9	–

Data are shown by mean ± standard deviation. *P*-value shows the result of multiple regression test.
†BMI > 25 is not included in the screening major items.
AHI, apnea–hypopnea index; BD, bipolar disorder; BDI, Beck Depression Inventory; BMI, body mass index; EDS, excessive daytime sleepiness; ESS, Epworth Sleepiness Scale; HAM-D, Hamilton Rating Scale for Depression; MDD, major depressive disorder; ODI, oxygen desaturation index.; OSAS, obstructive sleep apnea syndrome; PSQI, Pittsburgh Sleep Quality Index.

nosed in 59.1% (13/22) of patients with BMI > 25. All patients with mild symptoms plus ODI 4% of ≥5 times/h by pulse oximeter (4/4) were diagnosed with OSAS. By sex, OSAS was diagnosed in 69.6% (16/23) of men and 28.6% (2/7) of women. After excluding witnessed apnea during sleep and ODI 4% because there were too few of these items for statistical analysis, no significant difference was seen in the percentage of OSAS diagnosis between the three risk factors BMI > 25, severe snoring, and excessive daytime sleepiness ($P = 0.638$). OSAS was diagnosed in 57.9% (11/19) of patients who fulfilled only one of the inclusion criteria of severe snoring, excessive daytime sleepiness, witnessed apnea, and ODI 4%, and in 61.5% (8/13) of patients who fulfilled two or more; no significant difference was seen in these rates of diagnosis ($P = 0.836$). Adding BMI > 25 as a risk factor, which was not one of the inclusion criteria, the rate of diagnosis became 62.5% (5/8) of patients who fulfill only one criteria, and 58.3% (14/24) of patients who fulfill two or more; also no significant difference was seen between these two diagnostic rates ($P = 1.00$; Fisher's exact test).

In the multiple regression analysis, no significant relation was seen between AHI and any of the other clinical characteristics (Table 1).

DISCUSSION

Of the 32 mood disorder patients who met the subject conditions in the present study, 59.4% had OSAS. It has been reported that the prevalence of OSAS complications in depression patients is higher than that in healthy people. Kayukawa and Okada conducted PSG in 119 depression patients, and found that 11.8% had OSAS when the diagnostic criterion was AHI of ≥10.[1] It was also reported, from a cross-sectional study using telephone surveys of 18 980 subjects in England and four other European countries, that complications with sleep-related breathing disorders including OSAS were present in 18% of depression patients diagnosed according to DSM-IV.[2] In the screening conducted in the present study we were able to identify OSAS in 46.9% of subjects even using the criteria of AHI ≥ 10, which is remarkably and significantly greater than even a high estimate of a 20% complication rate with OSAS in mood disorder patients, based on the above previous reports. Therefore, we may consider that the screening criteria with the risk factors we employed in this study was an effective method of detecting OSAS in up to about half of mood disorder cases in a psychiatric clinical setting. Factors other than these risk

factors that may have contributed to raising the diagnosis rate are the inclusion of patients with bipolar disorder, and having ≥10 points on HAM-D as inclusion criteria. It is thought that a high proportion of bipolar disorder patients are overweight, and the possibility cannot be ruled out that the complication rate of OSAS is higher than in major depressive disorder patients. However, in our results there was no significant difference even though the OSAS diagnosis rate for bipolar disorder was slightly higher than that for monopolar major depressive disorder in this study (data not shown). Additionally, in subgroups of both major depressive disorder and bipolar disorder the OSAS diagnosis rate was significantly higher than 20% based on the criterion of AHI ≥ 10 (data not shown). Therefore, it is difficult to conclude that including bipolar disorder patients by itself led to a higher rate of OSAS diagnosis. In addition, one may consider that there would be more complications with OSAS in patient groups with high HAM-D scores, because of the possibility that the HAM-D score increases with the occurrence of arousals during sleep or other sleep disturbance in OSAS patients, or the possibility that depression itself is exacerbated by OSAS. However, in our results no significant correlation was seen between the HAM-D score and the AHI or OSAS diagnosis (described below), and so it seems unlikely that having a score of ≥10 on the HAM-D would greatly increase the diagnosis rate of OSAS. We have no direct verification of this, however, as we do not have data for the HAM-D of <10.

From the above, one may conclude that establishing the risk factors used in the present screening was effective in significantly raising the diagnosis rate. For the past several years we have had a special sleep clinic in the psychiatry department, and patients strongly suspected of OSAS have already undergone PSG and been treated before this study began. We conducted this screening excluding the patients already diagnosed with OSAS. Screening with the present risk factors revealed latent OSAS for which treatment may be required in about half of patients, which suggests that it may be worthwhile to conduct similar screening for OSAS in other psychiatric clinical settings as well.

Previous studies have indicated that risk factors for OSAS include sex, age, snoring, witnessed apnea during sleep, obesity, pharyngeal abnormalities, and cephalometric features.[6–10] We selected risk factors from among these with the purpose of thorough and effective screening in daily clinical practice in the psychiatric department. Excessive daytime sleepiness is a main symptom of OSAS, and it has been shown to be closely related to OSAS.[13] Because it is used conventionally in daily clinical practice as a subjective symptom that does not require observation by others, we adopted it as the major item in the present screening. The indicators of severe snoring and witnessed apnea during sleep have been given as risk factors for OSAS in previous reports, which claim that they are the main symptoms of OSAS.[8,10] Therefore, they were also adopted as the major items for screening. However, checking these items depends greatly on a patient's individual sleeping environment, particularly whether or not the patient has a bed partner, and so it would be very difficult to conduct screening with these items alone. Obesity, meanwhile, has been reported to have little usefulness as a risk factor for OSAS in the Asian population including the Japanese,[14,15] and it was not included in the major screening items in this study. It has been noted that cephalometric features can replace obesity as a risk factor in the Japanese population,[7,9] but cephalograms were not conducted as they are inconvenient for screening in the psychiatry clinic. Sex and age have been given as risk factors for OSAS,[6,8,10,12] but OSAS cannot be suspected because of these factors alone and so they were not included as the major items in the present screening.

When these items were included in the screening, the OSAS diagnosis rate, in the order from the highest, was 83.3% (5/6) with witnessed apnea during sleep, 61.9% (13/21) with severe snoring, and 46.7% (7/15) with excessive daytime sleepiness. Although BMI > 25 was not included in the inclusion criteria, the diagnosis rate with it was 59.1% (13/22). No significant differences were seen between these diagnosis rates. Therefore, no evidence was discovered for arguing that the weight of the items should be changed for a more effective screening. However, there were only a small number of patients with witnessed apnea during sleep, so they were excluded from the statistical testing. It is possible that with more cases the diagnosis rate would be higher than with the other risk factors. Recent studies reported less predictive value of excessive daytime sleepiness for OSAS,[8,16] and nonspecific daytime sleepiness from such as psychotropic drugs or the underlying disease itself may be stronger in mood disorder patients than in the general population. Although the diagnosis rate with excessive daytime sleepiness was lower than with other present risk factors, the difference was not

significant: even so, the possibility cannot be ruled out that with a greater number of cases it would be significantly lower.

ODI measured with a pulse oximeter has been reported to correlate with AHI to a certain extent, and there have also been several reports on methods to predict OSAS using pulse oximetry. However, there is a lack of agreement in the findings on the utility of this method. For example, one study supported the usefulness of pulse oximetry showing that the extent of the decline in SpO2 and ODI were sufficiently comparable to PSG;[17] however, other studies showed that the device succeeded to affirm the diagnosis of severe OSAS but overlooked mild or moderate OSAS.[18,19] There is also a report on the difficulty of diagnosis by evaluation with pulse oximetry alone.[20] In the present study pulse oximetry was conducted for patients who did not have the above major risk factors but had at least one of the conditions of mild snoring or mild sleep disturbance (arousal during sleep, unrefreshing sleep or mild daytime sleepiness), headache, high blood pressure; so it was not performed in all cases. All of the present patients who exhibited ODI 4% $\geq$5 times per h were diagnosed with OSAS, but as it was only seen in four included cases its efficacy cannot be compared with other risk factors. While it is possible that screening with pulse oximetry would detect a greater number of OSAS cases, regardless of the presence or absence of other risk factors and symptoms for OSAS, it would be virtually impossible to conduct pulse oximetry in almost all mood disorder patients in an actual clinical psychiatric practice. Conversely, it may be that the diagnostic efficiency could be raised by conducting pulse oximetry and excluding patients with an ODI below a certain level among candidates who already have other major risk factors. However, considering an increase in false negatives among patients with comparatively mild OSAS in the light of the device's measurement principles and the above reported results, it does not suit the purpose of screening, which is to reduce the number overlooked. Therefore, the present screening method, in which pulse oximetry was used in cases with weak symptoms of suspected OSAS other than the major risk factors, is thought to have a certain validity.

Whether the presence of multiple risk factors is more efficient for OSAS prediction than a single risk factor is of interest. Some studies have presented a predictive model for OSAS with combined risk factors;[6,8,11,12] however, they still lack consensus and the models are still inconvenient for daily clinical practice. Unexpectedly, our present results showed no significant difference in the diagnosis rate between cases with only one of the three major risk factors for inclusion and cases with more than one. Therefore, the presence of only one of these risk factors in screening is thought to have a uniform validity in strongly suspecting OSAS. Nor was there a significant difference between cases with one or multiple risk factors when the factor of BMI was included as a risk factor among the present cases, however no patients were included in the present study because of high BMI only, and so we cannot comment on the value of BMI alone as a major risk factor.

In a multiple regression analysis with the other clinical items as the independent variables and AHI as the dependent variable, no items showed a significant relation with AHI. This finding argues against the possibility that other specific factors contributed strongly to a diagnosis of OSAS in the cases included in the present study. It is worth noting that no correlation was seen between AHI and depressive severity according to BDI or HAM-D in the present subjects. Opinions are divided on the role of OSAS in depression,[21–26] but the present results did not strongly support such a role.

One limitation in the present study was the small number of subjects. As mentioned above, the possibility cannot be ruled out that with a greater number of subjects some significant differences would be revealed among risk factors in their level of contribution to diagnosis. In this study we evaluated OSAS only in a group with risk factors; therefore, we cannot evaluate the sensitivity and specificity of this screening model. Pulse oximetry was conducted in only a small part of the patients, as noted above, and cephalograms were not obtained, so we could not evaluate these potential risk factors: However, to verify a screening model for 'real world' psychiatric clinical settings, it would seem to be meaningful that the evaluation was made using a rather practical form.

In summary, the present results showed that OSAS can be detected at a higher rate by considering appropriate OSAS risk factors in mood disorder patients, and suggested that there is a high rate of undetected and therefore untreated OSAS among mood disorder patients. Therapists involved in treating mood disorders should not overlook OSAS and at the same time need to be cautious in using drugs with a muscle relaxant effect such as benzodiazepines. They also need to pay attention to obesity, which can exacer-

bate OSAS. In the future we will need to conduct investigations with greater numbers of patients and verify whether or not improvements in depressive symptoms can be expected with treatment of detected OSAS.

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ORIGINAL PAPER

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

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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

Taro Kishi and Tsuyoshi Kitajima participated equally in this work.

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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,

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.

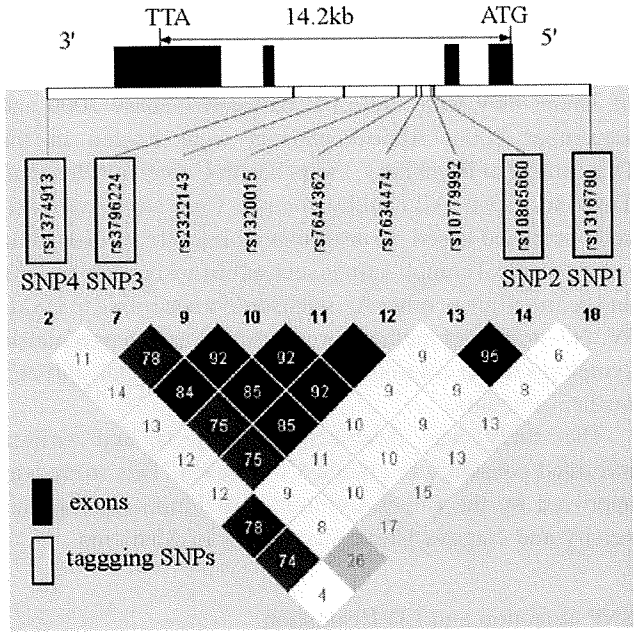

Fig. 1 LD evaluation and tagging SNPs in *PROK2* ATG is the start codon and TAA is the stop codon. *Vertical bars* represent exons. Tagging SNPs selected from the HapMap database are represented by *black boxes*. Color scheme is based on r^2 value. Other information can be seen at the Haploview website

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.

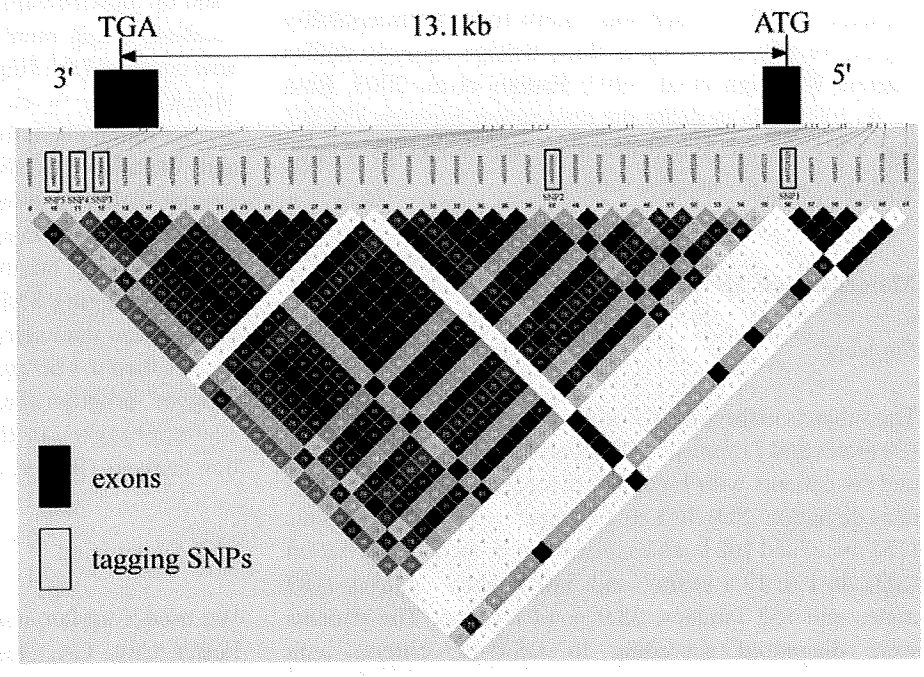

Fig. 2 LD evaluation and tagging SNPs in *PROKR2* ATG is the start codon and TGA is the stop codon. *Vertical bars* represent exons. Tagging SNPs selected from the HapMap database are represented by *black boxes*. Color scheme is based on r^2 value. Other information can be seen at the Haploview website

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