Synonymous SNPs, rs2254357 (exon 6), and rs2254358 (exon 6) that were associated with TD might affect mRNA decay rates. Unfortunately, the mechanism of the association between these SNPs and HSPG2 expression levels could not be elucidated in this study.

From findings in human postmortem brain samples, we speculated that increased expression of *HSPG2* is a risk factor for TD and interpreted that decreased expression of *Hspg2* in mouse brains after chronic administration of HDL was a compensatory or adaptive response to neuroleptic drugs. We, therefore, hypothesized that decreased expression level of *HSPG2* is protective for TD. We examined our hypothesis using hetero-knockout mice and confirmed it after finding lower numbers of VCMs in hetero-knockout mice than in the wild-type littermates after chronic administration of HDL and reserpine. We carried out the experiment using only female mice; therefore, we do not have the data on the sex difference.

The mechanism behind our hypothesis that increased expression levels of *HSPG2* may induce a susceptibility to neuroleptic-induced TD is not known at present. A potential efficacy of cholinergic drugs in the treatment of TD has been reported (Caroff *et al*, 2001; Tammenmaa *et al*, 2004). AChE terminates neurotransmission at cholinergic synapses by hydrolyzing acetylcholine. At the neuromuscular junction, AChE is in the basal lamina, where AChE tetramers bind the collagen ColQ, which interacts in turn with the dystroglycan complex through perlecan (Peng *et al*, 1999). Perlecan is an essential component of the ColQ–AChE localization in neuromuscular junction (Rotundo *et al*, 2005). At central synapses, AChE tetramers bind directly to the PRiMA (Perrier *et al*, 2002). Although ColQ also anchors AChE in brain and heart in addition to skeletal muscle (Feng *et al*, 1999), the role of perlecan in acetylcholine receptor signaling in central synapses is unclear. In this study, we tested the effect of the AChE inhibitor, physostigmine, on HDL- and reserpon-induced VCMs in mice. We found significant reduction in the number of VCMs only in wild-type mice and the number of VCMs was not reduced in hetero-knockout mice. These findings indicate that perlecan may be involved in the role of AChE in TD and the genotyping and/or levels of *HSPG2* may provide useful information about the effectiveness of treatment of TD with AChE.

The other important molecule to which perlecan and TD may be related is FGF2. Perlecan promotes FGF2–FGFR1 binding (Whitelock *et al*, 1996) and HSPGs including perlecan were upregulated by responding to injury and may have a role in intracellular trafficking of FGF2 in neurons and glia in the adult rat cerebral cortex (Leadbeater *et al*, 2006). Clozapine increases FGF2 expression and, on the basis of the neuroprotective activity of FGF2, a potential use of clozapine in TD was proposed (Riva *et al*, 1999).

Perlecan is expressed at the capillary endothelial cells in the brain and perlecan at the blood–brain barrier (BBB) may have a role in maintaining the blood–brain barrier function because of acceptance of the FGF2 secreted from astrocytes (Deguchi *et al*, 2002). It is reported that neuroleptics, such as HDL and chlorpromazine, alter the blood–brain barrier function and increase brain iron levels, which affect neuroleptic-induced dopamine receptor supersensitivity (Ben-Shachar *et al*, 1993).

Although the exact mechanisms of the association between HSPG2 and TD are unclear, this study identified the role of *HSPG2* in neuroleptic-induced TD.

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DISCLOSURE

The authors declare that no financial support or compensation has been received from any individual or corporate entity over the past 3 years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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ORIGINAL INVESTIGATION

Effect of aripiprazole, risperidone, and olanzapine on the acoustic startle response in Japanese chronic schizophrenia

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Abstract
Background Studies have also shown that differences in the kind of the antipsychotics influenced disruption of the sensorimotor gating system, including prepulse inhibition (PPI), acoustic startle reflex (ASR), and habituation (HAB). We investigated the influence on startle response in chronic schizophrenia in 20 patients with schizophrenia taking risperidone, 21 patients with schizophrenia taking olanzapine, and 20 patients with schizophrenia taking aripiprazole.
Method The patients who participated in this study were on maintenance therapy with only one antipsychotic drug for 4 months. We performed the test for the association between all PPI measures (ASR, HAB, and PPI at prepulse sound pressure intensities of 82, 86, and 90 dB) and each the risperidene, olanzapine, and aripiprazole groups, with analysis of covariance (ANCOVA; using age, duration of illness, and daily dose of the antipsychotic as covariates). Also, when significant difference was detected in ANCOVA, the differences of PPI measures between every pairs of two drug groups were tested as a post hoc analysis with the use of *t* test and Bonferroni's correction of multiple tests.
Result We found that PPI90 showed significant differences with ANCOVA among patients with schizophrenia taking each of the antipsychotics. When we performed a post hoc analysis for PPI90, the value was higher in the aripiprazole group than in the olanzapine group and higher in the risperidone group than in the olanzapine group.
Conclusion Aripiprazole and risperidone may improve PPI90. ASR, HAB, PPI82, and PPI86 were no different among the Japanese schizophrenic patient groups with different antipsychotics.

Keywords Acoustic startle response · Risperidone · Aripiprazole · Olanzapine · Schizophrenia · Prepulse inhibition · Antipsychotic

Abbreviations

PPI	Prepulse inhibition
ASR	Acoustic startle reflex
HAB	Habituation
OLZ	Olazapine
RIS	Risperidone
HPD	Haloperidol
ARP	Aripiprazole
SD	Standard deviation
SCID-1	Structured Clinical Interview for DSM-IV disorders
LI	Lead interval
PA trial	Pulse alone trials
PP trials	Trials of pulse with prepulse
ANOVA	Analysis of variance

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ANCOVA	Analysis of covariance
5-HT1A receptor	Serotonin 1A receptor
5-HT1B receptor	Serotonin 1B receptor
α2c receptor	Adrenergic alpha2c receptor
MDMA	Methylenedioxy-substituted phenalkylamines 3,4-methylenedioxy-*N*-methylamphetamine
M5 receptors	Muscarinic acetylcholine 5 receptors

Introduction

Disruption of the sensorimotor gating system, including prepulse inhibition (PPI) deficit, the acoustic startle reflex (ASR), and habituation (HAB), is suggested to be one of involved in the pathophysiology of schizophrenia (Braff et al. 1992; Kunugi et al. 2007; Takahashi et al. 2008; Walters and Owen 2007). These abnormalities are also considered to be endophenotypes for schizophrenia (Braff et al. 1992; Kunugi et al. 2007; Takahashi et al. 2008; Walters and Owen 2007). Recently, we detected significant differences in ASR, HAB, and each PPI (82, 86, and 90 dB) between patients with schizophrenia and controls (Moriwaki et al. 2009). Several investigations have reported that the ASR and PPI are influenced by factors such as gender and smoking state (Kumari et al. 1996, 1997, 2004; Swerdlow et al. 1999). However, we found no correlation between gender or current smoking state and ASR, HAB, or any PPI in a multiple regression analysis (Moriwaki et al. 2009).

It has also been reported that several measures of the startle response were influenced by the kind of antipsychotic. Wynn and colleagues also reported that olazapine (OLZ) improved the startle acoustic response significantly compared with risperidone (RIS) and haloperidol (HPD) in people with schizophrenia (Wynn et al. 2007). The antipsychotic aripiprazole (ARP) is known to be a dopamine system stabilizer, the pharmacological mechanism of which is a unique partial agonistic action on dopamine 2 receptors (Burris et al. 2002; Jordan et al. 2002). To our knowledge, there is no investigation on the influence ARP in the startle response in people with schizophrenia. Therefore, we investigated whether some antipsychotics, including ARP, RIS, and OLZ, influenced the startle response in chronic schizophrenia.

Materials and methods

Subjects

One hundred forty-one patients with schizophrenia (91 males and 50 females: mean age standard deviation (SD) 49.8±15.6 years) were recruited. This study increased 26 patients compared with our previous study (Moriwaki et al. 2009). However, because 17 patients were nonresponders, these patients excluded the further analysis. Detailed information about exclusion criteria can be seen in the paper of Takahashi et al. (2008). Among 124 patients, 71 patients were taken monotherapy antipsychotics. Also, 53 patients were taken polytherapy antipsychotics. 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 met the following inclusion criteria: (1) age 25 to 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) use of only one antipsychotic drug therapy for 4 months. All patients were hospitalized at the time of measurement. 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.

Startle response measurement

Apparatus and stimuli

We measured startle response 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 two 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 of 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 in the study of Takahashi and colleagues (Takahashi et al. 2008). Detailed information can be seen in their paper.

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

Measurements were made with startle paradigm constructed of three blocks with continuous background white noise of 70 dB SPL. 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, presented at three different intensities (82, 86, and 90 dB SPL). The 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 six 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 habituation. 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 the same method as Takahashi and colleagues (2008). Detailed information can be seen in their paper.

Statistical analysis

The numbers of patients, who took monotherapy antipsychotics, were 20 in the RIS group, 21 in the OLZ group, 20 in the ARP group, four in the quetiapine group, one in the perospirone group, two in the blonanserin group, two in the HPD group, and one in the timiperone group. Because our samples of quetiapine, perospirone, blonanserin, HPD, and timiperone group were small, we included only the RIS, OLZ, and ARP groups in this study. All demographic data were analyzed by one-way analysis of variance (ANOVA). As described in the "Results" section, the mean age of ARP group was youngest among three drug groups; meanwhile, the duration of illness was shortest, and the daily dose of the antipsychotic was the largest in ARP group. So we performed analyses of covariance (ANCOVA) for comparing the PPI measures among the three drug groups, using the above three parameters as covariates to adjust possible confounding. When significant difference was detected in ANCOVA, the differences of PPI measures between every pairs of two drug groups were tested as a post hoc analysis with the use of *t* test and Bonferroni's correction of multiple tests. The significance level for all statistical tests was 0.05.

All statistical analyses were performed using SPSS (SPSS 12.0, SPSS Japan Inc., Tokyo, Japan).

Results

The mean age of ARP group was youngest among three drug groups; meanwhile, the duration of illness was shortest, and the daily dose of the antipsychotic was the largest in ARP group. Although one-way ANOVA did not show significant difference as to these three parameters among the three drug groups (Table 1), we conducted ANCOVA for comparing the PPI measures among three drug groups, using the above three parameters as covariates to adjust possible confounding. We found that PPI90 were significantly different among the ARP, RIS, and OLZ groups with the use of ANCOVA (P=0.0320; Table 2). We then performed a post hoc analysis of PPI90 and found that the ARP group had higher PPI90 than the OLZ group (P= 0.0178; Fig. 1). Also, PPI90 was higher in the RIS group than in the OLZ groups (P=0.0368; Fig. 1).

Discussion

We first investigated whether ARP influenced the startle response. Also, because the participating patients took only one antipsychotic, we considered the effect of each antipsychotic on acoustic startle response in people with

Table 1 Schizophrenic patients' demographics and disposition

	Aripiprazole	Risperidone	Olanzapine	*P* value
N	20	20	21	
Sex (males/females)	14/6	10/10	13/8	0.231
Age, years (mean±SD)	46.7±17.1	56.9±16.8	51.0±13.9	0.140
Current smoker/non-smoker, n (%)	8 (40.0%)	7 (47.6%)	10 (35.0%)	0.709
Clinical diagnosis, n (Dis/Res/Par)	2/14/4	1/15/4	0/17/4	0.551
Duration of illness (day, mean ± SD)	8,070±547	10,500±435	9,790±391	0.235
PANSS total score	84.7±20.6	76.3±19.5	76.3±19.5	0.220
Antipsychotics (mg/day)[a]	626±119	505±188	599±245	0.156
Anxiolytics/hypnoticse, *n* (%) (mg/day)[b]	10.3±12.2	12.1±10.5	7.56±6.46	0.337

Dis disorganized type, *Res* residual type, *Par* paranoid type

[a] Chlorpromazine-equivalent

[b] Diazepam-equivalent

Table 2 ANCOVA of startle measure with three antipsychotics groups

Startle measure	Aripiprazole	Risperidone	Olanzapine	*P* value[a]
ASR (μV, mean ± SD)	162±160	74.9±72.3	70.3±30.1	0.108
HAB (%, mean ± SD)	23.3±27.1	24.0±28.3	29.6±25.6	0.103
PPI82 (%, mean ± SD)	32.0±25.7	18.2±16.9	22.6±17.5	0.171
PPI86 (%, mean ± SD)	41.6±25.7	29.7±24.0	23.7±16.0	0.0685
PPI90 (%, mean ± SD)	39.9±31.1	34.9±22.9	21.1±15.9	**0.0320**

Bold represents significant *P* value

ASR acoustic startle reflex, *HAB* habituation, *PPI* prepulse inhibition

[a] Calculated by ANCOVA. *P* values were obtained by ANCOVA using age, duration of illness, and daily antipsychotic dose

schizophrenia directly. In this study, the ARP group had higher scores than the OLZ group in PPI90. Also, the RIS group showed significant difference than the OLZ group in PPI90. We found that PPI86 showed marginal difference among RIS, OLZ, and ARP groups with the use of ANCOVA (P=0.0685). When we performed a post hoc analysis for PPI86, the value was higher in the ARP group than in the OLZ groups (P=0.0634). From this result, we considered that ARP and RIS may improve abnormalities in PPI. We found large differences in the pharmacological profiles of ARP, RIS, and OLZ as follows: first, ARP has stronger affinity with the serotonin 1A (5-HT1A) receptor than RIS and OLZ (Burris et al. 2002; Jordan et al. 2002; Roth et al. 2004). Gogos and colleagues reported that the activation of 5-HT1A receptors increased PPI (Gogos et al. 2008). Also, RIS has stronger affinity with the serotonin 1B (5-HT1B) receptor, serotonin 7 receptor, adrenergic alpha2c (α2c) receptor, adrenergic alpha1a receptor, and adrenergic alpha1b receptors than ARP and OLZ (Roth et al. 2004). Dulawa and colleagues reported that a5-HT1B receptors have relation with ASR, HAB, and PPI in mice (Dulawa et al. 1997, 1998). RU24969, which is 5-HT1A/1B agonist, reduced PPI in WT mice (Shanahan et al. 2009). Dulawa and colleagues reported that the methylenedioxy-substituted phenalkylamines 3,4-methylenedioxy-*N*-methylamphetamine (MDMA) increase PPI in 5-HT1B knockout mice, but not WT mice (Dulawa et al. 2000). These authors suggested that the activation of 5-HT1B receptors by 5-HT decreases PPI (Dulawa et al. 2000). Also, the adrenergic α2c receptor knockout mice showed increased ASR reduced PPI (Sallinen et al. 1998). On the other hand, OLZ has stronger affinity with serotonin 6 receptor, hisitamine 1 receptor, and muscarinic acetylcholine 5 (M5) receptors than RIS and ARP (Roth et al. 2004). Several animal studies using the M5 receptor knockout mice have shown significantly decreased PPI compared to wild-type (Thomsen et al. 2007); however, results have been rather inconsistent (Wang et al. 2004). Second, the antipsychotic aripiprazole is known to be a dopamine system stabilizer, the pharmacological mechanism of which is a unique partial agonistic action on dopamine 2 receptors. Several animal studies using mice reported that ARP restored the abnormalities in the PPI induced by apomorphine (Auclair et al. 2006; Nakai et al. 2008; Nordquist et al. 2008). These mechanisms may be involved in the different effects seen among the antipsychotic groups. However, this has, in seeming contrast to findings by Wynn and colleagues (2007), who reported that OLZ improved the startle acoustic response significantly, compared with RIS in people with schizophrenia. We consider that a replication study using larger samples or samples of other populations will be required for conclusive results.

Our previous study reported significant differences in ASR, HAB, and each PPI (82, 86, and 90 dB) between 115 patients with schizophrenia and 111 controls in multiple regression analysis (there were 15 nonresponders in the patient group and four in the control group; therefore, we performed the analysis of startle measure with 100 patients and 107 controls) (Moriwaki et al. 2009). When all PPI measures (except HAB) in the ARP group were compared with our 107 healthy control subjects with the use of ANCOVA (using age and sex as covariates), there was no statistical difference between the ARP group and controls

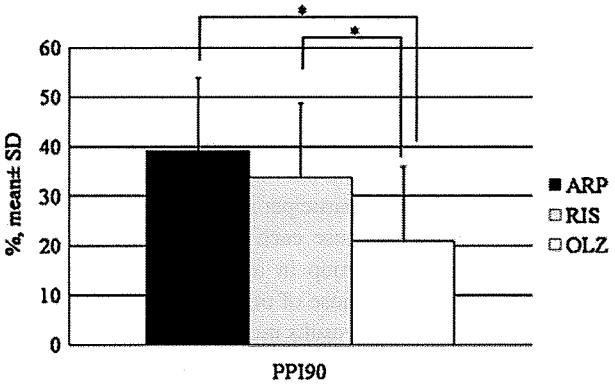

Fig. 1 Bonferroni's *post hoc* analysis of PPI90 in three antipsychotic groups *P<0.05, (μV, mean ± SD). ARP group=39.9±31.1, RIS group=33.7±22.9, and OLZ group=21.0±17.9

(detailed information about startle response on controls can be seen in our previous paper and Supplementary Table 1; P_{ASR}=0.686, P_{PPI82}=0.903, P_{PPI86}=0.703, and P_{PPI90}= 0.141) (Moriwaki et al. 2009). In addition, when all PPI measures (except HAB) in the RIS group were compared with our 107 healthy control subjects with the use of ANCOVA (using age and sex as covariates), there was no statistical difference between the RIS group and controls (Supplementary Table 1; P_{ASR}=0.485, P_{PPI82}=0.267, P_{PPI86}=0.900, and P_{PPI90}=0.331) (Moriwaki et al. 2009). On the other hand, when HAB in the ARP or RIS group were compared with our healthy control subjects with the use of ANCOVA (using age and sex as covariates), ARP or RIS group data were significantly different than that of control subjects (Supplementary Table 1; P_{ARP}= 0.00000109 and P_{RIS}=0.00840). Also, we performed ANCOVA for comparing the PPI measures among three groups (ARP, RIS, and healthy control groups), using above two parameters (age and sex) as covariates to adjust possible confounding. We found that HAB were significantly different among the ARP, RIS, and healthy control groups with the use of ANCOVA (P=0.00000736). However, we did not detect that other PPI measures were significantly different among the ARP, RIS, and healthy control groups with the use of ANCOVA (P_{ASR}=0.514, P_{PPI82}=0.327, P_{PPI86}=0.705, and P_{PPI90}=0.288). HAB was not statistically different between the antipsychotic groups among Japanese patients with schizophrenia (Table 2). This lack of difference in HAB among the Japanese patients with schizophrenia using different antipsychotics suggested that HAB was a common endophenotype of schizophrenia. Although ASR, PPI82, and PPI86 were not different in each of the antipsychotic groups, there was no statistical difference between the ARP or RIS groups and controls when ASR, PPI82, PPI86, and PPI90 in the ARP or RIS group were compared with our 107 healthy control subjects (Supplementary Table 1). Also, PPI90 was significantly different among the antipsychotic groups. Because it was possible that ARP and RIS led to the improvement of disruption of PPI90 to the level of healthy controls, we considered that ASR, PPI82, PPI86, and PPI90 might not be an endophenotype of schizophrenia.

There are a few limitations to this study. First, there is no control in this study. We did not measure the acoustic startle response when patients participating in this study did not take antipsychotics. Because each of the startle responses measured in the ARP group in a drug-naïve state might have higher scores than those of other antipsychotic groups in a drug-naïve state, our results must be interpreted careful. However, measuring the startle response of people with schizophrenia in the drug-naïve state is very difficult. Second, the positive association may be due to a small sample size. Third, because our samples were small, the statistical errors are possible in the results of these statistical association analyses. To overcome this limitation, a replication study using larger samples or samples of other populations will be required for conclusive results.

In conclusion, ARP and RIS may improve PPI90. ASR, HAB, PPI82, and PPI86 were no different among the Japanese schizophrenic patient groups with different antipsychotics. Since HAB showed no difference between the antipsychotic groups of Japanese patients with schizophrenia, we suggest that HAB may not be influenced by several clinical factors. However, since our samples are small, it will be necessary to conduct a replication study using larger samples.

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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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ORIGINAL PAPER

HTR2A is Associated with SSRI Response in Major Depressive Disorder in a Japanese Cohort

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Abstract Several recent investigations reported that the serotonin 2A receptor gene (*HTR2A*) was associated with selective serotonin reuptake inhibitors (SSRIs) in major depressive disorder. There have also been two reported association analyses of *HTR2A* with SSRI response in Japanese MDD patients, but the results were rather inconsistent and both studies had the problem of small sample sizes. Therefore, we conducted a replication association study using a sample larger than those in the two original Japanese studies (265 MDD patients), and found that four SNPs, two functional SNPs (-A1438G: rs6311 and T102C: rs6313) and two SNPs (rs7997012 and rs1928040) in *HTR2A*, were associated with the therapeutic response to SSRIs. *HTR2A* was associated with the therapeutic response SSRIs in Japanese MDD patients in a haplotype-wise analysis ($P_{\text{all markers}} = 0.0136$), and a significant association between rs1928040 in *HTR2A* and SSRI response was detected in MDD ($P_{\text{allele-wise analysis}} = 0.0252$). However, this significance disappeared after Bonferroni correction ($P_{\text{allele-wise analysis}} = 0.101$). In conclusion, we suggest that *HTR2A* may play an important role in the pathophysiology of the therapeutic response to SSRIs in Japanese MDD patients. However, it will be important to replicate and confirm these findings in other independent studies using large samples.

Keywords Serotonin 2A receptor gene (*HTR2A*) · SNPs · Major depressive disorder · Selective serotonin reuptake inhibitor (SSRI) response

Introduction

Several investigations have suggested that serotonin 2A receptor gene (*HTR2A*) might be a factor in the therapeutic response in major depressive disorder (MDD). The evidence for this relation is discussed in more detail in the reviews (Kato and Serretti 2008; Kato 2007; Serretti and Artioli 2004a, b; Serretti et al. 2007a, b; Serretti and Mandelli 2008). Other recent investigations reported that *HTR2A* was associated with selective serotonin reuptake inhibitors (SSRIs) treatment response in MDD. McMahon et al. (2006) reported an association between rs7997012 and rs1928040 in *HTR2A* and the outcome of citalopram treatment in a very large sample of outpatients with MDD. Peters et al. (2009) replicated those findings in a study showing that rs7997012 was associated with citalopram response in MDD. However, Perlis et al. (2009) reported that rs7997012 and rs1928040 were not associated with duloxetine treatment outcome in MDD. In Japan, there have been two reported association analyses of *HTR2A* with SSRIs response in MDD patients, but the results were rather inconsistent and both studies had the problem of small sample sizes (Kato et al. 2006; Sato et al. 2002).

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A recent meta-analysis reported that -A1438G (rs6311), which is known to be a functional SNP in *HTR2A*, was associated with SSRI response in Asian MDD patients (Kato and Serretti 2008).

In our previous study, we found no association between *HTR2A* and mood disorders, including MDD and bipolar disorder, in the Japanese population (Kishi et al. 2009c). Here, we conducted a replication association study using a sample larger than those in the two Japanese original studies (265 MDD patients), and found that four SNPs, two functional SNPs (-A1438G: rs6311 and T102C: rs6313) and two SNPs (rs7997012 and rs1928040) in *HTR2A*, were associated with the therapeutic response to SSRIs.

Materials and Methods

Subjects

Two hundred and sixty-five MDD patients participated in this study. These patients had been diagnosed according to DSM-IV criteria with the consensus of at least two experienced psychiatrists on the basis of a review of medical records and assessment with the Structured Interview Guide for Hamilton Rating Scale for Depression (SIGH-D) (Williams 1988). None had severe medical complications such as cirrhosis, renal failure, heart failure, or other Axis-I disorders according to DSM-IV.

Participating patients took fluvoxamine two or three times a day and sertraline and paroxetine one or two times a day for 8 weeks. Fluvoxamine, sertraline, and paroxetine were increased gradually to a maximum of 150, 100, and 40 mg, respectively, depending on the patients' condition. Patients with insomnia and severe anxiety were prescribed benzodiazepine drugs, but no other psychotropic drugs were permitted during the study. 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 University of Occupational and Environmental Health.

Data Collection

The scores of the 265 MDD patients in this study on the 17 items of the SIGH-D were 12 or higher (Peveler and Kendrick 2005). We defined a clinical response as a decrease of more than 50% in baseline SIGH-D within 8 weeks, and clinical remission as a SIGH-D score of less than 7 at 8 weeks. Detailed information on data collection was described in a previous article (Saito et al. 2006). The clinical characteristics of the patients in this study, classified according to these definitions, can be seen Table 1.

Table 1 Clinical characteristics of the patients in both definition groups

	N			Patients permitted SSRIs, *n* (%)[c]			Age (mean ± SD)	Baseline SIGH-D (avg ± SD)	Number of previous episodes (avg ± SD)	Patients permitted anxiolytics/hypnotics, *n* (%)
	Total	Male	Female	FLV	STL	PAX				
Overall	265	121	144	129 (48.7)	72 (27.2)	64 (24.2)	48.2 ± 16.3	20.6 ± 5.16	1.77 ± 0.787	116 (43.9)
Clinical response group[a]										
Responders	150	75	75	68 (25.7)	47 (17.7)	35 (13.2)	48.6 ± 15.6	21.3 ± 5.30	1.76 ± 0.750	70 (26.5)
Nonresponders	115	46	69	61 (23.0)	25 (9.43)	29 (10.9)	47.7 ± 17.2	19.7 ± 4.87	1.79 ± 0.842	46 (17.4)
P value	0.105			0.208			0.662	**0.0161**	0.745	0.305
Clinical remission group[b]										
Remitters	103	53	50	53 (20.0)	32 (12.1)	18 (6.79)	48.4 ± 15.9	19.6 ± 4.47	1.67 ± 0.686	42 (15.9)
Nonremitters	162	68	94	76 (28.7)	40 (15.1)	46 (17.4)	48.1 ± 16.6	21.2 ± 5.48	1.84 ± 0.843	74 (28.0)
P value	0.131			0.109			0.880	**0.0136**	0.122	0.407

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

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

[c] *FLV* fluvoxamine, *STL* sertraline, *PAX* paroxetine

SNP Selection and Linkage Disequilibrium (LD) Evaluation

We selected two biologically functional SNPs (T102C: rs6313 and -A1438G: rs6311; Myers et al. 2007; Spurlock et al. 1998). Because we detected r^2 less than 0.800 for all phenotypes (r^2 = healthy controls: 0.719 and MDD: 0.709; Kishi et al. 2009c), we selected two biologically functional SNPs (-A1438G: rs6311 and T102C: rs6313) in this study (Myers et al. 2007; Spurlock et al. 1998). In addition, we also included rs7997012 and rs1928040 in *HTR2A* because McMahon et al. (2006) reported an association between these two SNPs and outcome of citalopram treatment in a very large sample of outpatients with MDD. These four SNPs were used in the following association analysis. Detailed information about SNP selection was described in our previous article.

SNP Genotyping

We used TaqMan assays (Applied Biosystems, Inc., Foster City, CA,) for all SNPs. One allelic probe was labeled with FAM dye and the other with fluorescent VIC dye. The plates were heated 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, including primer sequences, and reaction conditions, is available on request.

Statistical Analysis

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

Marker-trait association analysis was used to evaluate allele- and genotype-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 analysis, we determined that the cutoff for testing haplotype frequency was 0.05. We used the permutation test option as provided in the haplotype analysis to avoid spurious results and correct for multiple testing. Permutation test correction was performed using 1,000 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 individual haplotype-wise analysis. For Bonferroni correction, we employed the following numbers of multiple tests: 4 for each sample set in allele- and genotype analysis (4 examined SNPs); and 3 for each sample set in the individual haplotype-wise analysis (3 common haplotypes).

The significance level for all statistical tests was 0.05. Power calculation was performed using the Genetic Power Calculator (Purcell et al. 2003).

Results

Among the clinical characteristics of patients in this pharmacogenetic study, significant differences between either responders or nonresponders and remitters or nonremitters were detected in total SIGH-D score at the baseline ($P_{\text{response}} = 0.0161$ and $P_{\text{remission}} = 0.0136$; Table 1). Genotype frequencies of all SNPs were in HWE (Table 2). We found *HTR2A* to be associated with SSRI therapeutic response and remission in Japanese MDD patients in an all markers haplotype-wise analysis ($P_{\text{response}} = 0.0136$ and $P_{\text{response}} = 0.0400$) (Tables 3 and 4). When we performed a haplotype-wise analysis using the sliding window fashion method, a three-marker haplotype (rs6311-rs6313-rs1928040) showed the strongest association with the SSRI therapeutic response in MDD (P value = 0.000707; Tables 3 and 5). Also, this three-marker haplotype (rs6311-rs6313-rs1928040) showed the strongest association with remission in MDD (P value = 0.0324) (Tables 4 and 6). We also detected a significant association between rs1928040 in *HTR2A* and SSRI response and remission in MDD in an allele-wise analysis ($P_{\text{response}} = 0.0252$ and $P_{\text{remission}} = 0.0418$), but the significance disappeared after Bonferroni correction ($P_{\text{response}} = 0.101$ and $P_{\text{remission}} = 0.167$) (Table 2).

In addition, regarding genotyping quality control measures, we added 32 randomly selected samples that were genotyped again as a measure of genotyping quality control, and the genotype consistency rates for all four SNPs were 100%.

We obtained power of more than 80% for the detection of association when we set the genotype relative risk at 1.65–1.78 in all 265 samples, under a multiplicative model of inheritance (Purcell et al. 2003).

Discussion

We performed an association study for the SSRI therapeutic response in Japanese MDD patients using a larger sample than in two original Japanese studies. In one of those studies, Kato et al. (2006) reported an association between -A1438G (rs6311) and the SSRI therapeutic response in Japanese MDD, whereas Sato et al. (2002) found no such association. In this study, we found an association between *HTR2A* and the SSRI therapeutic response and remission in MDD in the haplotype-wise analysis.

Table 2 Genotype and allele distributions of *HTR2A* in both definition groups

SNPs[a]	Phenotype	MAF[b]	*N*	Genotype distribution[c]			*P* value[e]			Corrected *P* value[f]	
				M/M	M/m	m/m	HWE[d]	Genotype	Allele	Genotype	Allele
rs6311	Responders	0.410	150	47	83	20	0.0784				
(-1438A/G)	Nonresponders	0.428	115	40	53	22	0.743	0.567	0.670		
Intronl	Remitters	0.389	103	36	54	13	0.293				
	Nonremitters	0.432	162	51	82	29	0.690	0.502	0.319		
rs6313	Responders	0.493	150	35	82	33	0.252				
(102T/C)	Nonresponders	0.487	115	31	56	28	0.875	0.624	0.884		
Exonl	Remitters	0.495	103	24	56	23	0.375				
	Nonremitters	0.488	162	42	82	38	0.869	0.827	0.867		
rs 1928040	Responders	0.323	150	64	75	11	0.0806				
T>C	Nonresponders	0.235	115	66	44	5	0.487	0.0540	**0.0252**		0.101
Intron2	Remitters	0.335	103	42	53	8	0.116				
	Nonremitters	0.253	162	88	66	8	0.323	0.0910	**0.0418**		0.167
rs7997012	Responders	0.177	150	99	49	2	0.132				
G>A	Nonresponders	0.186	115	74	39	2	0.215	0.938	0.761		
Intron2	Remitters	0.189	103	65	37	1	0.0840				
	Nonremitters	0.176	162	108	51	3	0.275	0.664	0.696		

[a] Major allele > minor allele, SNP position

[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 by Bonferroni's correction

Table 3 Haplotype-wise analysis between *HTR2A* and SSRIs response in MDD

	Global *P* value[a]		
	2 window	3 window	4 window
rs6311	0.518		
rs6313	**0.0101**	**0.000707**	**0.0136**
rsl928040	0.0535	0.106	
rs7997012			

[a] Bold numbers represent significant *P* value

Table 4 Haplotype-wise analysis between *HTR2A* and SSRIs remission in MDD

	Global *P* value[a]		
	2 window	3 window	4 window
rs6311	0.736		
rs6313	**0.0451**	**0.0324**	**0.0400**
rsl928040	0.0604	**0.0423**	
rs7997012			

[a] Bold numbers represent significant *P* value

Haplotype analysis to investigate SSRI response and resmissin in MDD indicated three common haplotypes (rs6311- rs6313-rs1928040: A-T-T, G-C-T and G-C–C). The G-C-T haplotype was less prevalent in subjects with an SSRI therapeutic response (corrected $P = 0.00723$), while G-C–C was very prevalent in subjects with an SSRI therapeutic response (corrected $P = 0.00864$). Therefore, we considered that *HTR2A* was associated with SSRI therapeutic response in MDD in the Japanese population. On the other hand, The G-C-T haplotype was less prevalent in subjects with remission on SSRIs (uncorrected $P = 0.0200$). This significance disappeared after Bonferroni correction (corrected $P = 0.0600$). As a result, there are possibilities of type I errors in an association between *HTR2A* and SSRI therapeutic remission in MDD of the haplotype-wise analysis statistically.

In this study, we detected a marginal association between rs1928040 and SSRI therapeutic response in Japanese MDD in the allele-wise analysis (uncorrected $P_{response} = 0.0252$ and uncorrected $P_{remission} = 0.0418$). Therefore, we considered that an association between haplotype in *HTR2A* and SSRI response in this study might

Table 5 Haplotype-wise analysis between rs6311-rs6313-rs1928040 in *HTR2A* and SSRIs response in MDD

rs6311-rs6313-rs1928040	Phenotype	Individual haplotype frequency	OR[a]	95% CI[b]	Individual *P* value[c]	Corrected *P* value[d]
A-T-T	Responders	0.551	1.00	1.00–1.00	0.816	
	Nonresponders	0.539				
G-C-T	Responders	0.267	1.84	1.07–3.15	**0.00241**	**0.00723**
	Nonresponders	0.142				
G-C-C	Responders	0.182	0.558	0.337–0.924	**0.00288**	**0.00864**
	Nonresponders	0.319				

[a] *OR* odds ratio

[b] *95% CI* 95% confidence interval

[c] Bold numbers represent significant *P* value

[d] Calculated by Bonferroni's correction (3 tests)

Table 6 Haplotype-wise analysis between rs6311-rs6313-rs1928040 in *HTR2A* and remission in MDD

rs6311-rs6313-rs1928040	Phenotype	Individual haplotype frequency	OR[a]	95% CI[b]	Individual *P* value[c]	Corrected *P* value[d]
A-T-T	Remitters	0.538	1.00	1.00–1.00	0.741	
	Nonremitters	0.556				
G-C-T	Remitters	0.237	1.76	3.16–5.41	**0.0200**	0.0600
	Nonremitters	0.139				
G-C-C	Remitters	0.225	0.759	0.466–1.24	0.0791	
	Nonremitters	0.306				

[a] *OR* odds ratio

[b] *95% CI* 95% confidence interval

[c] Bold numbers represent significant *P* value

[d] Calculated by Bonferroni's correction (3 tests)

be reflected rs1928040. According to the HapMap database, MAFs of rs7997012 and rs1928040 in Caucasians were different to those in Japanese. Haplotype frequencies and LD between rs6313, rs6311, rs1928040 and rs7997012 in Caucasians were significantly different than in Japanese.

Because we detected r^2 less than 0.800 for all phenotypes (r^2 = Control 0.719 and MDD 0.709) (Kishi et al. 2009c), we selected two biologically functional SNPs (T102C: rs6313 and -A1438G: rs6311) in this study (Myers et al. 2007; Spurlock et al. 1998). Although Wilkie and colleagues recently reported an association between rs6314 (C1354T) in *HTR2A* and both response and remission to paroxetine in MDD (Wilkie et al. 2008), this SNP was shown to have "minor allele frequencies: 0%" in the HapMap database (Japanese population).

A few points of caution should be noted in interpreting our results. First, our sample sizes were small, and there is a possibility of statistical errors in our results. Secondly, because we did not perform an 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. Thirdly, we measured plasma levels of administered sertraline and paroxetine excepting fluvoxamine. However, these effects should be minimal because no correlation between plasma SSRI concentration and clinical response has been reported (Kasper et al. 1993; Saito et al. 2006). Fourthly, because we investigated SSRIs response in MDD patients who were able to take each SSRIs without side effects during the treatment protocol, we did not examine the number of drop out patients due to side effects in this study. Fifthly, we did not investigate several demographic informations (education, income, etc.) of the participated patients in this study. Finally, 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; Stensland et al. 2008). Also, we did not perform a screening to exclude Axis II disorders. However, in this study patients were carefully diagnosed according to DSM-IV criteria with consensus of at least two experienced

psychiatrists on the basis of a review of medical records (Kishi et al. 2008, 2009a, b, c, d). In addition, when we found a misdiagnosis, we promptly excluded the misdiagnosed case in consideration of the precision of our sample.

In conclusion, we suggest that *HTR2A* may play an important role in the pathophysiology of the SSRI therapeutic response in Japanese MDD patients. However, it will be important to replicate and confirm these findings in other independent studies using large samples.

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Abstract Several recent investigations reported that the serotonin 2A receptor gene (*HTR2A*) was associated with selective serotonin reuptake inhibitors (SSRIs) in major depressive disorder. There have also been two reported association analyses of *HTR2A* with SSRI response in Japanese MDD patients, but the results were rather inconsistent and both studies had the problem of small sample sizes. Therefore, we conducted a replication association study using a sample larger than those in the two original Japanese studies (265 MDD patients), and found that four SNPs, two functional SNPs (-A1438G: rs6311 and T102C: rs6313) and two SNPs (rs7997012 and rs1928040) in *HTR2A*, were associated with the therapeutic response to SSRIs. *HTR2A* was associated with the therapeutic response SSRIs in Japanese MDD patients in a haplotype-wise analysis ($P_{\text{all markers}} = 0.0136$), and a significant association between rs1928040 in *HTR2A* and SSRI response was detected in MDD ($P_{\text{allele-wise analysis}} = 0.0252$). However, this significance disappeared after Bonferroni correction ($P_{\text{allele-wise analysis}} = 0.101$). In conclusion, we suggest that *HTR2A* may play an important role in the pathophysiology of the therapeutic response to SSRIs in Japanese MDD patients. However, it will be important to replicate and confirm these findings in other independent studies using large samples.

Keywords Serotonin 2A receptor gene (*HTR2A*) · SNPs · Major depressive disorder · Selective serotonin reuptake inhibitor (SSRI) response

Introduction

Several investigations have suggested that serotonin 2A receptor gene (*HTR2A*) might be a factor in the therapeutic response in major depressive disorder (MDD). The evidence for this relation is discussed in more detail in the reviews (Kato and Serretti 2008; Kato 2007; Serretti and Artioli 2004a, b; Serretti et al. 2007a, b; Serretti and Mandelli 2008). Other recent investigations reported that *HTR2A* was associated with selective serotonin reuptake inhibitors (SSRIs) treatment response in MDD. McMahon et al. (2006) reported an association between rs7997012 and rs1928040 in *HTR2A* and the outcome of citalopram treatment in a very large sample of outpatients with MDD. Peters et al. (2009) replicated those findings in a study showing that rs7997012 was associated with citalopram response in MDD. However, Perlis et al. (2009) reported that rs7997012 and rs1928040 were not associated with duloxetine treatment outcome in MDD. In Japan, there have been two reported association analyses of *HTR2A* with SSRIs response in MDD patients, but the results were rather inconsistent and both studies had the problem of small sample sizes (Kato et al. 2006; Sato et al. 2002).

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