All tests were administered by experienced examiners within 2 weeks of the evaluation of psychopathological symptoms.

Statistical analysis

In order to study the relationship between subjective QOL and clinical variables (age, living situation, duration of disorder, number of hospital admissions for schizophrenia, type of antipsychotics (one type of atypical antipsychotics or more than two types of atypical or typical antipsychotics), dose of antipsychotics, scores on each of the five PANSS areas, CA and PEN on WCST, and d′ on CPT), Spearman rank correlation coefficients were calculated. Because the range of each PANSS subscore was narrow and the SD was small, we used non-parametric analysis.

In order to examine the extent of the effect of clinical variables on subjective QOL, multiple regression analysis using a stepwise forward selection method was performed. Clinical variables that were statistically significant or nearly significant ($P < 0.1$) were regarded as independent variables, and scores on each of the three JSQLS areas were considered dependent variables.

Kruskal–Wallis *H*-test was used to analyze psychopathological characteristics of samples, and a post-hoc analysis was performed using the Mann–Whitney *U*-test with Bonferroni correction.

SPSS version 10.0 (SPSS, Chicago, IL, USA) was used for the analysis, and the level of significance was set at 5%.

RESULTS

Results of subjective QOL evaluation, psychopathological symptom evaluations, and cognitive function examination are given in Table 2. According to the Lindenmayer *et al.* five-factor model[32,33], the score for the excitement factors was significantly lower than the scores for other factors in the present participants.

The correlation matrix of the scores for each of the three JSQLS areas and clinical variables is given in Table 3. To determine the extent of the effects of clinical variables on the three JSQLS areas, multiple regression analysis was performed using the stepwise forward selection method. As a result, the models had a good fit with the data (psychosocial conditions area, $F = 10.548$, $P < 0.001$; motivation/energy area, $F = 9.285$, $P < 0.01$; symptoms/side-effects area, $F = 4.239$, $P < 0.05$). Excluded variables are not reported herein. The psychosocial conditions area of the JSQLS was predicted independently on the basis of the duration of disorder and the PANSS depression/anxiety factors. The motivation/energy area of the JSQLS was predicted by the PANSS depression/anxiety factors. The symptoms/side-effects area of the JSQLS was predicted by the WCST CA (Table 4).

Table 2. QOL, psychopathological symptoms and cognitive functioning

		Mean	SD
JSQLS	Psychosocial	48.7	19.8
	Motivation/energy	48.8	17.1
	Symptoms/side effect	34.0	18.6
PANSS	Total score	83.2	17.6
	Negative	2.9	1.1
	Excitement	2.1	0.8
	Cognitive	2.7	0.8
	Positive	3.1	0.8
	Depression/anxiety	2.8	1.0
WCST	CA	4.4	1.4
	PEN	3.5	3.8
CPT	d′	1.4	0.8

CA, Categories Achieved; CPT, Continuous Performance Test; JSQLS, Japanese Schizophrenia Quality Life of Scale; PANSS, Positive and Negative Syndrome Scale; PEN, Perseverative Errors of Nelson; QOL, quality of life; WCST, Wisconsin Card-Sorting Test.

DISCUSSION

In the present study, depression/anxiety factors, age, living situation, and duration of disorder correlated with the score for the psychosocial conditions area. Stepwise regression analysis indicated that the psychosocial condition worsens with an aggravation of the depression/anxiety factors and improves with an increase in the duration of disorder. Negative factors, depression/anxiety factors, and number of admissions for schizophrenia correlated with the scores for motivation/energy area. Stepwise regression analysis also indicated that with an increase in the depression/anxiety factors, the scores for motivation/energy area deteriorate.

With regard to psychopathological symptoms, some areas of subjective QOL were not influenced by positive factors or negative factors but were significantly affected by depression/anxiety factors. This

Table 3. JSQLS scores and clinical variables

	JSQLS		
	Psychosocial	Motivation/energy	Symptoms/side-effect
PANSS			
Negative	0.101	0.315**	0.145
Positive	−0.153	−0.034	0.035
Cognitive	0.031	0.032	0.090
Excitement	0.111	0.162	0.068
Depression/anxiety	0.407***	0.391***	0.088
WCST			
CA	0.120	−0.123	0.268*
PEN	−0.122	0.120	−0.279**
CPT			
d′	0.060	0.138	0.079
Age	−0.348**	−0.069	−0.071
Living situation†	−0.286**	−0.195	−0.056
Duration of disorder	−0.334**	−0.024	−0.201
No. admissions	−0.164	−0.360***	−0.016
Type of antipsychotics‡	−0.049	0.183	0.071
Dose of antipsychotics	−0.100	−0.078	0.163

*$P < 0.10$; **$P < 0.05$; ***$P < 0.01$, Spearman correlations

†Outpatients = 0, Inpatient = 1. ‡One type of atypical antipsychotic = 0; more than two kinds of atypical or typical antipsychotics = 1.

CA, Categories Achieved; CPT, Continuous Performance Test; JSQLS, Japanese Schizophrenia Quality Life of Scale; PANSS, Positive and Negative Syndrome Scale; PEN, Perseverative Errors of Nelson; WCST, Wisconsin Card-Sorting Test.

finding supports those of other reports on the relationship between depression/anxiety symptoms and subjective QOL.[11–13] Because the psychosocial condition area of JSQLS addresses various emotional problems, patients with schizophrenia appear to be able to validly express their emotions. The motivation/energy area of JSQLS addresses various problems of activity rather than emotion, and such issues might be associated with negative factors, but depression/anxiety factors rather than negative factors affect this area. It is suggested that the better that emotional problems are controlled, the more energy/motivation patients with schizophrenia feel, even if their activity levels are actually poor. Several studies have reported that objective QOL, which is evaluated with QLS, has a close relationship with negative symptoms.[25,37] The fact that QLS was developed for measuring defect symptoms in schizophrenia might explain this relationship with negative symptoms. Subjective QOL, however, is not determined by a therapist's evaluations but by how the patient with schizophrenia feels.

Table 4. Multiple regression of psychopathological symptoms and cognitive functioning

Outcome variable: JSQLS	Predictor	Adjusted R^2	β
Psychosocial	PANSS: Depression/anxiety	0.272	0.390**
	Duration of disorder		−0.391**
Motivation/energy	PANSS: Depression/anxiety	0.140	0.396**
Symptoms/side effect	WCST: CA	0.060	0.280*

*$P < 0.05$, **$P < 0.01$.

CA, Categories Achieved; JSQLS, Japanese Schizophrenia Quality Life of Scale; PANSS, Positive and Negative Syndrome Scale; WCST, Wisconsin Card-Sorting Test.

Until recently, depression and anxiety have tended not to be seen as important as treatment targets for patients with schizophrenia. Ginsberg *et al.*, however, reported that 50% of patients with schizophrenia suffered from depression, and that this was a major risk factor for suicide.[38] Given that subjective QOL correlated with depression/anxiety factors rather than other factors of the PANSS, which is an objective symptom evaluation, we may have to target improvements in depression/anxiety factors in order to improve subjective QOL. Consequently, it is possible that the patients feel the effects of treatments, leading to improvements in adherence. Taniguchi *et al.* reported that replacement of antipsychotic drugs with quetiapine improved clinical symptoms, including depression/anxiety, and the psychosocial conditions score on JSQLS.[39] Treatment plans focusing on the improvement of depression/anxiety will lead to patients feeling the effects of treatment and, consequently, to increased adherence to treatment. In the future, trends in areas such as objective psychopathological symptoms and subjective QOL, as well as treatment adherence, must be examined before and after drug therapy or psychosocial treatments such as cognitive behavioral therapy. This could help identify treatments that are compatible with patient needs and could lead to increased adherence.

The present findings suggested that the longer the duration of disorder, the better the psychosocial condition. As the disorder progresses, patients with schizophrenia might become acclimated to their condition and may not be troubled by their emotional problems. Yamauchi *et al.*, however, reported a nonsignificant correlation between the psychosocial conditions of the JSQLS and the duration of illness,[40] therefore further investigations are necessary to clarify this aspect.

With regard to the relationship between the cognitive function and subjective QOL, the correlation of WCST CA and PEN with the symptoms/side-effects area was evaluated. Stepwise regression analysis suggested that the worse the executive functioning, the better the score for the symptoms/side-effects area. Most of the items in the JSQLS symptoms/side-effects area concern side-effects of drug therapy. The lower the executive functioning, the more indifferent patients are to side-effects and, as a result, patients might rate their QOL higher. We did not assess the objective side-effects. Yamauchi *et al.* reported that objective side-effects predicted the symptoms/side-effects area of JSQLS.[40] It might be necessary to investigate the correlation between the objective QOL and executive functioning and how these factors predict subjective QOL. Matsui *et al.* reported that there was no significant relationship between executive functioning and subjective QOL using the abbreviated version of SQLS.[22] Hofer *et al.* used the same cognitive function survey, and reported no relationship between executive functioning and subjective QOL.[30] The fact that these results are inconsistent with the present results might be explained by the fact that executive functioning in the Matsui *et al.* study was not measured using the WCST and that subjective QOL in the Hofer *et al.* study was measured with the World Health Organization Quality of Life Assessment–Short Form (WHOQOL-Bref),[41] which is not a QOL scale specific to schizophrenia. Some insight measure might be useful to investigate in this area. Patients with schizophrenia exhibit significantly impaired sustained attention.[18,20] Cornblatt *et al.* reported that attentional deficits using CPT-IP resulted in a schizophrenia spectrum with a sensitivity of 67% and specificity of 79%,[42] and that the mean d' in normal adults was 1.720 (SD = 0.778).[36] In the present study sustained attention in subjects would be lower than that in the normal population, and this did not affect subjective QOL. Prouteau *et al.* reported that poorer sustained attention predicted better subjective QOL,[43] and Wegener *et al.* reported that sustained attention had a negative effect on subjective QOL.[24] The inconsistency of these findings with the present findings might result from the fact that each study used different instruments to measure subjective QOL assessment and sustained attention. In the future, there is a need for methodology to be standardized in further investigations into the relationship between cognitive function and subjective QOL.

The present study had several limitations. First, the average total PANSS score for the subjects in the present study was 85.2 ± 19.3; thus, psychopathological symptoms were relatively mild. In particular, excitement symptoms had subsided. Moreover, the subjects were chronically ill patients who were not in acute exacerbation. Verification in severely ill and acute patients is insufficient, therefore it is difficult to assume that these results can be generalized to schizophrenia patients as a group. If possible, future investigations should examine subject groups that include the severely and acutely ill.

If we include improvement of subjective QOL as well as reduction of psychopathological symptoms in

treatment goals for schizophrenia, the present findings indicate a need to develop treatments that focus on symptoms of depression/anxiety. Such treatments lead to patients really feeling the effects of treatments and can improve treatment adherence. In the future, longitudinal research is needed into how psychopathological symptoms and cognitive function affect subjective QOL.

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ORIGINAL PAPER

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

Taro Kishi · Reiji Yoshimura · Tsuyoshi Kitajima · Tomo Okochi · Takenori Okumura · Tomoko Tsunoka · Yoshio Yamanouchi · Yoko Kinoshita · Kunihiro Kawashima · Hiroshi Naitoh · Jun Nakamura · Norio Ozaki · Nakao Iwata

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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).

Taro Kishi and Reiji Yoshimura have contributed equally to this work.

T. Kishi (✉) · T. Kitajima · T. Okochi · T. Okumura · T. Tsunoka · Y. Yamanouchi · Y. Kinoshita · K. Kawashima · H. Naitoh · N. Iwata
Department of Psychiatry, Fujita Health University School of Medicine, Toyoake, Aichi 470-1192, Japan
e-mail: tarok@fujita-hu.ac.jp

R. Yoshimura · J. Nakamura
Department of Psychiatry, University of Occupational and Environmental Health, Kitakyushu, Fukuoka 807-8555, Japan

N. Ozaki
Department of Psychiatry, Nagoya University Graduate School of Medicine, Nagoya 466-8850, Japan

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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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Copy Number Variation in Schizophrenia in the Japanese Population

Masashi Ikeda, Branko Aleksic, George Kirov, Yoko Kinoshita, Yoshio Yamanouchi, Tsuyoshi Kitajima, Kunihiro Kawashima, Tomo Okochi, Taro Kishi, Irina Zaharieva, Michael J. Owen, Michael C. O'Donovan, Norio Ozaki, and Nakao Iwata

Background: Copy number variants (CNVs) have been shown to increase the risk to develop schizophrenia. The best supported findings are at 1q21.1, 15q11.2, 15q13.3, and 22q11.2 and deletions at the gene neurexin 1 (*NRXN1*).

Methods: In this study, we used Affymetrix 5.0 arrays to investigate the role of rare CNVs in 575 patients with schizophrenia and 564 control subjects from Japan.

Results: There was a nonsignificant trend for excess of rare CNVs in schizophrenia ($p = .087$); however, we did not confirm the previously implicated association for very large CNVs (>500 kilobase [kb]) in this population. We provide support for three previous findings in schizophrenia, as we identified one deletion in a case at 1q21.1, one deletion within *NRXN1*, and four duplications in cases and one in a control subject at 16p13.1, a locus first implicated in autism and later in schizophrenia.

Conclusions: In this population, we support some of the previous findings in schizophrenia but could not find an increased burden of very large (>500 kb) CNVs, which was proposed recently. However, we provide support for the role of CNVs at 16p13.1, 1q21.1, and *NRXN1*.

Key Words: Deletion, duplication, *NRXN1*, 16p13.1, 1q21.1, schizophrenia

Copy number variations (CNVs) are deletions and duplications of DNA ranging from a kilobase (kb) to several megabases (Mb). Recently, rare CNVs were shown to play a role in the etiology of a number of neuropsychiatric disorders, particularly schizophrenia, autism, and mental retardation (1).

Several studies have reported a greater prevalence of rare CNVs in people with schizophrenia (2-4). However, some have found no such excess (5,6) and even among the positive studies, there is marked variation in the magnitude of the observed effect. For example, in the International Schizophrenia Consortium (ISC) study (4), cases had only a 1.15-fold excess of rare CNVs, rising to 1.67-fold for deletions greater than 500 kb. An increase only among very large CNVs (>1 Mb) in cases was found by Kirov *et al.* (7). Another study showed an odds ratio of 3.37 for CNVs, rising to 4.82 for early-onset schizophrenia (2). This may, in part, reflect differences in the sensitivity of CNV assays, definitions of low-frequency CNVs, or variation in the phenotypic composition of the samples, as cases with early onset or lower IQ were particularly enriched for CNVs in one study (2).

In addition to increased CNV burden, a number of specific CNVs have been associated with schizophrenia (4,7,8). There is strong replicated evidence for deletions at 1q21.1, 15q11.2, 15q13.3, and 22q11.2 and emerging evidence for duplications at 16p13.1 (4,7). Deletions of the neurexin 1 gene (*NRXN1*) have also been reported in multiple studies on schizophrenia (2,6,7,9,10). Given the discrepancy in estimates of the effect size of CNV burden as a risk factor for schizophrenia and in particular the absence of association in the only Asian sample reported to date (5), we aimed to test for an excess burden of CNVs in a population from Japan. We also sought supportive evidence for a contribution for the specific loci listed above.

Methods and Materials

We analyzed 1139 age- and gender-matched unrelated subjects of Japanese ethnicity (575 schizophrenic patients and 564 control subjects). Control subjects were members of the general public who had no personal history of mental disorders. This was ascertained during face-to-face interviews where subjects were asked if they had suffered an episode of depression, mania, or psychotic experiences or if they had received treatment for any psychiatric disorder. Patients were entered into the study if they 1) met DSM-IV criteria for schizophrenia; 2) were physically healthy and had normal routine laboratory tests; and 3) had no mood disorders, substance abuse, neurodevelopmental disorders, epilepsy, or known mental retardation. Consensus diagnoses were made by at least two experienced psychiatrists according to DSM-IV criteria on the basis of unstructured interviews with patients and families and review of medical records. After description of the study, written informed consent was obtained from each subject. This study was approved by the ethics committees of each participating university.

We used Affymetrix 5.0 Arrays (Affymetrix, Santa Clara, California), following the manufacturer's protocols (http://www.affymetrix.com). This array includes 470K single nucleotide polymorphism (SNP) probes and 420K nonpolymorphic probes. The CNVs discussed below in more detail (at *NRXN1*, 1q21.1, and 16p13.1) were validated using the Illumina HumanHap 660W- or 610-quad bead arrays (Illumina, San Diego, California), following the manufacturer's protocols (http://www.illumina.com).

Copy number variations were called using the Birdsuite program (http://www.broadinstitute.org/science/programs/medical-and-

From the Medical Research Council (MRC) Centre for Neuropsychiatric Genetics and Genomics (MI, GK, IZ, MJO, MCO), Department of Psychological Medicine and Neurology, School of Medicine, Cardiff University, Cardiff, United Kingdom; Department of Psychiatry (MI, YK, YY, TKit, KK, TO, TKis, NI), Fujita Health University School of Medicine, Toyoake, Aichi; Department of Psychiatry (BA, NO), Nagoya University Graduate School of Medicine, Nagoya; and Core Research for Environmental Science and Technology (CREST) (BA, YK, TKit, KK, TO, TKis, NO, NI), Japan Science and Technology Agency, Kawaguchi, Saitama, Japan.

Address correspondence to George Kirov, M.D., MRC Centre for Neuropsychiatric Genetics and Genomics, Department of Psychological Medicine and Neurology, School of Medicine, Cardiff University, Cardiff CF14 4XN, UK; E-mail: kirov@cardiff.ac.uk.

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Table 1. Global CNV Burden Analysis

CNV Type	Size	CNV Burden				CNVs Intersecting Genes			
		SCZ	CON	CNV Rate SCZ/CON	*p* Value	SCZ	CON	CNV Rate SCZ/CON	*p* Value
Deletions and Duplications	All	567	485	1.1/.95	.087	382	320	.74/.62	.084
	100–200 kb	285	229	.55/.45	.046	182	145	.35/.28	.074
	200–500 kb	221	192	.43/.37	.20	150	134	.29/.26	.30
	500 kb–1 Mb	48	52	.09/.10	.72	38	32	.07/.06	.31
	>1 Mb	13	12	.025/.023	.52	12	9	.023/.018	.35
Deletions Only	All	174	157	.34/.31	.30	91	87	.18/.17	.46
	100–200 kb	98	84	.19/.16	.26	52	47	.10/.09	.38
	200–500 kb	65	60	.13/.12	.42	29	35	.06/.07	.79
	500 kb–1 Mb	8	8	.015/.016	.62	8	3	.015/.006	.12
	>1 Mb	3	5	.006/.010	.86	2	2	.004/.004	.69
Duplications Only	All	393	328	.76/.64	.10	291	233	.56/.45	.075
	100–200 kb	187	145	.36/.28	.070	130	98	.25/.19	.071
	200–500 kb	156	132	.30/.26	.21	121	99	.23/.19	.18
	500 kb–1 Mb	40	44	.077/.086	.73	30	29	.058/.057	.53
	>1 Mb	10	7	.019/.014	.33	10	7	.019/.014	.33

p values are one-tailed and based on 10,000 permutations.
CNV, copy number variation; CON, control; kb, kilobase; Mb, megabase; SCZ, schizophrenia.

population-genetics/birdsuite/birdsuite-0) (11). The software first assigns copy number across regions of known copy number polymorphisms, then calls SNP genotypes (for samples and SNPs believed to have two copies of the locus), then searches for novel CNVs via a hidden Markov model, and generates an integrated sequence and copy number genotype at every locus. It takes into account genotypes within CNVs, e.g., A-null, AAB, and BBB, in addition to AA, AB, and BB calls (11).

We observed a batch effect, similar to what we reported in our previous study (7): arrays from different batches gave poor results if analyzed together. Therefore, we identified the batches and analyzed together samples within the same batch, as recommended in the Birdsuite manual (11). After initial filtering for quality control, using the standard criteria implemented in the Genotyping Console software (www.affymetrix.com), including quality control call rate (>86%), SNP call rate (>95%), and population stratification based upon principal components analysis, 1107 samples (560 cases and 547 control subjects) were retained for further analysis. They had 16,466 CNVs (eight subjects showed no CNVs). We then excluded low-confidence CNVs (logarithm of odds <10), CNVs <100 kb, and those with the lowest 1% density for probe coverage (52 segments). We removed 50 samples that had high sample-specific measures of noise (variance >2), as those had a mean of 175 CNV segments, indicating they were false-positives. We also removed 17 samples that had more than 20 apparent CNVs (the mean number of CNVs for these samples was 156), as such samples are also likely to be false-positives (4,7). The filtering left 1032 samples: 519 cases aged 43.4 ± 14.7 years (258 male and 261 female cases) and 513 control subjects aged 43.8 ± 14.5 years (252 male and 261 female control subject). They had a total of 5180 CNVs (~5 per person). Finally, following previous studies (4,7), we filtered common CNVs (found in >1% of the total sample), leaving 1052 rare and larger than 100 kb CNVs for the analysis (~1 per person). This filtering was also performed for CNVs found at >5% in the total sample, resulting in 2081 CNVs. All CNVs that passed filtering and were present in <1% of the samples are available as an University of California, Santa Cruz (UCSC)-friendly file in Supplement 1.

Copy number variations were considered to colocalize if they overlapped by at least 50% of their length, as implemented in PLINK ver1.0.4 (http://pngu.mgh.harvard.edu/~purcell/plink/) (12) as used for the analysis of CNV loci in previous datasets (4,7).

Results

The numbers of rare CNVs stratified by size in cases and control subjects are listed in Table 1. Overall, we found an excess of CNVs in subjects with schizophrenia (case-control ratio = 1.16). Although not significant ($p = .087$, one-tailed permutation test), this is similar to that reported by the largest CNV study (4) where the case-control ratio was 1.15. The effect in that study (4) was coming mostly from deletions >500 kb and duplications in the 100 kb to 200 kb range. No subcategory of CNV defined by size or nature (deletion or duplication) was significantly associated with disease in the current study. Copy number variations in the 100 kb to 200 kb range were more common in cases than in control subjects, ratio = 1.23, $p = .046$; however, this does not survive correction for the multiple testing of four size ranges and two types of CNVs. Duplications (but not deletions) within the same size range were the most significantly associated general category in the ISC study ($p = 1 \times 10^{-4}$) with virtually an identical effect (case-control ratio = 1.26). However, no specific duplications of this size overlapped between the two studies (4). We did not replicate the finding of an excess of large deletions (>500 kb) that was reported in the ISC study (4) or of deletions and duplications >1 Mb reported in the study by Kirov *et al.* (7).

Analysis of the burden of CNVs intersecting genes revealed no significant excess of genes disrupted in subjects with schizophrenia, either overall or for any size range, with similar trends to the results from the general burden analysis (Table 1).

We repeated the same analysis for CNVs <5% in the sample. This resulted in 388 and 368 deletions and 698 and 627 duplications in cases and control subjects, respectively. The trends between cases and control subjects were virtually identical to those in Table 1 (data not presented).

Although we found no enrichment of large CNVs in schizophrenia, we present the details of large CNVs (>1 Mb) in Table S1 in Supplement 2 because these have been most consistently implicated by others (4,7). Of those, one case but no control subjects had a deletion on 1q21.1, one of the most convincingly

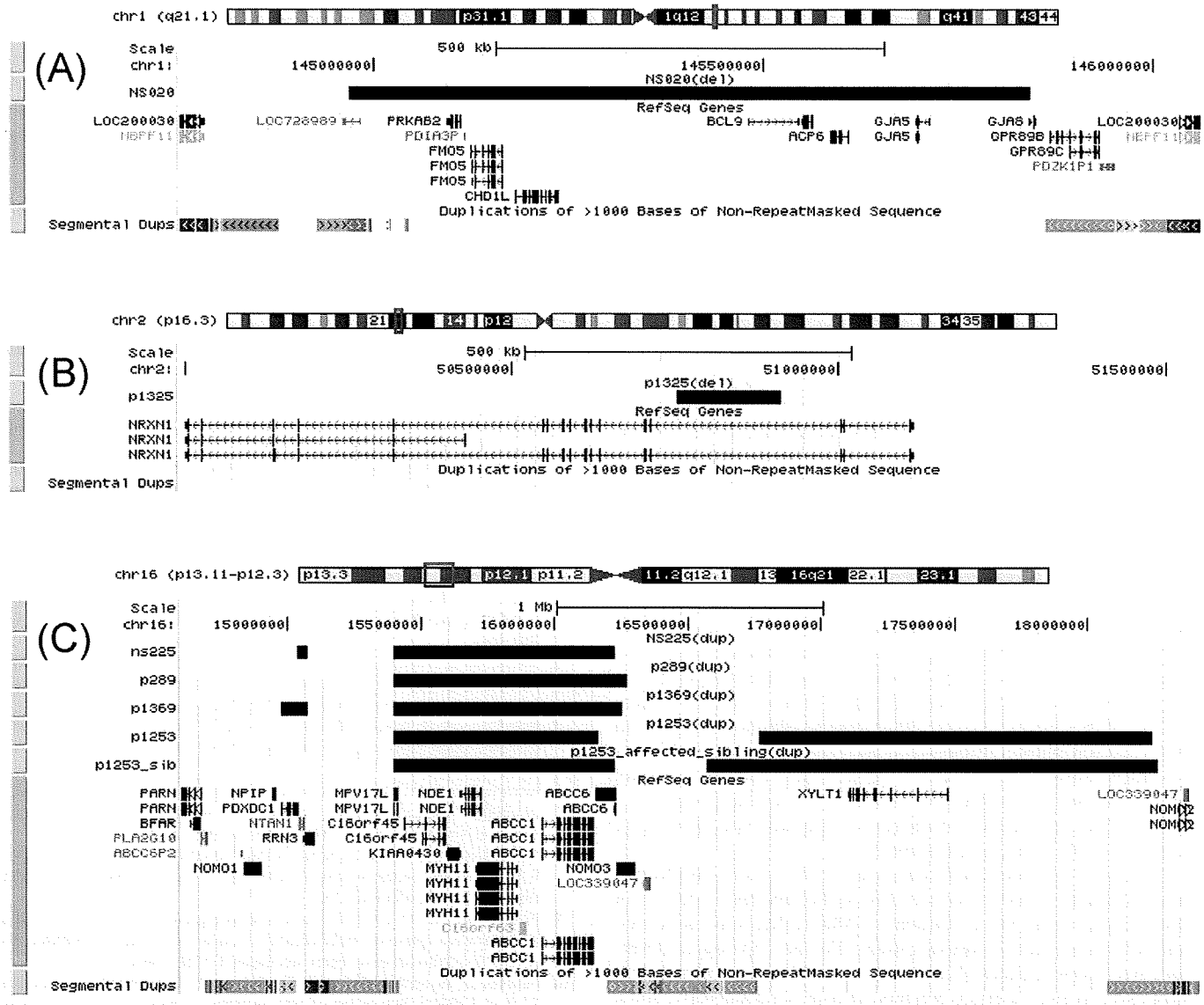

Figure 1. Positions of CNVs according to the validation experiments. CNV validation was undertaken using Illumina HumanHap 660W quad bead arrays (for CNVs at 1q21.1 and *NRXN1*) or 610-quad bead arrays (for CNVs at 16p13.1). Figures are produced on the UCSC Genome Browser according to NCBI Build 36.1, March 2006, hg18 (http://www.genome.ucsc.edu/) and indicate the positions of the CNVs: **(A)** 1q21.1; **(B)** *NRXN1*; and **(C)** 16p13.1: the last trace is that of the affected sibling of "p1253." CNV, copy number variation; NCBI, National Center for Biotechnology Information; UCSC, University of California, Santa Cruz.

implicated CNV risk factors for schizophrenia (4,8). Among large duplications, the most notable is that on 16p13.1, which was found in four cases and one control subject, while one more control subject had the reciprocal deletion (Fisher exact test p = .19, one-tailed). These CNVs in cases were confirmed using Illumina arrays (Figure S1 and Tables S1 and S2 in Supplement 2). One of the patients with 16p13.1 duplication had an affected sibling and unaffected mother who had also provided DNA. The duplication was found in the affected sibling but not the unaffected mother (DNA from the father was not available and there is no indication that he suffers with mental illness). The duplication in this family extends further on the centromeric side compared with the region usually included in CNVs of this region (Figure 1).

Of the remaining susceptibility loci reported in the recent studies (4,7,8), we found no deletions at 22q11.2 or 15q13.3. We also find no support for the 15q11.2 locus, where three deletions were found in control subjects and only one in a case (Fisher exact test p = .37, two-tailed, a trend in the opposite direction).

We also searched for CNVs that intersected genes and were present only in cases, reasoning as have others (2,3) that such CNVs are good candidates (Tables S3 and S4 in Supplement 2). One of the singleton deletions was in *NRXN1*, a gene implicated in previous studies (2,6,7,9,10) (Figure S1 and Table S2 in Supplement 2). Several more contain intriguing candidate genes (e.g., deletions in *PARK2, GRIK2, MAGEL2,* and *ATXN2L* and duplications in *CHRNA7* and *NRG4*), which have been implicated in neurodegenerative disorders or have possible functional relevance for neurodevelopment.

Discussion

In this study, we do not find a significant increase in the burden of CNVs in schizophrenia, either overall or for any

specific size range of CNVs, as proposed in previous studies (2–4,7). We did, however, find several trends in the same direction and of a similar magnitude as the largest global CNV survey of schizophrenia (4). Not all research has found such an increased burden, e.g., no evidence was obtained from a study in the Chinese population (5). It is possible that genuine population differences might drive this discrepancy between Caucasian and Asian samples, as might our exclusion of subjects with mental retardation or epilepsy. Sample size could also have played a role. Our sample had a modest power of ~.65 to detect a single CNV in a case for the following very strong candidate loci: 1q21.1, 15q13.3, and 22q11.2 and *NRXN1*, where approximately .2% of affected persons have deletions. In fact, we did find one deletion each in two of these loci (1q21.1 and *NRXN1*).

We found stronger support for association with duplications at 16p13.1, which contain the candidate gene *NDE1*. It is within the interval duplicated in all patients (Figure 1). Deletions and duplications of this region were implicated in autism (13) and schizophrenia (7), while deletions have been implicated in mental retardation (14). The most recent study surveying children with unexplained intellectual disability also reported significant association for both deletions and duplications at this locus ($p = 4.7 \times 10^{-5}$) (15), suggesting that this duplication is also pathogenic for a broad range of neuropsychiatric disorders. Our result for an excess of duplications in schizophrenic probands does not reach statistical significance; however, the frequency of the duplication is fourfold higher in cases than in control subjects (.8% vs. .2%), which is very similar to the rate found in our previous study from the United Kingdom (.6% vs. .2%) (7) and in the ISC study (.4% vs. .2%) (4). We found an identical duplication in an affected sibling. Larger CNVs in this locus, as in one of our probands, were also found in three cases and two control subjects in the ISC (4). The four probands in our study who carry 16p13.1 duplications do not appear to share any specific clinical features (Table S2 in Supplement 2).

We also found one deletion in a case at 1q21.1 and *NRXN1* and none in control subjects, which is close to the reported frequency of .2% in cases. Unlike those deletions of *NRXN1* that were associated with schizophrenia in a previous study (10), the CNV reported here does not intersect exons (10). However, it is large compared with most exon-sparing deletions reported in control subjects (10), and a new reanalysis of all *NRXN1* deletions shows that large (>100 kb) deletions in this gene might be almost as relevant as those affecting exons (16). The relevance to schizophrenia of the other CNVs found only in cases can only be assessed in future meta-analyses of such studies, but we note here that the three deletions we found in *PARK2* are of particular interest, as they have been implicated as a susceptibility factor for autism (17).

In summary, we provide support for the role of CNVs at 16p13.1, 1q21.1, and *NRXN1* in the etiology of schizophrenia. Although we find similar, but not significant, trends for an increased overall burden of CNVs, as well as for the involvement of duplications in the 100 kb to 200 kb range as proposed in the ICS study (4), in this population we could not find an increased burden of very large CNVs (>500 kb) in schizophrenia, which has been the main finding in recent studies (4,7). The discrepancy with previous studies could be due to our exclusion of patients with neurodevelopmental disorders, epilepsy, or known mental retardation, as such features are found in many of the carriers of large CNVs, e.g., 15q13.3 (15). Given the rarity of the CNVs that have been implicated so far in schizophrenia, there is a need for more large studies, studies in non-European populations, and meta-analyses.

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ORIGINAL PAPER

Translin-Associated Factor X Gene (*TSNAX*) may be Associated with Female major Depressive Disorder in the Japanese Population

Akiko Okuda · Taro Kishi · Tomo Okochi · Masashi Ikeda · Tsuyoshi Kitajima · Tomoko Tsunoka · Takenori Okumukura · Yasuhisa Fukuo · Yoko Kinoshita · Kunihiro Kawashima · Yoshio Yamanouchi · Toshiya Inada · Norio Ozaki · Nakao Iwata

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Abstract Several investigations have reported that the translin-associated factor X gene (*TSNAX*)/disrupted-in-schizophrenia-1 gene (*DISC1*) was associated with major psychiatric disorders including schizophrenia, bipolar disorder (BP), and major depressive disorder (MDD). *TSNAX* is located immediately upstream of *DISC1*, and has been shown to undergo intergenic splicing with *DISC1*. It thus may also be influenced by translocation. To our knowledge, there are no reported gene-based association analyses between *TSNAX* and mood disorders in the Japanese population. We conducted a case–control study of Japanese samples (158 bipolar patients, 314 major depressive disorder patients, and 811 controls) with three tagging SNPs in *TSNAX*, selected using HapMap database. In addition, we performed an association analysis between *TSNAX* and the efficacy of fluvoxamine treatment in 120 Japanese patients with MDD. The MDD patients in this study had scores of 12 or higher on the 17 items of the Structured Interview Guide for Hamilton Rating Scale for Depression (SIGH-D). We defined a clinical response as a decrease of more than 50% in baseline SIGH-D within 8 weeks, and clinical remission as an SIGH-D score of less than 7 at 8 weeks. We found an association between rs766288 in *TSNAX* and female MDD in the allele/genotype analysis. However, we did not find any association between *TSNAX* and BP or the fluvoxamine therapeutic response in MDD in the allele/genotype analysis or haplotype analysis. Our results suggest that rs766288 in *TSNAX* may play a role in the pathophysiology of female MDD in the Japanese population. A replication study using larger samples may be required for conclusive results, since our sample size was small.

Keywords Major depressive disorder · Bipolar disorder · Translin-associated factor X gene (*TSNAX*) · Disrupted-in-schizophrenia-1 gene (*DISC1*) · Linkage disequilibrium · Tagging SNP

Akiko Okuda, Taro Kishi participated equally in this work.

A. Okuda · T. Kishi (✉) · T. Okochi · M. Ikeda · T. Kitajima · T. Tsunoka · T. Okumukura · Y. Fukuo · Y. Kinoshita · K. Kawashima · Y. Yamanouchi · N. Iwata
Department of Psychiatry, Fujita Health University School of Medicine, Toyoake, Aichi 470-1192, Japan
e-mail: tarok@fujita-hu.ac.jp

A. Okuda
Okehazama Hospital, Toyoake, Aichi 470-1168, Japan

M. Ikeda
Department of Psychological Medicine, School of Medicine, Cardiff University, Heath Park, Cardiff CF14 4XN, UK

T. Inada
Neuropsychiatric Research Institute, Seiwa Hospital, Tokyo 162-0851, Japan

N. Ozaki
Department of Psychiatry, Nagoya University Graduate School of Medicine, Nagoya 466-8850, Japan

Introduction

The translin-associated factor X gene (*TSNAX*) and disrupted-in-schizophrenia-1 gene (*DISC1*) are located at 1q 42. These genes are associated with major psychiatric disorders, such as schizophrenia, bipolar disorder (BP), and major depressive disorder (MDD).

TSNAX (OMIM * 602964, 7 exons in this genomic region spanning 38.672 bp and 1q42), is located immediately upstream of *DISC1*, and has been shown to undergo

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

Materials and Methods

Subjects

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

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

Data Collection

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

SNP Selection and LD Evaluation

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

Table 1 Clinical characteristics of the patients in both definition groups

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

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

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

SNP Genotyping

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

Statistical Analysis

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

Results

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

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

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

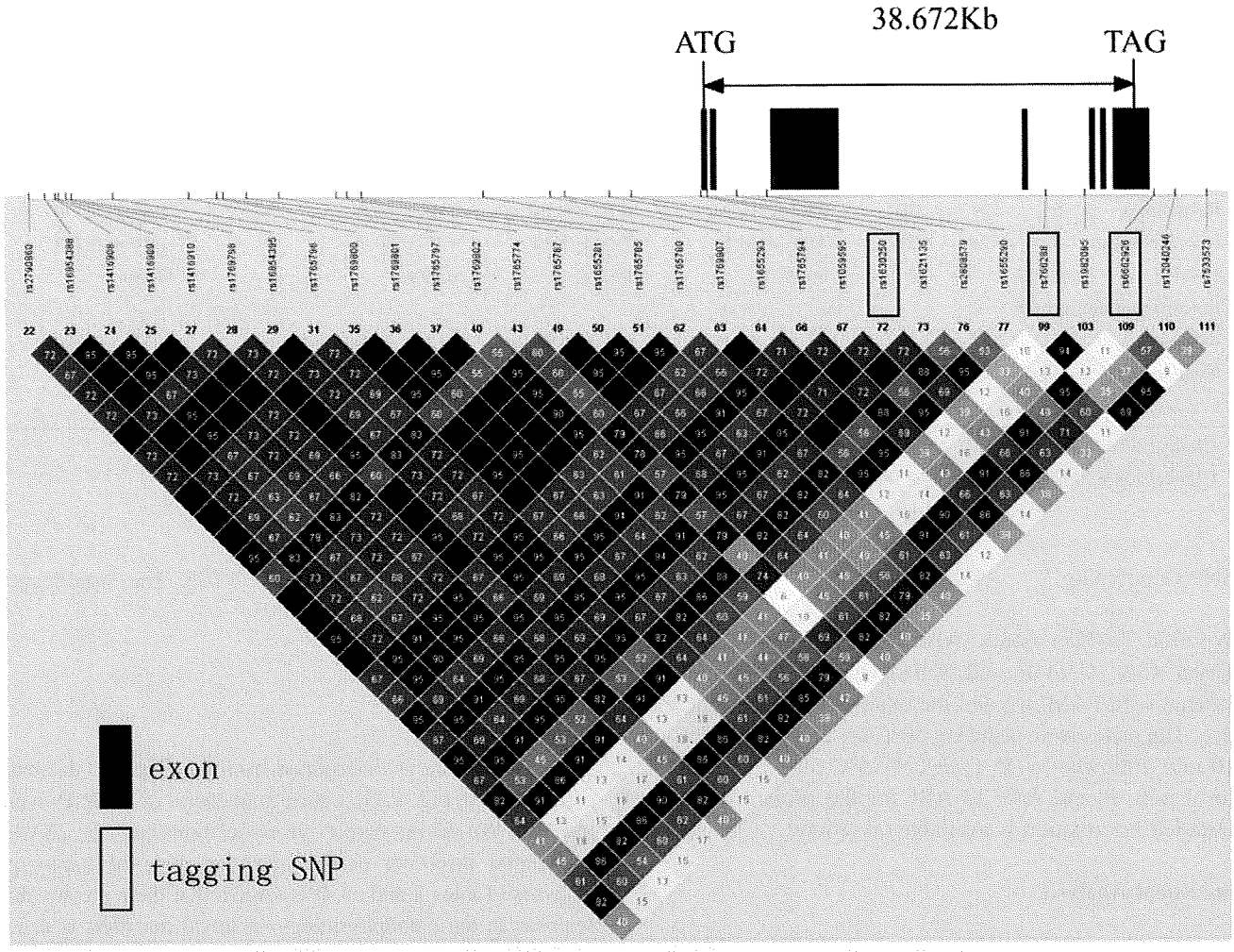

Fig. 1 LD evaluation and tagging SNPs in *TSNAX* ATG is the start codon and TAG is the stop codon. *Vertical bars* represent exons. Tagging SNPs selected from the HapMap database are represented by *black boxes*. Color scheme is based on r^2 value. Other information can be seen at the Haploview website

Table 2 Tagging SNPs and association analysis of *TSNAX*

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

[a] Major allele > minor allele

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

[c] Hardy–Weinberg equilibrium

Table 3 Haplotype analysis of tagging SNPs in *TSNAX*

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

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

[b] Bold numbers represent significant *P* value

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

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

[a] Major allele > minor allele

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

[c] Hardy–Weinberg equilibrium

[d] Bold represents significant *P* value

[e] Calculated by Bonferroni's correction

Discussion

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