Uno Y, Uchiyama T, Kurosawa M, Aleksic B, Ozaki N	The combined measles, mumps, and rubella vaccines and the total number of vaccines are not associated with development of autism spectrum disorder: The first case-control study in Asia.	Vaccine	30 (28)	4292-8	2012
Ohi K, Hashimoto R, Yasuda Y, Fukumoto M, Yamamori H, Umeda-Yano S, Okada T, Kamino K, Morihara T, Iwase M, Kazui H, Numata S, Ikeda M, Ohmura T, Iwata N, Ueno S, Ozaki N, Ohmori T, Arai H, Takeda M	Functional genetic variation at the NRGN gene and schizophrenia: evidence from a gene-based case-control study and gene expression analysis.	American Journal of Medical Genetics Part B: Neuropsychiatric Genetics	159B(4)	405-13	2012
Ohi K, Hashimoto R, Nakazawa T, Okada T, Yasuda Y, Yamamori H, Fukumoto M, Umeda-Yano S, Iwase M, Kazui H, Yamamoto T, Kano M, Takeda M.	The p250GAP Gene is Associated with Risk for Schizophrenia and Schizotypal Personality Traits.	PLoS One	7(4)	e35696	2012
Noda T, Yoshida S, Matsuda T, Okamoto N, Sakamoto K, Koseki S, Numachi Y, Matsushima E, Kunugi H, Higuchi T	Frontal and right temporal activations correlate negatively with depression severity during verbal fluency task: A multi-channel near-infrared spectroscopy study	Journal of Psychiatric Research	46	905-912	2012
野田隆政，中込和幸	シンポジウム　精神疾患の診断ツールとしての光トポグラフィー	認知神経科学	14	35-41	2012
野田隆政	光トポグラフィによるうつ病診断	医学のあゆみ	244	425-431	2013
野田隆政，安藤 久美子, 平林直次，大森まゆ，黒木規臣，岡田幸之	医療観察法における ECT のインフォームド・コンセント	精神神経学雑誌	114　第107回学術総会特別号	SS385-SS390	2013

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
野田隆政，安藤久美子，清水悠	精神症状への対応	山村隆	多発性硬化症（ＭＳ）診療のすべて	診断と治療社	東京	2012	91-98
野田隆政，松田太郎，蟹江絢子，安藤久美子.	頭蓋内疾患によるせん妄	八田耕太郎、岸泰宏	病棟・ICUで出会うせん妄の診かた	中外医学社	東京	2012	20-32
野田隆政	精神症状	村田美穂	やさしいパーキンソン病の自己管理（改訂版）	医薬ジャーナル社	大阪	2012	31-36

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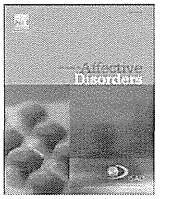

Research report

Difference in Temperament and Character Inventory scores between depressed patients with bipolar II and unipolar major depressive disorders

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ABSTRACT

Background: Although some core personality variables are known to be characteristic of unipolar or bipolar depression, few studies have compared the personality profile between these two disorders.
Methods: Temperament and Character Inventory (TCI) was employed to assess the personality of 36 depressed patients with bipolar II disorder (BPII), 90 patients with unipolar major depressive disorder (UP), and 306 healthy controls. The TCI was administered during the depressive episode in BPII and UP patients so that the results can be applied in a clinical setting.
Results: Significantly higher scores in harm avoidance ($p<0.0001$) and lower scores in self-directedness ($p<0.0001$) and cooperativeness ($p<0.05$) were observed in both BPII and UP patients compared to controls. Lower novelty seeking in UP patients compared to BPII patients and controls was observed in females ($p<0.0001$, $p<0.01$, respectively). A significant difference in self-transcendence score was observed between BPII and UP patients in females ($p<0.0005$), with higher scores in BPII ($p=0.009$) and lower scores in UP ($p=0.046$) patients compared to controls. A logistic regression model predicted BPII in depressed females based on novelty seeking and self-transcendence scores with a sensitivity of 89% and a specificity of 73%, but did not accurately predict BPII in males.
Limitations: Patients in our study were limited to those receiving outpatient treatments, and bipolar patients were limited to those with BPII.
Conclusions: Novelty seeking and self-transcendence scores of TCI might be useful in the differentiation of UP and BPII in female patients.

1. Introduction

Differentiating unipolar and bipolar depression is of great clinical importance since the treatment of the two disorders differs substantially. However, the differentiation may be difficult due to the similar depressive symptomatology in these two disorders. One study reported that 69% of bipolar disorder (BP) had been misdiagnosed, with the most frequent

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misdiagnosis being unipolar depression (Hirschfeld et al., 2003). Some clinical characteristics such as hypersomnia and psychotic features have been suggested to be more common in bipolar depression than in unipolar depression (Forty et al., 2008; Mitchell et al., 2001). However, there are no clear-cut clinical features that distinguish the two disorders, and clinicians must use all available information to predict the possibility of bipolarity in depressed patients.

A few studies have attempted to examine differences in personality between unipolar and bipolar depression. Two studies (Mendlowicz et al., 2005; Nowakowska et al., 2005) used the Temperament Evaluation of Memphis, Pisa, Paris, and San Diego-Autoquestionnaire (TEMPS-A) and found that cyclothymic subscale score was significantly elevated in BP patients. Another study (Lovdahl et al., 2010), which used Temperament and Character Inventory (TCI) (Cloninger et al., 1993) to compare bipolar II disorder (BPII) with recurrent brief depression (RBD; defined as intermittent depressive episodes fulfilling the diagnostic criteria for major depressive episodes except for duration, which is less than 14 days), failed to find definitive difference between the two disorders. Evans, et al. (Evans et al., 2005) used both TEMPS-A and TCI to find higher dysthymic, cyclothymic, irritable, anxious, and novelty seeking temperaments and lower self-directedness and cooperativeness characters in BP patients. A study using a battery including 17 conventional personality scales has reported sanguine in patients with bipolar I disorder, labile or cyclothymic in patients with BPII, and subanxious and subdepressive in patients with unipolar depression (Akiskal et al., 2006).

Although above studies report a number of findings showing different personality in UP and BP patients, few have assessed the personality of the two disorders during depressive state (Mendlowicz et al., 2005). Since the severity of depressive symptoms impact on how the subject describes their personality (Spittlehouse et al., 2010), it is important that the mood state at the time of assessment is taken into account. If the personality assessment is to be used as an aid for differentiating unipolar and bipolar depression in the real world setting, studies are required to compare personality measures during depressed mood.

In the present study, TCI was used to assess the personality difference between depressed BPII and UP patients. TCI is a 240 item true/false questionnaire measuring four dimensions of temperament (novelty seeking (NS), harm avoidance (HA), reward dependence (RD), and persistence (P)) and three dimensions of character (self-directedness (SD), cooperativeness (C), and self-transcendence (ST)), developed on the basis of a psychobiological model of personality. Although TCI has been frequently used in the studies of mood disorders (Akiskal et al., 2005; Celikel et al., 2009; de Winter et al., 2007; Engstrom et al., 2004; Farmer et al., 2003; Hansenne et al., 1999; Hirano et al., 2002; Kimura et al., 2000; Loftus et al., 2008; Marijnissen et al., 2002; Matsudaira and Kitamura, 2006; Naito et al., 2000; Richter et al., 2000; Smith et al., 2005), no studies to date have compared TCI score profiles of patients with unipolar and bipolar depression during their depressed states. We aimed to identify personality profiles specific to either unipolar or bipolar depression, which could aid in the differentiation of the two disorders.

2. Methods

2.1. Subjects

Subjects were 36 patients with BPII (18 men, 18 women; age ± S.D. (standard deviation) = 36.3 ± 11.1 years), 90 patients with UP (45 men, 45 women; age ± S.D. = 36.7 ± 10.2 years), and 306 healthy volunteers (153 men, 153 women; age ± S.D. = 36.4 ± 11.0 years), matched for age distribution in each gender group, recruited from the outpatient clinic of the National Center of Neurology and Psychiatry Hospital, Tokyo, Japan or through advertisements in local free magazines, website announcement, notices posted in the hospital, flyers, and word of mouth. Consensus diagnoses by at least two research psychiatrists were made according to the DSM-IV criteria (American Psychiatric Association, 1994) for BPII or UP for enrollment in the study. Those recruited from the outpatient clinic (BPII: 24 patients (67%); UP: 66 patients (73%)) were also assessed with the Structured Clinical Interview for DSM-IV by a trained psychiatrist to confirm the diagnosis. BPII and UP patients with Hamilton Rating Scale for Depression (17-item version) score greater than 7 were enrolled in the study. Healthy participants were interviewed using the Japanese version of the Mini-International Neuropsychiatric Interview (Otsubo et al., 2005; Sheehan et al., 1998) by a research psychiatrist, and only those who demonstrated no history of psychiatric illness or contact to psychiatric services were enrolled as healthy controls. Participants were excluded from both the patient and control groups if they had a prior medical history of central nervous system disease or severe head injury, or if they met DSM-IV criteria for mental retardation, substance dependence, or substance abuse. All subjects were biologically unrelated Japanese who resided in the Western part of Tokyo. Written informed consent was obtained from all subjects prior to their inclusion in the study and the study was approved by the ethics committee of the National Center of Neurology and Psychiatry, Japan.

2.2. Measures

All participants were administered the TCI (Cloninger et al., 1993), a 240 item true/false self-report questionnaire measuring four dimensions of temperament and three dimensions of character. The Japanese version of the TCI (Kijima et al., 1996; Kijima et al., 2000) was used in the present study. Each subject was allowed to take as much time as needed to complete the questionnaire. Depressive symptoms were assessed by an experienced research psychiatrist using the Japanese version of the GRID Hamilton Rating Scale for Depression, 17-item version (HDRS) (Hamilton, 1967), which has been demonstrated to show excellent inter-rater reliability (Tabuse et al., 2007).

2.3. Statistical analyses

Gender differences concerning the temperament and the character dimensions have been reported previously (Gutierrez-Zotes et al., 2004; Hansenne et al., 2005; Pelissolo and Lepine, 2000), and thus is one of the major potential confounding factors

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in TCI studies. Therefore, the data for male and female subjects were analyzed separately to avoid gender-dependent influence.

Statistical differences of demographic data among groups were evaluated by the chi-squared test for categorical variables and one-way analysis of variance (ANOVA) for continuous variables. Student t-test was used for comparisons of clinical variables between BPII and UP patients. The scores of the dimensions of TCI were compared among the three diagnostic groups using Kruskal–Wallis test, and thereafter, pairwise comparisons between each group were done using Mann–Whitney tests. Bonferroni method was used to correct for multiple comparisons among the three diagnostic groups. However, since the scale scores of each TCI dimension are intercorrelated and thus are not completely independent measures, we did not apply Bonferroni method for the number of TCI dimensions. Correlations between TCI scores and HDRS scores were assessed using Spearman's rank correlation coefficients. A stepwise logistic regression analysis was used in patients with BPII and UP to determine the optimal model for the prediction of BPII in depressive patients. Prediction models were developed separately for males, females, and both genders combined. The stepwise analysis was conducted as a forward stepping procedure based on a likelihood ratio test, with $p<0.05$ for variable inclusion and $p>0.2$ for exclusion from the model. Variables used as potential predictor variables were age, gender, HDRS score, and scores of 7 dimensions of TCI. Nagelkerke's R^2 was used to approximate the percent of variance explained by the model (Nagelkerke, 1991). The area under the receiver-operating characteristic (ROC) curve (AUC) was also used to determine the predictive power of the logistic model. The predicted probability with the highest Youden index was selected as the optimal cut-off point. Statistical significance was set at two-tailed $p<0.05$. Analyses were performed using the SPSS version 11.0 (SPSS Japan, Tokyo).

3. Results

Table 1 shows demographic and clinical characteristics of the subjects. Age distribution did not differ across the three diagnostic groups. Although the average years of education were significantly higher in the male controls, there was no significant difference between BPII and UP patients. Patients with BPII and UP did not differ significantly in age at onset or in HDRS scores.

The mean scores for the 7 dimensions of TCI are presented in Table 2. Three-group comparisons of BPII, UP patients, and controls revealed differences in all dimensions except for RD in both genders, P in females, and ST in males. Significantly higher HA and lower SD and C scores in BPII and UP patients compared with control subjects were observed in both males and females. Significantly higher P scores in BPII and UP patients were detected only in females. Lower NS in UP patients compared to BPII patients and healthy controls was observed only in females. ST scores in female subjects showed the opposite directions between BPII and UP patients, i.e. BPII patients scored higher and UP patients scored lower in ST compared to controls. The comparison of ST scores in female subjects between the UP patients and controls nearly reached the Bonferroni-corrected significance of $p<0.017$, and the comparisons of BPII patients with control subjects and with UP patients remained significant after Bonferroni correction.

The HDRS scores of UP patients were significantly correlated positively with P in female patients ($rho=0.327$, $p<0.05$) and negatively with SD in male patients ($rho=-0.407$, $p<0.01$). The HDRS in male BPII patient group was significantly correlated positively with C score ($rho=0.497$, $p<0.05$). No other significant correlations were found between TCI and HDRS scores.

Results of the stepwise logistic regression analysis are shown in Table 3. The Nagelkerke R^2 values show that 8.7%, 40.3%, and 24.1% of the variance are explained by the models for males, females, and both genders combined, respectively. The total AUC was significantly greater than 0.5 in models for females and for both genders combined but not for the model for males. These results indicate that the logistic regression model for female patients and for both genders combined appropriately fit the data while the model for male patients does not accurately predict BPII and UP. The following prediction model for female patients was derived:

$$\textit{Predicted Probability} = 1/\{1+\exp(-5.8143+0.129601\times(\textit{NS score})+0.20554\times(\textit{ST score}))\}$$

At the optimal cut-off point of 0.782 determined by the Youden index, the sensitivity and the specificity of differentiating BPII from UP were 89% and 73%, respectively.

4. Discussion

The main findings of the present study could be summarized as follows. Higher HA, lower SD, and lower C scores were observed in both BPII and UP patients when compared with controls. In females, ST scores significantly

Table 1
Demographic and clinical characteristics.

	Male				Female			
	Controls (n=153)	BPII (n=18)	UP (n=45)	Statistical difference	Controls (n=153)	BPII (n=18)	UP (n=45)	Statistical difference
Demographics								
Age, years: mean (S.D.)	35.6 (12.0)	36.1 (8.4)	34.5 (10.8)	F=0.20, p=0.82	37.3 (9.9)	36.5 (13.6)	39.0 (12.3)	F=0.52, p=0.60
Education, years: mean (S.D.)	16.7 (2.9)	16.1 (2.5)	15.5 (2.4)	F=3.5, p=0.032	15.0 (1.8)	14.7 (2.6)	14.5 (2.1)	F=1.6, p=0.21
Clinical features								
Age at onset, years: mean (S.D.)	–	28.5 (8.2)	28.1 (7.1)	t=0.19, p=0.85	–	28.0 (11.7)	30.5 (13.2)	t=0.70, p=0.49
HDRS score: mean (S.D.)	–	15.4 (4.7)	15.8 (5.9)	t=0.20, p=0.84	–	16.6 (5.4)	16.2 (5.8)	t=0.25, p=0.80

BPII: patients with bipolar disorder, UP: patients with unipolar major depressive disorder, S.D.: standard deviation, HDRS: Hamilton Depression Rating Scale.

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Table 2
Comparisons of TCI scores across diagnostic groups.

TCI dimension	Number of items	Gender	Mean score (S.D.)			Kruskal–Wallis test p values	Mann–Whitney test (uncorrected P values)		
			Ctrl (n=153)	BPII (n=18)	UP (n=45)		BPII vs Ctrl	UP vs Ctrl	BPII vs UP
NS	40	Male	20.8 (4.5)	19.5 (5.0)	17.7 (4.3)	<0.001	n.s.	<0.0005	n.s.
		Female	21.0 (4.5)	22.4 (6.3)	17.2 (5.0)	<0.0001	n.s.	<0.0001	0.007
HA	35	Male	17.8 (6.3)	25.4 (6.0)	28.1 (4.0)	<0.0001	<0.0001	<0.0001	n.s.
		Female	18.8 (6.0)	26.6 (4.2)	27.6 (4.9)	<0.0001	<0.0001	<0.0001	n.s.
RD	24	Male	14.7 (3.8)	14.0 (2.6)	14.5 (3.3)	n.s.	n.s.	n.s.	n.s.
		Female	15.8 (3.3)	16.6 (3.2)	15.2 (3.2)	n.s.	n.s.	n.s.	n.s.
P	8	Male	4.8 (1.8)	4.9 (1.7)	4.7 (1.8)	n.s.	n.s.	n.s.	n.s.
		Female	4.1 (1.8)	5.6 (1.7)	5.2 (1.9)	<0.0001	0.001	<0.0005	n
SD	44	Male	28.3 (6.5)	18.7 (8.6)	18.8 (7.2)	<0.0001	<0.0001	<0.0001	n.s.
		Female	28.8 (6.7)	17.3 (7.1)	20.2 (7.6)	<0.0001	<0.0001	<0.0001	n.s.
C	42	Male	28.2 (5.6)	24.4 (6.3)	24.3 (5.7)	<0.0001	0.016	<0.0001	n.s.
		Female	28.9 (4.7)	24.3 (7.5)	26.0 (6.3)	0.002	0.011	0.005	n.s.
ST	42	Male	10.2 (5.0)	11.4 (6.3)	8.7 (4.1)	n.s.	n.s.	n.s.	n.s.
		Female	11.2 (5.6)	14.9 (5.8)	9.1 (4.4)	0.002	0.009	0.046	<0.0005

Ctrl: control subjects, BPII: patients with bipolar II disorder, UP: patients with unipolar major depressive disorder, S.D.: standard deviation, NS: novelty seeking, HA: harm avoidance, RD: reward dependence, P: persistence, SD: self-directedness, C: cooperativeness, ST: self-transcendence.

differed between BPII and UP patients, with higher scores in BPII patients and lower scores in UP patients compared to controls. Patients with UP showed lower NS scores compared to controls in both genders and also to BP patients in females. BPII in depressed females could be predicted using the NS and ST scores.

Consistent with our results, several previous studies have shown higher scores on HA and lower scores on SD and C in patients with UP (Farmer et al., 2003; Hansenne et al., 1999) or in those with BP (Engstrom et al., 2004; Evans et al., 2005) compared to healthy controls. Higher NS (Evans et al., 2005; Nowakowska et al., 2005) and ST (Evans et al., 2005; Loftus et al., 2008; Nowakowska et al., 2005) have also been reported in BP patients compared to controls, though most studies comparing UP patients and controls have shown no significant difference in these two dimensions (Celikel et al., 2009; Evans et al., 2005; Farmer et al., 2003; Hansenne et al., 1999; Kimura et al., 2000; Marijnissen et al., 2002; Nowakowska et al., 2005; Smith et al., 2005). Although the comparison between BPII and UP patients showed similar trends in both genders (i.e., higher NS and ST in BPII), statistical significance between these two disorders was reached only in females. This suggests that, despite the similar tendency in both genders, the differences between diagnostic groups in TCI profiles are more evident in women than in men.

The mood state affects how the subjects describe their personality. Therefore, most previous studies on mood

Table 3
Stepwise logistic regression analyses in patients with BPII and those with UP.

Step and variable	Stepwise analysis							ROC	
	Beta	S.E.	Wald	p value	OR	95% CI	Nagelkerke R^2	AUC	95% CI
Male subjects									
Step 1									
HA	−0.12	0.06	3.8	0.05	0.89	0.79–1.00	0.09	0.61	0.45–0.78
Constant	2.18	1.60	1.8	0.18					
Female subjects									
Step 1									
ST	0.24	0.07	11.1	0.0009	1.27	1.10–1.45	0.32		
Constant	−3.70	0.93	15.7	<0.0001					
Step 2									
NS	0.13	0.06	4.4	0.04	1.14	1.01–1.29	0.40	0.83	0.72–0.95
ST	0.20	0.07	7.8	0.005	1.23	1.06–1.42			
Constant	−5.81	1.49	15.3	<0.0001					
All subjects									
Step 1									
ST	0.16	0.04	14.8	0.0001	1.17	1.09–1.28	0.19		
Constant	−2.70	0.53	26.0	<0.0001					
Step 2									
NS	0.10	0.04	5.3	0.02	1.11	1.02–1.20	0.24	0.74	0.64–0.83
ST	0.14	0.05	9.8	0.002	1.15	1.05–1.27			
Constant	−4.39	0.95	21.3	<0.0001					

S.E.: standard error, Wald: Wald statistic, OR: odds ratio, ROC: receiver-operating characteristic, AUC: area under the curve, CI: confidence interval, NS: novelty seeking, HA: harm avoidance, ST: self-transcendence.

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disorders assessed the personality in euthymic period. However, in order to be used as an aid for differentiating unipolar and bipolar depression in a real world clinical setting, it is required to evaluate TCI scores during depressed period. It has been reported that depressed individuals accurately portray their vulnerability to stress, their joylessness, and their lack of motivation, and that depression-caused changes in the assessed personality trait may reflect their current condition of the individual (Costa et al., 2005). Previous studies reported that severity of depression positively correlates with HA and negatively with SD scores (Farmer et al., 2003; Hansenne et al., 1999; Naito et al., 2000; Richter et al., 2000; Spittlehouse et al., 2010). In our study, however, correlation coefficients of HA and SD scores with HDRS scores did not reach statistical significance except for SD in male UP patients. This discrepancy might be due in part to the fact that we did not include patients with a HDRS score of 7 or less.

The fact that TCI scores are influenced by the severity of depression complicates the interpretation of the findings. However, the prediction model of BPII for female depressed patients in the present study is unlikely to be greatly biased by the severity of depression for several reasons. First, the mean HDRS scores were similar in BPII and UP patients. Secondly, HA and SD, which are previously reported to be influenced by depression severity, were not included in the prediction model for females. Thirdly, the correlation coefficients relating HDRS scores to each TCI score did not significantly differ between female patients with BPII and UP.

The present study is the first to use personality profiles to create a logistic regression model to predict BPII in depressed patients. Previously, Perlis, et al. (Perlis et al., 2006) made a logistic regression prediction model accurately distinguishing BP and UP by including age at onset, number of previous depressive episodes, family history, Montgomery Åsberg Depression Rating Scale (MADRS) scores, and Hamilton Anxiety Scale scores. Their model predicted bipolarity in depressed patients with a sensitivity of 69.0% and a specificity of 94.9%, with the total area under the ROC curve of 0.914. Combining their model with the present one may result in a more accurate prediction model with a wide clinical application.

A major strength of this study was that patients with BPII and UP were both in depressed state with similar severity of depressive symptoms. To our knowledge, this study is the first to compare the TCI score profiles in BPII and UP patients during depressed states. Knowing the differences in TCI profiles in their depressed states could help clinicians to predict bipolarity in depressed patients.

There are several limitations to this study. First, the cross-sectional design did not allow any definitive conclusions as to whether the TCI score profiles of the BPII and UP patients were premorbid or the results of illness onset. Whether the TCI profiles observed here can be generalized to recovered patients needs further investigation. Some UP subjects in this study may go on to experience a manic/hypomanic episode and be rediagnosed as BP, and thus follow-ups are necessary for accurate diagnosis. Secondly, the subjects were recruited through methods such as advertisements and notices, and therefore sampling biases may exist. Thirdly, bipolar patients in our study were limited to BPII. Larger studies are needed to compare the TCI scores between different subtypes of BP or UP. Fourthly, as the BPII and UP patients were limited to those receiving outpatient treatments, our subjects might have been overrepresented by milder forms of illness.

In conclusion, we assessed personality profiles in patients with BPII and UP during depressed period and confirmed that both UP and BPII patients have characteristic personality profiles in common: higher HA, lower SD, and lower C scores assessed with TCI when compared to controls. However, BPII and UP patients differ in some personality profiles, i.e., higher NS and ST in BPII than in UP patients particularly in female patients. Logistic regression analyses showed that BPII and UP could be predicted based on NS and ST scores in female patients. On the other hand, TCI scores were not very helpful for predicting BPII and UP in male patients. Our findings suggest that assessment of personality profiles using TCI in depressed female patients may serve as a useful tool to conveniently differentiate UP and BPII.

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Conflict of interest

The authors declare no conflicts of interest.

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References

Akiskal, H.S., Mendlowicz, M.V., Jean-Louis, G., Rapaport, M.H., Kelsoe, J.R., Gillin, J.C., Smith, T.L., 2005. TEMPS-A: validation of a short version of a self-rated instrument designed to measure variations in temperament. Journal of Affective Disorders 85, 45–52.

Akiskal, H.S., Kilzieh, N., Maser, J.D., Clayton, P.J., Schettler, P.J., Traci Shea, M., Endicott, J., Scheftner, W., Hirschfeld, R.M., Keller, M.B., 2006. The distinct temperament profiles of bipolar I, bipolar II and unipolar patients. Journal of Affective Disorders 92, 19–33.

American Psychiatric Association, 1994. DSM-IV: Diagnostic and Statistical Manual of Mental Disorders, 4th Edition. . Washington D.C.

Celikel, F.C., Kose, S., Cumurcu, B.E., Erkorkmaz, U., Sayar, K., Borckardt, J.J., Cloninger, C.R., 2009. Cloninger's temperament and character dimensions of personality in patients with major depressive disorder. Comprehensive Psychiatry 50, 556–561.

Cloninger, C.R., Svrakic, D.M., Przybeck, T.R., 1993. A psychobiological model of temperament and character. Archives of General Psychiatry 50, 975–990.

Costa Jr., P.T., Bagby, R.M., Herbst, J.H., McCrae, R.R., 2005. Personality self-reports are concurrently reliable and valid during acute depressive episodes. Journal of Affective Disorders 89, 45–55.

de Winter, R.F., Wolterbeek, R., Spinhoven, P., Zitman, F.G., Goekoop, J.G., 2007. Character and temperament in major depressive disorder and a highly anxious-retarded subtype derived from melancholia. Comprehensive Psychiatry 48, 426–435.

Engstrom, C., Brandstrom, S., Sigvardsson, S., Cloninger, R., Nylander, P.O., 2004. Bipolar disorder: I. Temperament and character. Journal of Affective Disorders 82, 131–134.

Evans, L., Akiskal, H.S., Keck Jr., P.E., McElroy, S.L., Sadovnick, A.D., Remick, R.A., Kelsoe, J.R., 2005. Familiality of temperament in bipolar disorder: support for a genetic spectrum. Journal of Affective Disorders 85, 153–168.

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Farmer, A., Mahmood, A., Redman, K., Harris, T., Sadler, S., McGuffin, P., 2003. A sib-pair study of the Temperament and Character Inventory scales in major depression. Archives of General Psychiatry 60, 490–496.

Forty, L., Smith, D., Jones, L., Jones, I., Caesar, S., Cooper, C., Fraser, C., Gordon-Smith, K., Hyde, S., Farmer, A., McGuffin, P., Craddock, N., 2008. Clinical differences between bipolar and unipolar depression. The British Journal of Psychiatry 192, 388–389.

Gutierrez-Zotes, J.A., Bayon, C., Montserrat, C., Valero, J., Labad, A., Cloninger, C.R., Fernandez-Aranda, F., 2004. Temperament and Character Inventory Revised (TCI-R). Standardization and normative data in a general population sample. Actas Españolas de Psiquiatría 32, 8–15.

Hamilton, M., 1967. Development of a rating scale for primary depressive illness. The British Journal of Social and Clinical Psychology 6, 278–296.

Hansenne, M., Reggers, J., Pinto, E., Kjiri, K., Ajamier, A., Ansseau, M., 1999. Temperament and character inventory (TCI) and depression. Journal of Psychiatric Research 33, 31–36.

Hansenne, M., Delhez, M., Cloninger, C.R., 2005. Psychometric properties of the temperament and character inventory-revised (TCI-R) in a Belgian sample. Journal of Personality Assessment 85, 40–49.

Hirano, S., Sato, T., Narita, T., Kusunoki, K., Ozaki, N., Kimura, S., Takahashi, T., Sakado, K., Uehara, T., 2002. Evaluating the state dependency of the Temperament and Character Inventory dimensions in patients with major depression: a methodological contribution. Journal of Affective Disorders 69, 31–38.

Hirschfeld, R.M., Lewis, L., Vornik, L.A., 2003. Perceptions and impact of bipolar disorder: how far have we really come? Results of the national depressive and manic-depressive association 2000 survey of individuals with bipolar disorder. The Journal of Clinical Psychiatry 64, 161–174.

Kijima, N., Saito, R., Takeuchi, M., Yoshino, A., Ono, Y., Kato, M., Kitamura, T., 1996. Cloninger's seven-factor model of temperament and character and Japanese version of Temperament and Character Inventory (TCI) Article in Japanese Archives of Psychiatric Diagnostics and Clinical Evaluation 7, 379–399.

Kijima, N., Tanaka, E., Suzuki, N., Higuchi, H., Kitamura, T., 2000. Reliability and validity of the Japanese version of the Temperament and Character Inventory. Psychological Reports 86, 1050–1058.

Kimura, S., Sato, T., Takahashi, T., Narita, T., Hirano, S., Goto, M., 2000. *Typus melancholicus* and the Temperament and Character Inventory personality dimensions in patients with major depression. Psychiatry and Clinical Neurosciences 54, 181–189.

Loftus, S.T., Garno, J.L., Jaeger, J., Malhotra, A.K., 2008. Temperament and character dimensions in bipolar I disorder: a comparison to healthy controls. Journal of Psychiatric Research 42, 1131–1136.

Lovdahl, H., Boen, E., Falkum, E., Hynnekleiv, T., Malt, U.F., 2010. Temperament and character in patients with bipolar II disorder and recurrent brief depression. Comprehensive Psychiatry 51, 607–617.

Marijnissen, G., Tuinier, S., Sijben, A.E., Verhoeven, W.M., 2002. The temperament and character inventory in major depression. Journal of Affective Disorders 70, 219–223.

Matsudaira, T., Kitamura, T., 2006. Personality traits as risk factors of depression and anxiety among Japanese students. Journal of Clinical Psychology 62, 97–109.

Mendlowicz, M.V., Akiskal, H.S., Kelsoe, J.R., Rapaport, M.H., Jean-Louis, G., Gillin, J.C., 2005. Temperament in the clinical differentiation of depressed bipolar and unipolar major depressive patients. Journal of Affective Disorders 84, 219–223.

Mitchell, P.B., Wilhelm, K., Parker, G., Austin, M.P., Rutgers, P., Malhi, G.S., 2001. The clinical features of bipolar depression: a comparison with matched major depressive disorder patients. The Journal of Clinical Psychiatry 62, 212–216 quiz 217.

Nagelkerke, N., 1991. A note on a general definition of the coefficient of determination. Biometrika 2.

Naito, M., Kijima, N., Kitamura, T., 2000. Temperament and Character Inventory (TCI) as predictors of depression among Japanese college students. Journal of Clinical Psychology 56, 1579–1585.

Nowakowska, C., Strong, C.M., Santosa, C.M., Wang, P.W., Ketter, T.A., 2005. Temperamental commonalities and differences in euthymic mood disorder patients, creative controls, and healthy controls. Journal of Affective Disorders 85, 207–215.

Otsubo, T., Tanaka, K., Koda, R., Shinoda, J., Sano, N., Tanaka, S., Aoyama, H., Mimura, M., Kamijima, K., 2005. Reliability and validity of Japanese version of the Mini-International Neuropsychiatric Interview. Psychiatry and Clinical Neurosciences 59, 517–526.

Pelissolo, A., Lepine, J.P., 2000. Normative data and factor structure of the Temperament and Character Inventory (TCI) in the French version. Psychiatry Research 94, 67–76.

Perlis, R.H., Brown, E., Baker, R.W., Nierenberg, A.A., 2006. Clinical features of bipolar depression versus major depressive disorder in large multicenter trials. The American Journal of Psychiatry 163, 225–231.

Richter, J., Eisemann, M., Richter, G., 2000. Temperament and character during the course of unipolar depression among inpatients. European Archives of Psychiatry and Clinical Neuroscience 250, 40–47.

Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., Dunbar, G.C., 1998. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. The Journal of Clinical Psychiatry 59 (Suppl. 20), 22–33 quiz 34–57.

Smith, D.J., Duffy, L., Stewart, M.E., Muir, W.J., Blackwood, D.H., 2005. High harm avoidance and low self-directedness in euthymic young adults with recurrent, early-onset depression. Journal of Affective Disorders 87, 83–89.

Spittlehouse, J.K., Pearson, J.F., Luty, S.E., Mulder, R.T., Carter, J.D., McKenzie, J.M., Joyce, P.R., 2010. Measures of temperament and character are differentially impacted on by depression severity. Journal of Affective Disorders 126, 140–146.

Tabuse, H., Kalali, A., Azuma, H., Ozaki, N., Iwata, N., Naitoh, H., Higuchi, T., Kanba, S., Shioe, K., Akechi, T., Furukawa, T.A., 2007. The new GRID Hamilton Rating Scale for Depression demonstrates excellent inter-rater reliability for inexperienced and experienced raters before and after training. Psychiatry Research 153, 61–67.

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Resequencing and Association Analysis of the *KALRN* and *EPHB1* Genes And Their Contribution to Schizophrenia Susceptibility

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Background: **Our genome-wide association study of schizophrenia found association signals at the Kalirin gene (*KALRN*) and EPH receptor B1 gene (*EPHB1*) in a Japanese population. The importance of these synaptogenic pathway genes in schizophrenia is gaining independent supports. Although there has been growing interest in rare (<1%) missense mutations as potential contributors to the unexplained heritability of schizophrenia, there are no population-based studies targeting rare (<1%) coding mutations with a larger effect size (eg, OR >1.5) in *KALRN* or *EPHB1*. *Methods and Results:*** **The present study design consisted of 3 phases. At the discovery phase, we conducted resequencing analyses for all exon regions of *KALRN* and *EPHB1* using a DNA microarray–based method. Seventeen rare (<1%) missense mutations were discovered in the first sample set (320 schizophrenic patients). After the prioritization phase based on frequencies in the second sample set (729 cases and 562 controls), we performed association analyses for each selected mutation using the third sample set (1511 cases and 1517 controls), along with a combined association analysis across all selected mutations. In *KALRN*, we detected a significant association between schizophrenia and P2255T (OR = 2.09, corrected *P* = .048, 1 tailed); this was supported in the combined association analysis (OR = 2.07, corrected *P* = .006, 1 tailed). We found no evidence of association of *EPHB1* with schizophrenia. *In silico* analysis indicated the functional relevance of these rare missense mutations.** ***Conclusion:*** **We provide evidence that multiple rare (<1%) missense mutations in *KALRN* may be genetic risk factors for schizophrenia.**

Key words: synaptogenic pathway/rare missense mutations/GWAS/Japanese population

Introduction

Schizophrenia is a genetically heterogeneous disorder with heritability estimated at up to 80%.[1] According to a recent simulation based on genome-wide association study (GWAS) datasets, a highly polygenic model involving a number of common variants of very small effect may explain more than one-third of the total variation in risk of schizophrenia.[2] On the other hand, interest has been growing in rare variants as potential contributors to the unexplained heritability of schizophrenia.[3] This is partly triggered by recent studies establishing an important role for rare genomic copy number variants (CNVs) in the etiology of schizophrenia.[4] Another potential genetic variation to explain the remaining heritability is rare missense mutations. Kryukov et al[5] reported that ~20% of new (de novo) missense mutations in humans result in a loss of function, whereas ~53% have mildly deleterious effects and ~27% are effectively neutral with respect to phenotype by a combined analysis of mutations causing human Mendelian diseases, mutations driving human-chimpanzee sequence divergence, and systematic data on human genetic variation. Their results were supported by an independent study.[6] Because the pressure of purifying selection acting on the mildly deleterious mutations is weak, their cumulative high frequency in the human population is being maintained

by "mutation-selection balance." This provides support to a speculation that the accumulation of mildly deleterious missense mutations in individual human genomes can be a genetic basis for complex diseases.[5] The importance of rare missense mutations in schizophrenia is demonstrated by a study of the *ABCA13* gene in which multiple rare (<1%) coding variants were associated with schizophrenia.[7]

We recently performed a GWAS for schizophrenia in a Japanese population.[8] Although single locus analysis did not reveal genome-wide support for any locus, a shared polygenic risk of schizophrenia between the Japanese and the Caucasian samples was confirmed. In our GWAS, association signals were detected at the regions of the Kalirin gene (*KALRN*) on 3q21.2 and the EPH receptor B1 gene (*EPHB1*) on 3q21-q23, both of which are in the same synaptogenic pathway[9] (supplementary figure S1). Associations of each gene with schizophrenia have recently received support from independent GWASs in different populations.[10,11] Furthermore, a rare de novo CNV overlapping with the *EPHB1* gene locus was detected in a patient with schizophrenia.[12]

KALRN is a large neuronal dual Rho guanine nucleotide exchange factor (GEF) that activates small guanosine triphosphate–binding proteins of the Rho family, including Rac1.[13] This activation enables KALRN to regulate neurite initiation, axonal growth, dendritic morphogenesis, and spine morphogenesis. Consistent with its biological function, KALRN is a key factor responsible for reduced densities of dendritic spines on pyramidal neurons in the dorsolateral prefrontal cortex (DLPFC)[14] observed in postmortem brains from schizophrenic patients. The messenger RNA expression level of *KALRN* is significantly reduced in DLPFC of patients with schizophrenia and strongly correlated with spine density.[15] In addition, *KALRN*-knockout mice not only exhibit spine loss and reduced glutamatergic transmission in the frontal cortex but also schizophrenia-like phenotypes including robust deficits in working memory, sociability, prepulse inhibition, and locomotor hyperactivity reversible by clozapine, an atypical antipsychotic.[16] These synaptic and behavioral dysfunctions are apparent during young adulthood in mice (12 weeks old), which coincides with the onset of schizophrenia in patients. Notably, Disrupted-in-Schizophrenia 1, a prominent schizophrenia risk factor, was shown to be involved in the maintenance of spine morphology and function by regulating access of KALRN to Rac1.[17] *EPHB1* belongs to a receptor tyrosine kinase family and controls multiple aspects of neuronal development, including synapse formation and maturation, as well as synaptic structural and functional plasticity. In neurons, activation of EphB receptors by its ligand B-type ephrins induces the rapid formation and enlargement of dendritic spines, as well as rapid synapse maturation. One of the downstream effectors of ephrinB/EphB signaling is KALRN. In young hippocampal neurons, KALRN is reported to play an important role in the maturation of synapses induced by trans-synaptic ephrinB/EphB signaling.[18]

According to the above-mentioned study,[5] most missense mutations with a frequency of <1% are mildly deleterious, indicating that a low frequency of missense mutation per se can serve as a strong predictor of a deleterious effect of variants. Therefore, the working hypothesis of the present study is that rare (<1%) missense or nonsense mutations with a larger effect size (eg, OR >1.5) in *KALRN* and *EPHB1* may be genetic risk factors for schizophrenia. Recently, a DNA microarray–based resequencing method has been developed to enable accurate and rapid resequencing analysis of candidate genes.[19] Using this system, we conducted resequencing analyses for all exon regions of *KALRN* and *EPHB1* in 320 schizophrenic patients and found evidence that rare (<1%) missense mutations in *KALRN* are significantly associated with schizophrenia using the 3-phase study design.

Methods and Materials

Subjects

Three sample sets were used in this study. The first sample set, comprising 320 schizophrenic patients (mean age, 54.2 ± 14.1 years, 49.1% male), with long-term hospitalization for severe symptoms, was used to search for rare missense or nonsense mutations. We used the first sample set for mutation screenings because patients with extreme phenotypes (severe symptoms) can be expected to carry more deleterious mutations.[20] The second sample set, including 729 cases (45.4 ± 15.1 years, 52.2% male) and 562 controls (44.0 ± 14.4 years, 49.8% male), was used to prioritize detected functional variants for subsequent association analyses. The third sample set, including 1511 cases (45.9 ± 14.0 years, 49.6% male) and 1517 controls (46.0 ± 14.6 years, 49.6% male), was used for association analyses. Age and gender were matched in the second and third sample sets, respectively. All patients were diagnosed according to *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*, criteria, and controls were evaluated using unstructured interviews to exclude individuals with history of mental disorders. Detailed information regarding diagnostic procedures is available elsewhere.[21] All subjects were ethnically Japanese and provided written informed consent. This study was approved by the ethics committees at each participating university.

Array Design for Resequencing Analyses

We used the Affymetrix GeneChip CustomSeq Resequencing Array (Affymetrix, Santa Clara, California) for exon sequencing in the first sample set. These arrays rely on allele-specific hybridization for determining DNA

sequence.[19] Each individual nucleotide of both the sense and the antisense DNA strands is interrogated with four 25-mer probes that differ only with respect to the central position (A, C, G, and T). According to Affymetrix's Custom-Seq Array Design Guide, we designed arrays covering all exon regions of *KALRN* and *EPHB1* (Ensembl release 52 [Human CCDS set]; Transcript: ENST00000360013, ENST00000240874, and ENST00000291478 for *KALRN*; ENST00000398015 for *EPHB1*). Because the principle of the resequencing arrays is based on hybridization, it is necessary to avoid cross-hybridization for accurate resequencing. For this purpose, we removed repetitive elements and highly homologous sequences from the array design.

Array-Based Resequencing

The experiments were conducted according to the manufacturer's instructions (supplementary figure S2). Genomic DNA was extracted from peripheral blood using standard methods. To generate enough target-enriched subject material for hybridization to the arrays, we generated 47 and 14 amplicons per sample for *KALRN* and *EPHB1*, respectively, using long-range polymerase chain reaction (PCR). The PCR conditions were as follows: 94°C for 2 minutes followed by 30 cycles consisting of 94°C for 15 seconds, 68°C for 3 minutes, followed by a final extension of 68°C for 8 minutes, using TaKaRa LA Taq™ (Takara Bio, Otsu, Shiga, Japan). Each PCR product was quantified using PicoGreen (Molecular Probes, Eugene, Oregon), pooled in an equimolar fashion. The PCR products were then purified, fragmented, labeled, and hybridized to the arrays, following the protocol. Finally, the arrays were washed and stained using the GeneChip Fluidics Station 450 (Affymetrix) and scanned using the GeneChip Scanner 3000 (Affymetrix). The data were analyzed using the GeneChip Operating Software (GCOS; Affymetrix), the GeneChip Sequence Analysis Software (GSEQ; Affymetrix), and SeqC (JSI Medical Systems, Kippenheim, Germany; http://www.jsi-medisys.de/html/products/SeqC/SeqC.htm) to automate the generation of sequence and genotype calls from the intensity data. In this study, around 17 kb was sequenced per sample, meaning that more than 5.4 Mb was sequenced in total. All missense mutations presented in this study were confirmed using both Sanger sequencing and Custom TaqMan SNP genotyping assays (Applied Biosystems, Foster City, California).

Association Analysis of Each Missense Mutation

Although the rare (<1%) missense mutations were originally discovered among 320 schizophrenic patients, it was possible that a portion of them might have neutral or protective effects.[5] In addition, it was necessary to reduce the number of statistical tests for multiple comparison problems. To accomplish this, we prioritized rare (<1%) deleterious variants for subsequent association analyses based on the frequencies in the second case-control sample set because rare deleterious variants relevant to schizophrenia can be assumed to have higher frequency in cases than in controls. The criteria for prioritization were as follows: (1) frequencies of mutations were <1% in controls and (2) frequencies of mutations were higher in cases (ie, OR > 1). Mutations not detected in the second sample set were not followed up in this analysis. The frequencies of such mutations can be so low (<0.0005) that the results of association analyses are unlikely to be statistically significant in our sample size. For mutations meeting the above criteria, we conducted association analyses with schizophrenia using the third sample set. Genotyping was conducted by Custom TaqMan SNP genotyping assays (Applied Biosystems). For quality control, samples with missing call rates of 10% or higher were excluded from the analyses.

Combined Association Analyses

In general, it is difficult to establish an association of a rare mutation with a phenotype because statistical power is limited by low population frequency and because the number of rare variants requires a strict multiple test correction. Therefore, we conducted combined association analyses across rare mutations observed in each gene in the third sample set, comparing the number of mutations in cases with the number in controls. The criteria for mutations included in these analyses were same as the above criteria with 1 exception: Mutations not detected in the second sample set were included in the combined association analyses.

In Silico Analysis

The potential influence of missense mutations was evaluated using PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) and[22] PMut (http://mmb2.pcb.ub.es:8080/PMut/)[23] softwares. PolyPhen-2 uses 8 sequence-based and 3 structure-based predictive features and compares a property of the wild-type allele and the corresponding property of the mutant allele. PolyPhen-2 trained on HumDiv datasets is reported to achieve true positive prediction rates of 92% with a false-positive rate of 20%.[22] A mutation is appraised qualitatively as benign, possibly damaging, or probably damaging based on naive Bayes posterior probability that a given mutation is damaging. PMut also allows the fast and accurate prediction (~80% success rate in humans) of the pathological character of missense mutations based on the use of neural networks. The final output is a pathogenicity index ranging from 0 to 1 (indexes >0.5 signal pathological mutations).

We also examined evolutionary conservation of the mutated residues and surrounding amino acids. Multiple sequence alignment of human *KALRN* or *EPHB1* with 6 orthologs was performed for this purpose.

Power Calculation

Power calculation was performed with a power calculator called CaTS (http://www.sph.umich.edu/csg/abecasis/CaTS/).[24] Power was estimated under the following parameter assumptions with respect to association test statistics: genetic relative risk = 2, prevalence of disease = 0.01, risk allele frequency = the values frequency observed in controls, and $\alpha = .05$; a multiplicative model was used.

Statistical Analysis

For the association analysis of each variant, Fisher exact test was used to examine whether rare deleterious variants were significantly overrepresented in the patient group rather than the control group.

A combined association test was performed following a previous study.[7] In brief, to account for variable sample size, sample size was adjusted to $N=n/(\sum(1/Ni))$, where Ni is the sample size at the ith variant, and n is the number of variants. The number of observed variants was adjusted as $\sum(pi)\times N$, where pi is the frequency of the ith variant. Fisher exact test was used in this test as well to examine an overrepresentation of rare deleterious missense mutations in the patient group rather than control group.

All statistical tests were 1 tailed, and a P value less than 0.05 was considered significant. Bonferroni correction was used for solving multiple testing problems.

Results

Discovery of Mutations

We detected 12 and 6 missense mutations with a frequency of <5% in *KALRN* and *EPHB1*, respectively, among 320 cases in the first sample set (table 1). All but 2 mutations (N2973S in *KALRN* and T981M in *EPHB1*) were novel. All mutations were validated by both Sanger sequencing and Custom TaqMan SNP genotyping assays. In the first sample set, 2 patients were compound heterozygotes for rare missense mutations in the 2 genes. One patient had R410H in *KALRN* and R905C in *EPHB1*. The other had A2382V in *KALRN* and D375N in *EPHB1*. There were no clinical characteristics shared between these patients. No nonsense mutations were identified in this study.

Association Analysis of Each Missense Mutation

In the prioritization phase using the second sample set, T1207M and P2255T in *KALRN* and R637C and R905C in *EPHB1* showed a higher frequency in cases than in controls (table 1). Seven missense mutations (R410H, Q770K, and A2382V in *KALRN* and F151S, D375N, D577N, and T981M in *EPHB1*) were not detected. The frequency of P1695Q was more than 4% both in cases and in controls. Based on our criteria, we selected 4 missense mutations (T1207M and P2255T in *KALRN* and R637C and R905C in *EPHB1*) for subsequent association analyses using the third sample set.

In the third phase, P2255T showed a nominally significant association with schizophrenia (OR = 2.09, P = .012) in the third sample set (table 2). This remained significant after correction for multiple testing of 4 variants (corrected P = .048). T1207M in *KALRN* and R637C and R905C in *EPHB1* were also more frequent in cases, although differences were not significant.

We excluded mutations not detected in the second sample set from this analysis. This was supported by a power analysis showing that the third sample set had only 10% power in analysis of very rare mutations.

Combined Association Analysis

In addition to 4 mutations (T1207M and P2255T in *KALRN* and R637C and R905C in *EPHB1*), 7 very rare mutations (R410H, Q770K, and A2382V in *KALRN* and F151S, D375N, D577N, and T981M in *EPHB1*), which were not detected in the second samples set, were included in the combined association analysis. A global comparison of the frequencies of 5 selected mutations in *KALRN* between cases and controls in the third sample set showed a significant increase in frequency in schizophrenic patients (OR = 2.07, P = .003) (table 3). This remained significant after correction for multiple testing (corrected P = .006). On the other hand, a global comparison of the frequencies of 6 selected mutations in *EPHB1* did not show a significant difference (OR = 1.09, P = .438).

In Silico Analysis

Results of *in silico* analysis are shown in table 4. All missense mutations but A2382V in *KALRN* were predicted to have functional relevance by PolyPhen-2 or PMut software.

A multiple alignment of the region of *KALRN* or *EPHB1* containing rare missense mutations with 6 orthologs is shown in table 4. Most of the rare missense mutations showed a high degree of amino acid conservation in different species.

Discussion

In this study, we conducted resequencing analyses for the 2 synaptogenic pathway genes (*KALRN* and *EPHB1*) in schizophrenia using a DNA microarray–based method. After resequencing more than 5.4 Mb, we discovered 17 rare (<1%) missense mutations in *KALRN* or *EPHB1* and detected a significant association between schizophrenia and P2255T in *KALRN*, as well as in the combined association analysis for *KALRN*. These findings are consistent with an estimation that most rare (<1%) missense mutations are mildly deleterious and are associated with a heterozygous fitness loss.[5]

Table 1. *KALRN* And *EPHB1* Missense Mutations Identified in The First Sample Set And Their Frequencies in The Second Sample Set

Gene	Genomic Position	Base Change	dbSNP Reference	AA Change	First Sample Set		Second Sample Set				
							Genotype Counts		Mutation Frequency		
					Homo	Hetero	SCZ	CONT	SCZ	CONT	OR >1
KALRN	125527659	G → A	ss250607852	R410H	0	1	0/0/701	0/0/541	0	0	
KALRN	125531474	T → A	ss250607853	L452Q	0	1	0/1/709	0/2/541	0.0007	0.0018	
KALRN	125600376	C → A	ss250607854	Q770K	0	1	0/0/706	0/0/544	0	0	
KALRN	125656787	C → T	ss250607855	T1207M	0	1	0/2/705	0/1/542	0.0014	0.0009	+
KALRN	125764534	C → A	ss250607856	P1695Q	0	1	0/59/636	1/44/492	0.0425	0.0428	
KALRN	125764599	A → T	ss250607857	M1717L	0	1	0/0/705	0/1/540	0	0.0009	
KALRN	125860927	G → A	ss250607858	R2049K	0	1	0/1/696	0/1/540	0.0007	0.0009	
KALRN	125873259	C → A	ss250607859	P2255T	0	7	1/14/684	0/7/536	0.0114	0.0064	+
KALRN	125873289	C → T	ss250607860	P2265S	1	0	0/6/701	0/7/533	0.0042	0.0065	
KALRN	125873382	G → T	ss250607861	G2296C	0	1	0/1/703	0/1/542	0.0007	0.0009	
KALRN	125876103	C → T	ss250607862	A2382V	0	1	0/0/697	0/0/540	0	0	
KALRN	125920964	A → G	rs16835896	N2973S	0	3	0/3/698	0/6/538	0.0021	0.0055	
EPHB1	136153231	T → C	ss252863894	F151S	0	1	0/0/710	0/0/543	0	0	
EPHB1	136334407	G → A	ss252863895	D375N	0	1	0/0/708	0/0/544	0	0	
EPHB1	136368508	G → A	ss252863896	D577N	0	1	0/0/707	0/0/544	0	0	
EPHB1	136394134	C → T	ss252863897	R637C	0	2	1/1/707	0/2/541	0.0021	0.0018	+
EPHB1	136450890	C → T	ss252863898	R905C	0	3	0/9/695	0/1/543	0.0064	0.0009	+
EPHB1	136460639	C → T	rs56186270	T981M	0	2	0/0/706	0/0/541	0	0	

Note: Genomic position based on NCBI build 36, chromosome 3. Amino acid changes based on NCBI Reference Sequence NP_001019831.2 (2986 aa) for KALRN and NP_004432.1 (984 aa) for EPHB1. All but N2973S (rs16835896) and T981M (rs56186270) are novel. AA change, amino acid change; dbSNP, Single Nucleotide Polymorphism Database; Homo, homozygote; Hetero, heterozygote; SCZ, schizophrenia; CONT, control; NCBI, National Center for Biotechnology Information.

Schizophrenia is a genetically heterogeneous disorder, with both very rare variants with a high effect size (eg, CNVs in 1q21.1, 15q13.3) and common variants with a low effect size (eg, rs1344706 in *ZNF804A*) involved in its genetic architecture. In this frequency-effect size spectrum, P2255T (OR: ~2, risk allele frequency in controls: ~0.005) is located between the CNV in 1q21.1 (OR: ~10, frequency in controls: ~0.0001)[25] and rs1344706[T] in *ZNF804A* (OR: ~1.1, risk allele frequency in controls: ~0.6),[26] both of which have been recently associated with schizophrenia. The relatively modest effect size of P2255T compared with that of the above CNVs can be attributable to the difference in the effect of each variant on gene(s): Although CNVs strongly influence the expression of multiple genes, missense mutations in *KALRN* are presumed to have limited effects on KALRN function. P2255T is located in the evolutionally conserved proline-rich region between the C-terminal GEF and SH3 domains[27] and is surrounded by 2 nearby phosphorylation sites (S2237 and S2262), according to Human Protein Reference Database (http://www.hprd.org/index_html)[28] (figure 1). *In silico* analysis with PhosphoMotif Finder[29] shows that T2255 itself can be recognized and phosphorylated by many kinases, suggesting functional implications of P2255T (figure 1). In addition, *in silico* analysis predicts that phosphorylation of T2255 will induce that of nearby S2253. Thus, P2255T may greatly change the phosphorylation status in a narrow

Table 2. Association Analyses of Each Missense Mutation in the Third Sample Set

	AA Change	Third Sample Set					
		Genotype Counts		Mutation Frequency			
		SCZ	CONT	SCZ	CONT	OR	*P* Value
KALRN	T1207M	0/7/1477	0/3/1482	0.0024	0.0010	2.34	.171
KALRN	P2255T	0/31/1448	0/15/1473	0.0105	0.0050	2.09	.012
EPHB1	R637C	0/4/1477	0/4/1478	0.0014	0.0014	1.00	.636
EPHB1	R905C	0/15/1458	0/12/1466	0.0051	0.0041	1.26	.347

Note: Abbreviations are explained in the first footnote to table 1. *P* values were calculated by Fisher exact test (1 tailed).

Table 3. Combined Association Analysis in The Third Sample Set

Gene	AA Change	Third Sample Set				Combined Analysis	
		Genotype Counts		Mutation Frequency		Gene Based	
		SCZ	CONT	SCZ	CONT	OR	*P* value
KALRN	R410H	0/0/1481	0/0/1484	0	0	2.07	.003
KALRN	Q770K	0/0/1486	0/0/1490	0	0		
KALRN	T1207M	0/7/1477	0/3/1482	0.0024	0.0010		
KALRN	P2255T	0/31/1448	0/15/1473	0.0105	0.0050		
KALRN	A2382V	0/7/1473	0/4/1480	0.0024	0.0013		
EPHB1	F151S	0/0/1478	0/0/1484	0	0	1.09	.438
EPHB1	D375N	0/0/1483	0/0/1490	0	0		
EPHB1	D577N	0/0/1486	0/2/1483	0	0.000673		
EPHB1	R637C	0/4/1477	0/4/1478	0.0014	0.0014		
EPHB1	R905C	0/15/1458	0/12/1466	0.0051	0.0041		
EPHB1	T981M	0/5/1481	0/4/1484	0.0017	0.0013		

Note: Abbreviations are explained in the first footnote to table 1. *P* values were calculated by Fisher exact test (1 tailed).

region between the C-terminal GEF and SH3 domain. A protein with multiple phosphorylated sites like KALRN can be assumed to have an exponential number of phospho-forms, and individual phospho-forms may have distinct biological effects. The diffuse distribution of these phospho-forms at steady state enables the phosphoproteome to encode information and flexibly respond to varying demands.[30] Thus, it is conceivable that P2255T may influence such plasticity in KALRN by changing the number of phosphorylated sites. Interestingly, detailed examination of clinical information from the first sample set, which was uniquely available to us, revealed that congenital or early-onset vascular disease was observed in 5 of 7 cases with P2255T (supplementary table S1). Because *KALRN* may represent a candidate gene for vascular diseases,[31,32] it is tempting to speculate that P2255T may be a potential risk factor for vascular disease.

In addition to P2255T, we detected multiple rare (<1%) missense mutations in *KALRN* or *EPHB1*. Such variants are not sufficiently frequent to be covered by GWAS nor do they have sufficiently large effect sizes to be detected by linkage analysis in family studies. For modest effect sizes, it is suggested that association testing may require composite tests of overall mutational load,

Table 4. Results of *In Silico*/Conservation Analysis

KALRN							
Analysis		R410H	Q770K	T1207M	P2255T	A2382V	
PolyPhen-2		Probably damaging	Probably damaging	Probably damaging	Benign	Benign	
PMut		Pathological	Neutral	Pathological	Pathological	Neutral	
Conservation analysis	Human (NP_001019831.2)	LDE**R**STI	IFL**Q**LRI	IHATE**I**R	RSQPAR**L**	SILAPL**T**	
	Chimpanzee (XP_516703.2)	LDE**R**STI	IFL**Q**LRI	IHATE**I**R	RSQPAR**L**	SILAPL**T**	
	Dog (XP_535768.2)	LDE**R**STI	IFL**Q**LRI	IHATE**I**R	RSQPS**R**V	SVLAPL**T**	
	Cattle (XP_001790302.1)	LDE**R**STI	IFL**Q**LRI	IHATE**I**R	RSQPAR**V**	SILTPL**T**	
	Mouse (XP_001481079.1)	LDE**R**STI	IFL**Q**LRI	IHATE**I**R	RSQPP**R**V	SILAPL**A**	
	Rat (NP_114451.1)	LDE**R**STI	IFL**Q**LRI	IHATE**I**R	RSQPP**R**V	SILAPL**T**	
EPHB1							
Analysis		F151S	D375N	D577N	R637C	R905C	T981M
PolyPhen-2		Benign	Probably damaging	Possibly damaging	Probably damaging	Probably damaging	Probably damaging
PMut		Pathological	Neutral	Neutral	Pathological	Pathological	Pathological
Conservation analysis	Human (NP_004432.1)	QVD**F**GGR	RCD**D**NVE	VYS**D**KLQ	YKG**R**LKL	LLD**R**SIP	QSPTAMA
	Chimpanzee (XP_001150963.1)	QVD**F**GGR	RCD**D**NVE	LLVEQWQ	YKG**R**LKL	LLD**R**SIP	QSPTAMA
	Dog (XP_542791.2)	QVD**F**GGR	RCD**D**NVE	VYS**D**KLQ	YKG**R**LKL	LLD**R**SIP	QSPTTMA
	Cattle (XP_614602.4)	QVD**F**GGR	RCD**D**NVE	VYS**D**KLQ	YKG**R**LKL	LLD**R**SIP	QSPTAMA
	Mouse (NP_775623.2)	QVD**F**GGR	RCD**D**NVE	AYS**D**KLQ	YKG**R**LKL	LLD**R**SIP	QSPSVMA
	Rat (XP_217250.1)	QVD**F**GGR	RCD**D**NVE	VYS**D**KLQ	YKG**R**LKL	LLD**R**SIP	QSPSVMA

Note: The bold are the mutated amino acids.

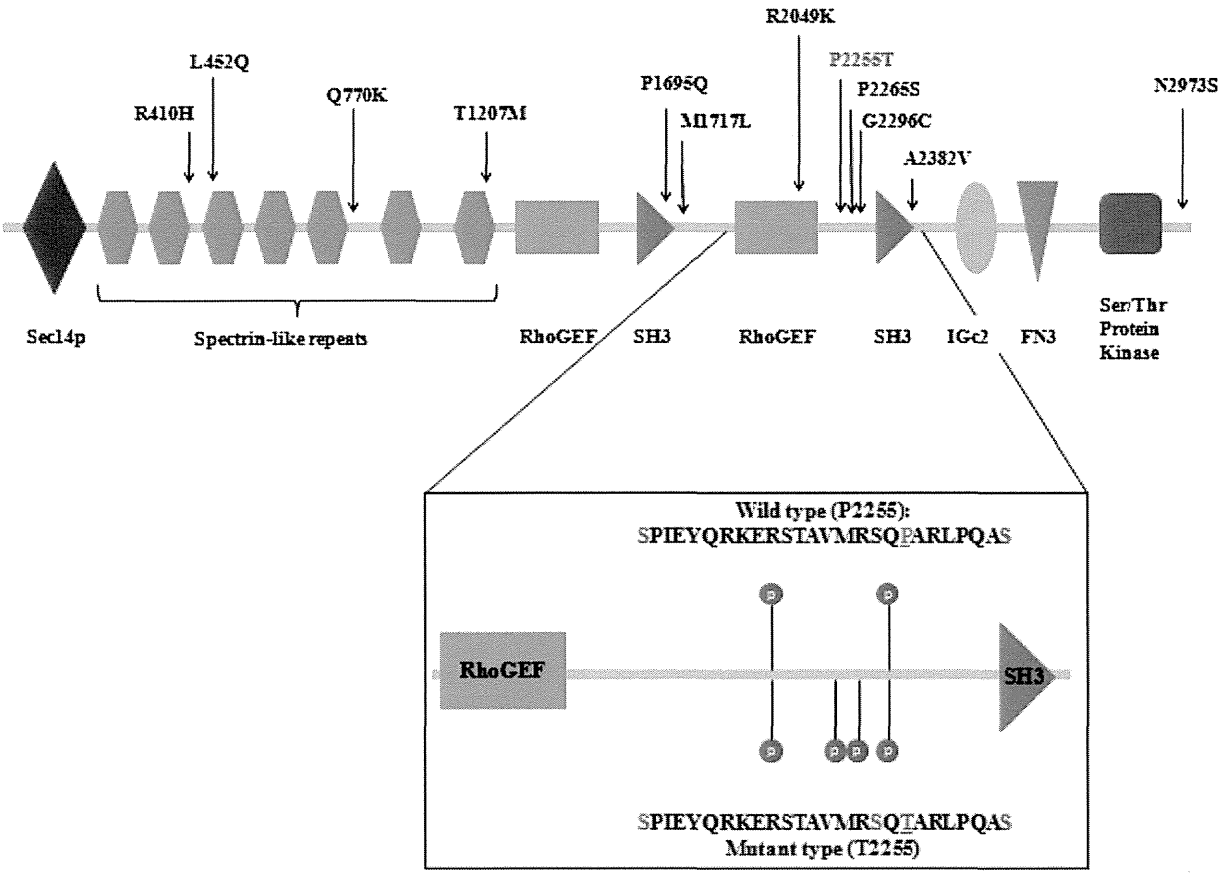

Fig. 1. Rare Missense Mutations in *KALRN* and Change in Phosphorylation Status by P2255T.

comparing frequencies of mutations of potentially similar functional effect in cases and controls. Thus, we also performed combined association analyses for *KALRN* or *EPHB1* and found evidence that multiple rare (<1%) missense mutations in *KALRN* as a whole are associated with schizophrenia. This finding is supported by *in silico* analyses showing that most of the mutations are predicted as being of functional relevance and that they are located in evolutionally conserved regions. In contrast, there were no significant differences in the cumulative frequencies of rare missense mutations in *EPHB1*. This might be due to a type II error. The cumulative frequency of rare mutations of *EPHB1* in controls is almost same as the one of *KALRN* in controls (0.0075 vs 0.0073), indicating that cumulative effect size of rare missense mutations in *EPHB1* may be smaller than the one in *KALRN*. In the mammalian genome, there are 5 different EphB receptors (EphB1, EphB2, EphB3, EphB4, and EphB6), with a high similarity at the amino acid level. Analysis of double and triple knockout mice lacking EphB1, EphB2, and EphB3 in different combinations revealed that EphBs have functional redundancy even though all these EphBs are responsible for spine morphogenesis and synapse formation to varying degrees.[33] This is in contrast with the drastic phenotypes observed in *KALRN*-knockout mice.[16] Therefore, biological effects of rare missense mutations in *EPHB1* may be compensated for by other intact *EPHBs*. This might lower the ORs of rare missense mutations in *EPHB1*. Given that all the mutations detected in *EPHB1* were predicted to have pathogenicity by PolyPhen-2 or PMut, a larger-scale case-control study with sufficient power may provide a significant result in a combined analysis for *EPHB1*.

One important aspect of the present study is that we found rare mutations associated with schizophrenia in the *KALRN* gene, in which GWASs detected association signals for schizophrenia. Several studies have recently reported the 1 gene may harbor both rare and common variants associated with the same diseases, including schizophrenia,[34] type 2 diabetes,[35] and hypertriglyceridemia.[36] Given that the cost of whole-genome sequencing is still high to search for rare mutations, resequencing analyses for genes with support from GWAS might be a better strategy for detection of rare mutations with larger effect size.

There are several limitations to this study. First, we could not conduct segregation analyses for mutations due to limited access to family members. Furthermore, given the modest risk (OR ~2), these mutations would show incomplete penetrance. In fact, it is reported that penetrance estimates of CNVs at 1q21.1 and 15q13.3,

both of which show higher ORs, are 0.061 and 0.074, respectively.[25] Therefore, a population-based study is a better choice to evaluate genetic associations for missense mutations with modest risk.[37] The second limitation is population stratification. Although a Japanese population is considered relatively homogenous, small population stratifications may have influenced our findings.[38] However, we believe that the recruitment of subjects in local regions minimized this concern. Third, we did not conduct functional analyses for detected missense mutations. The detailed effects of these mutations on the pathophysiology of schizophrenia need to be examined in a future study. Fourth, our resequencing analyses were not comprehensive in terms of the kind of variants and the number of genes. In other words, the present study did not cover indels or CNVs because of the methodological limitation of the DNA microarray–based method. Because these classes of variants could have a more profound effect on protein function, their genetic contribution to schizophrenia might be revealed in future studies. Also, as shown in *EPHB1*, it is assumed that a variety of molecules or pathways have a role in spine formation or synapse plasticity, which are impaired in patients with schizophrenia, to compensate for each other. A combined analysis of a large number of genes relevant for synaptic function might provide more robust evidence that rare missense mutations as a whole contribute to pathomechanisms of schizophrenia.

In conclusion, we provide the first evidence that multiple rare (<1%) missense mutations in *KALRN* may be genetic risk factors for schizophrenia. Further studies will be needed to examine the pathogenicity of these mutations from a biologic point of view.

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Supplementary Material

Supplementary material is available at http://schizophreniabulletin.oxfordjournals.org.

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References

1. Sullivan PF, Kendler KS, Neale MC. Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. *Arch Gen Psychiatry*. 2003;60:1187–1192.
2. Purcell SM, Wray NR, Stone JL, et al. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature*. 2009;460:748–752.
3. Manolio TA, Collins FS, Cox NJ, et al. Finding the missing heritability of complex diseases. *Nature*. 2009;461:747–753.
4. Stefansson H, Rujescu D, Cichon S, et al. Large recurrent microdeletions associated with schizophrenia. *Nature*. 2008; 455:232–236.
5. Kryukov GV, Pennacchio LA, Sunyaev SR. Most rare missense alleles are deleterious in humans: implications for complex disease and association studies. *Am J Hum Genet*. 2007; 80:727–739.
6. Boyko AR, Williamson SH, Indap AR, et al. Assessing the evolutionary impact of amino acid mutations in the human genome. *PLoS Genet*. 2008;4:e1000083.
7. Knight HM, Pickard BS, Maclean A, et al. A cytogenetic abnormality and rare coding variants identify ABCA13 as a candidate gene in schizophrenia, bipolar disorder, and depression. *Am J Hum Genet*. 2009;85:833–846.
8. Ikeda M, Aleksic B, Kinoshita Y, et al. Genome-wide association study of schizophrenia in a Japanese population [published online ahead of print September 10, 2010]. *Biol Psychiatry*. doi: 10.1016/j.biopsych.2010.07.010.
9. Penzes P, Beeser A, Chernoff J, et al. Rapid induction of dendritic spine morphogenesis by trans-synaptic ephrinB-EphB receptor activation of the Rho-GEF kalirin. *Neuron*. 2003; 37:263–274.
10. St Jean PL. Genes associated with schizophrenia identified using a whole genome scan. 2008. http://www.freepatentsonline.com/y2008/0176240.html. Accessed August 8, 2010.
11. Sullivan PF, Lin D, Tzeng JY, et al. Genomewide association for schizophrenia in the CATIE study: results of stage 1. *Mol Psychiatry*. 2008;13:570–584.
12. Xu B, Roos JL, Levy S, et al. Strong association of de novo copy number mutations with sporadic schizophrenia. *Nat Genet*. 2008;40:880–885.
13. Rabiner CA, Mains RE, Eipper BA. Kalirin: a dual Rho guanine nucleotide exchange factor that is so much more than the sum of its many parts. *Neuroscientist*. 2005;11:148–160.
14. Glantz LA, Lewis DA. Decreased dendritic spine density on prefrontal cortical pyramidal neurons in schizophrenia. *Arch Gen Psychiatry*. 2000;57:65–73.
15. Hill JJ, Hashimoto T, Lewis DA. Molecular mechanisms contributing to dendritic spine alterations in the prefrontal cortex of subjects with schizophrenia. *Mol Psychiatry*. 2006; 11:557–566.
16. Cahill ME, Xie Z, Day M, et al. Kalirin regulates cortical spine morphogenesis and disease-related behavioral phenotypes. *Proc Natl Acad Sci U S A*. 2009;106:13058–13063.
17. Hayashi-Takagi A, Takaki M, Graziane N, et al. Disrupted-in-Schizophrenia 1 (DISC1) regulates spines of the glutamate synapse via Rac1. *Nat Neurosci*. 2010;13:327–332.

18. Penzes P, Jones KA. Dendritic spine dynamics—a key role for kalirin-7. *Trends Neurosci.* 2008;31:419–427.
19. Kothiyal P, Cox S, Ebert J, et al. An overview of custom array sequencing. *Curr Protoc Hum Genet.* 2009;61:7.17.1–7.17.11.
20. Cirulli ET, Goldstein DB. Uncovering the roles of rare variants in common disease through whole-genome sequencing. *Nat Rev Genet.* 2010;11:415–425.
21. Ikeda M, Aleksic B, Kirov G, et al. Copy number variation in schizophrenia in the Japanese population. *Biol Psychiatry.* 2010;67:283–286.
22. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. *Nat Methods.* 2010;7:248–249.
23. Ferrer-Costa C, Gelpi JL, Zamakola L, et al. PMUT: a web-based tool for the annotation of pathological mutations on proteins. *Bioinformatics.* 2005;21:3176–3178.
24. Skol AD, Scott LJ, Abecasis GR, Boehnke M. Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. *Nat Genet.* 2006;38:209–213.
25. Vassos E, Collier DA, Holden S, et al. Penetrance for copy number variants associated with schizophrenia. *Hum Mol Genet.* 2010;19:3477–3481.
26. O'Donovan MC, Craddock N, Norton N, et al. Identification of loci associated with schizophrenia by genome-wide association and follow-up. *Nat Genet.* 2008;40:1053–1055.
27. Johnson RC, Penzes P, Eipper BA, Mains RE. Isoforms of kalirin, a neuronal Dbl family member, generated through use of different 5'- and 3'-ends along with an internal translational initiation site. *J Biol Chem.* 2000;275:19324–19333.
28. Keshava Prasad TS, Goel R, Kandasamy K, et al. Human Protein Reference Database—2009 update. *Nucleic Acids Res.* 2009;37:D767–D772.
29. Amanchy R, Periaswamy B, Mathivanan S, et al. A curated compendium of phosphorylation motifs. *Nat Biotechnol.* 2007;25:285–286.
30. Thomson M, Gunawardena J. Unlimited multistability in multisite phosphorylation systems. *Nature.* 2009;460:274–277.
31. Wang L, Hauser ER, Shah SH, et al. Peakwide mapping on chromosome 3q13 identifies the kalirin gene as a novel candidate gene for coronary artery disease. *Am J Hum Genet.* 2007;80:650–663.
32. Krug T, Manso H, Gouveia L, et al. Kalirin: a novel genetic risk factor for ischemic stroke. *Hum Genet.* 2010;127:513–523.
33. Henkemeyer M, Itkis OS, Ngo M, Hickmott PW, Ethell IM. Multiple EphB receptor tyrosine kinases shape dendritic spines in the hippocampus. *J Cell Biol.* 2003;163:1313–1326.
34. Steinberg S, Mors O, Borglum AD, et al. Expanding the range of ZNF804A variants conferring risk of psychosis [published online ahead of print January 05, 2010]. Mol Psychiatry. doi: 10.1038/mp.2009.149.
35. Voight BF, Scott LJ, Steinthorsdottir V, et al. Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. *Nat Genet.* 2010;42:579–589.
36. Johansen CT, Wang J, Lanktree MB, et al. Excess of rare variants in genes identified by genome-wide association study of hypertriglyceridemia. *Nat Genet.* 2010;42:684–687.
37. Bodmer W, Bonilla C. Common and rare variants in multifactorial susceptibility to common diseases. *Nat Genet.* 2008;40:695–701.
38. Yamaguchi-Kabata Y, Nakazono K, Takahashi A, et al. Japanese population structure, based on SNP genotypes from 7003 individuals compared to other ethnic groups: effects on population-based association studies. *Am J Hum Genet.* 2008;83:445–456.

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Serotonin 1A receptor gene, schizophrenia and bipolar disorder: An association study and meta-analysis

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ABSTRACT

Several investigations have reported associations between serotonin 1A (5-HT1A) receptor and major psychiatric disorders, such as schizophrenia and bipolar disorder (BP), making the 5-HT1A receptor gene (*HTR1A*) a good candidate gene for the pathophysiology of schizophrenia and BP. To evaluate the association between *HTR1A* and schizophrenia and BP, we conducted a case-control study of Japanese population samples with two single- nucleotide polymorphisms (SNPs), including rs6295 (C-1019G) in *HTR1A*. In addition, we conducted a meta-analysis of rs6295, which has been examined in other studies. Using one functional single- nucleotide polymorphism (SNP; rs6295) and one tagging SNP (rs878567), we conducted a genetic association analysis of case-control samples (857 schizophrenic patients, 1028 BP patients and 1810 controls) in the Japanese population. Two association studies for schizophrenia and three association studies for BP, including this study, met our criteria for the meta-analysis of rs6295. We found an association between *HTR1A* and Japanese BP in a haplotype-wise analysis, the significance of which remained after Bonferroni correction. In addition, we detected an association between rs6295 and BP in the meta-analysis (fixed model: $P(Z) = 0.000400$). However, we did not detect an association between *HTR1A* and schizophrenia in the allele/genotype-wise, haplotype-wise or meta-analysis. *HTR1A* may play an important role in the pathophysiology of BP, but not schizophrenia in the Japanese population. In the meta-analysis, rs6295 in *HTR1A* was associated with BP patients.

1. Introduction

Altered serotonergic neural transmission is hypothesised to be a susceptibility factor for schizophrenia (Meltzer et al., 2003; Geyer and Vollenweider, 2008).

The serotonin 1A (5-HT1A) receptor is present in various regions of the brain, including the cortex, hippocampus, amygdala, hypothalamus and septum (Barnes and Sharp, 1999; Aznar et al., 2003; Varnas et al., 2004; Le Francois et al., 2008), and several post-mortem studies reported increased 5-HT1A receptor in the prefrontal cortex of schizophrenic patients (Hashimoto et al., 1991; Hashimoto et al., 1993; Burnet et al., 1996; Simpson et al., 1996; Sumiyoshi et al., 1996). Some antipsychotic drugs, such as aripiprazole, clozapine and perospirone, have partial agonist effects on 5-HT1A receptors (Meltzer et al., 2003; Meltzer and Sumiyoshi, 2008; Sumiyoshi et al., 2008).

Sumiyoshi and colleagues conducted several studies of the effects of the addition of tandospirone, a 5-HT1A receptor agonist, on cognitive function in patients with schizophrenia being treated with antipsychotics (Sumiyoshi et al., 2001a,b). The addition of tandospirone (30 mg day^{-1}), but not placebo, to antipsychotic drugs for 4–6 weeks, was found to improve executive function in one study and verbal learning and memory in another (Sumiyoshi et al., 2007).

Mason and Reynolds reported that one of the major pharmacological therapeutic targets of clozapine is 5-HT1A receptors on cortical glutamatergic neurons (Mason and Reynolds, 1992). These authors suggested that clozapine binding to 5-HT1A receptors may contribute to the mechanism of the unique efficacy of clozapine in schizophrenic patients (Mason and Reynolds, 1992). Recent pharmacogenetics studies

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reported that a SNP (C-1019G: rs6295) in the promoter region of the 5-HT1A receptor gene (*HTR1A*), which regulates *HTR1A* transcription (Lemonde et al., 2003; Le Francois et al., 2008), is associated with improved response in negative symptoms with antipsychotics such as risperidone (Reynolds et al., 2006; Wang et al., 2008; Mossner et al., 2009).

These findings suggest a crucial relationship between the 5-HT1A receptor and schizophrenia, and that *HTR1A* is a good candidate for the aetiology of schizophrenia. *HTR1A* (OMIM * 109760, one exon in this genomic region spanning 2.069 kb) is located on 5q11. This genomic region has been shown to be a susceptibility region for schizophrenia (McGuffin et al., 1990; Amos et al., 1991; Hallmayer et al., 1992; Macciardi et al., 1992; Kalsi et al., 1999). Huang and colleagues reported that rs6295 in *HTR1A* was associated with Caucasian schizophrenia patients (108 schizophrenic patients and 107 controls) (Huang et al., 2004). However, their study had a small number of samples. We calculated the statistical power in this research using a genetic power calculator (Purcell et al., 2003), and obtained more than 80% power for the detection of association when we set the genotype relative risk at 2.4 in schizophrenia for rs6295 in *HTR1A* under a multiplicative model of inheritance. On the other hand, Kawanishi and colleagues reported no association between *HTR1A* and Japanese schizophrenic patients (Kawanishi et al., 1998). This study also had a small number of samples (61 schizophrenic patients and 100 controls). In addition, they performed a mutation scan with *HTR1A* and an association analysis between rare variants and schizophrenia. In a power analysis, we obtained more than 80% power for the detection of association when we set the genotype relative risk at 4.8–7.4 in schizophrenia for *HTR1A* under a multiplicative model of inheritance. Thus, it is difficult to evaluate the association of such extremely rare variants from the viewpoint of power (Kawanishi et al., 1998). Several whole genome association studies (GWAS) reported no association between *HTR1A* and schizophrenia in the Caucasian population (O'Donovan et al., 2008; Ng et al., 2009). However, to obtain adequate statistical power in GWAS between common variants and common complex disease, it is thought that more than 10 000 cases and control samples are necessary (Kong et al., 2009; Manolio et al., 2009). Therefore, we examined the association between *HTR1A* and Japanese schizophrenic patients using a sample larger than that in the two original studies (Kawanishi et al., 1998; Huang et al., 2004).

Several investigations reported that the translin-associated factor X gene (*TSNAX*)/disrupted-in-schizophrenia-1 gene (*DISC1*) was associated with schizophrenia and bipolar disorder (BP) (Hennah et al., 2003; Hennah et al., 2005; Thomson et al., 2005; Zhang et al., 2005; Hashimoto et al., 2006; Palo et al., 2007; Schosser et al., in press). We considered that BP and schizophrenia might have common susceptibility genes. Schizophrenia and BP have approximately 80% heritability. Recent whole genome studies have showed that a number of susceptibility regions overlap in schizophrenia and BP (1q32, 10p11-15, 13q32, 18p11.2 and 22q11-13). Schizoaffective disorder is known to be a disorder with both characteristics of schizophrenia and BP. The evidence for this is discussed in more detail in four reviews (Ivleva et al., 2010; Moskvina et al., 2009; O'Donovan et al., 2009; Purcell et al., 2009). Recent GWAS reported that zinc Wnger binding protein 804A (*ZNF804A*) and calcium channel, voltage-dependent, L type, alpha 1C subunits (*CACNA1C*) were associated with schizophrenia and BP (Consortium, 2007; O'Donovan et al., 2008; Green et al., in press; Moskvina et al., 2009; Purcell et al., 2009). This evidence is discussed in more detail in a review by O'Donovan (O'Donovan *et al.*, 2009). A recent GWAS reported that BP and schizophrenia have common susceptibility genes (Moskvina et al., 2009). Another GWAS using Japanese BP samples did not include *HTR1A* (Hattori et al., 2009). When GWAS between common variants and common complex disease are performed, it is thought that more than 10 000 cases and control samples are necessary to obtain adequate statistical power (Kong et al., 2009; Manolio et al., 2009). Because the main problem of these past association studies between *HTR1A* and schizophrenia and BP was small sample sizes, we conducted an analysis of the association of *HTR1A* with schizophrenia and BP using the recently recommended strategy of 'gene-based' association analysis (Neale and Sham, 2004) and larger samples than the original studies (Huang et al., 2004; Sullivan et al., 2009). Recently, it has been suggested that meta-analysis, in which larger samples are examined, is required for conclusive results in genetic studies (O'Donovan et al., 2008). Therefore, we conducted a meta-analysis of rs6295, which has been examined in other genetic research.

2. Materials and methods

2.1. Subjects

715 schizophrenic patients and 1017 BP patients were diagnosed according to Diagnostic and Statistical Manual for Mental Disorders (DSM)-IV criteria with the consensus of at least two experienced psychiatrists on the basis of unstructured interviews and a review of medical records. As many as 142 schizophrenic patients and 11 BP patients underwent the Structured Clinical Interview for DSM-IV disorders (SCID-1). Schizophrenic patients were grouped according to the following DSM-IV subtypes of schizophrenia: paranoid type ($n=221$), disorganised type ($n=224$), Catatonic type ($n=29$), residual type ($n=143$) and undifferentiated type ($n=125$). A total of 1633 controls were also diagnosed according to DSM-IV criteria with the consensus of at least two experienced psychiatrists on the basis of unstructured interviews, of which 46 and 131 controls underwent the Mini-International Neuropsychiatric Interview (MINI) and SCID-1, respectively. None had severe medical complications such as liver cirrhosis, renal failure, heart failure or other Axis-I disorders according to DSM-IV. Controls included hospital staff and medical students. Yamaguchi-Kabata and colleagues reported that different proportions of individuals from different regions of Japan in case and control groups can lead to statistical error (Yamaguchi-Kabata et al., 2008); however, another recent study confirmed that there is no population stratification in our control samples (Ikeda et al., 2010). However, our control samples may not be representative of the general population. The study was described to subjects and written informed consent was obtained from each. This study was approved by the Ethics Committee at Fujita Health University and Nagoya University School of Medicine.

2.2. SNPs selection and linkage disequilibrium (LD) evaluation

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

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

2.3. SNPs genotyping

We used TaqMan assays (ABI: Applied Biosystems, Inc., Foster City, CA, USA) for all SNPs. One allelic probe was labelled with FAM dye and the other with fluorescent VIC dye. The plates were heated for 2 min at 50 °C 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.

2.4. Statistical analysis

2.4.1. Case-control study

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). The distribution of patient characteristics in the schizophrenia group, BP group and healthy control group was analysed using a t test or a chi-square test. We found significant differences in gender distribution among these groups ($P_{\text{schizophrenia}}=0.00110$ and

$P_{BP}=0.512$); however, there was no difference in age among them ($P_{schizophrenia}<0.001$ and $P_{BP}<0.001$). We therefore performed logistic regression analysis to compare the phenotype of each of the examined SNPs genotypes to adjust for possible confounding. The phenotype (each disorder or healthy control) was the dependent variable, and gender, age at the time of recruitment and each of the examined SNP genotypes were set as the independent variables. The statistical package JMP for Windows was used for logistic regression analysis (JMP 5.0. 1 J, SAS Japan Inc., Tokyo, Japan). A haplotype-wise association analysis was done with a likelihood ratio test using the COCAPHASE2.403 program (Dudbridge, 2003). This software uses the expectation-maximisation (EM) algorithm to estimate the haplotype frequencies of unphased genotype data and standard unconditional logistic regression analysis, applying the likelihood-ratio test under a log-linear model to compare haplotype frequencies between cases and healthy controls. In order to avoid misleading results caused by rare haplotypes, all haplotypes with a frequency ≤5% in both the cases and the controls were declared rare and clumped together for a test of the null hypothesis, using the command line option 'rare 0.05'. This analysis was adjusted for age and gender. To avoid spurious results and correct for multiple testing, we used the permutation test option as provided in the haplotype-wise analysis. Permutation test correction was performed using 10 000 iterations (random permutations). In addition, Bonferroni's correction was used to control inflation of the type I error rate in the marker trait association analysis. For Bonferroni correction, we employed the following numbers of multiple tests: three for each sample set in allele, genotype and haplotype-wise analysis (two examined SNPs), and two for marker-trait association analysis (chi-square test and logistic regression analysis). Therefore, we performed 12 Bonferroni correction tests ($3\times2\times2$) to all P values. Power calculation was performed using a genetic power calculator (Purcell et al., 2003). We set each item in each value in the Genetic Power Calculator as follows: Prevalence: 0.01 in schizophrenia and BP, and user-defined: 0.025 (Two SNPs were examined in this study. Bonferroni's correction was used to control inflation of the type I error rate). When we calculated the statistical power using the genetic power calculator, we substituted MAFs of cases and healthy controls and number of cases and healthy controls (the MAFs used to calculate the statistical power are shown in Table 1). The significance level for statistical tests was 0.05.

2.4.2. Meta-analysis

To identify studies eligible for the meta-analysis, we searched PubMed citations through March 2009 using the terms '*HTR1A*,' 'serotonin 1A receptor gene,' 'schizophrenia,' 'bipolar disorder,' or 'BP' as keywords. In cases when we could not obtain detailed information about allele frequencies in the article, we referred to the 'SzGene database' (http://www.schizophreniaforum.org/res/sczgene/default.asp) (Allen et al., 2008).

We used the following criteria for selection of eligible studies: (1) be published in peer-reviewed journal, (2) contain independent data, (3) have distribution of genotypes in the control population that was in HWE, (4) have schizophrenia or BP patients diagnosed according to DSM and (5) use healthy individuals as controls in case–control studies.

Cochran's chi-square-based Q-statistic test was applied to assess between-study heterogeneity. The significance of the pooled odds ratio (OR) was determined using a Z-test. Overall ORs and their 95% confidence intervals (CIs) were estimated under both the Mantel–Haenszel fixed-effects (Mantel and Haenszel, 1959) and DerSimonian–Laird random-effects models (DerSimonian and Laird, 1986). The random-effects model is more conservative than the fixed-effects model and produces a wider CI. When there is no evidence of heterogeneity, the random-effects model will give results similar to the fixed-effects model. Therefore, if it is confirmed that there was no heterogeneity, we could calculate pooled ORs and P-values according to the Mantel–Haenszel fixed-effects model. If there was evidence of heterogeneity, we could calculate pooled ORs and P-values according to the DerSimonian and Laird random-effects model. Publication bias was evaluated using a funnel plot asymmetry with Egger's test. The statistical significance was set at 0.05. All data were analysed using Comprehensive Meta-Analysis (Ver 2.0). More detailed information about the meta-analysis method is given in our previous articles (Kawashima et al., 2009; Okochi et al., 2009). The significance level for all statistical tests was 0.05.

3. Results

3.1. Case-control study

715 schizophrenic patients, 1017 BP patients and 1633 healthy controls did not undergo structured interviews (more detailed characteristic information about subjects can be seen in Section 2.1.). However, in this study, patients were carefully diagnosed according to DSM-IV criteria with consensus of at least two experienced psychiatrists on the basis of a review of medical records. In addition, when we found a misdiagnosis in a patient, we promptly excluded the misdiagnosed case to maintain the precision of our sample. Because the diagnosis of one patient in our BP sample was changed to schizoaffective disorder, we excluded this patient from the BP sample. There were no schizophrenia patients whose diagnoses were changed. Detailed information on our samples was provided in previous articles (Kishi et al., 2008a, b, 2009a).

We added 5 randomly selected samples that were genotyped again as a measure of genotyping quality control, and the genotype consistency rates for all two SNPs were 100%.

The LD from rs6449693, rs878567 and rs1423691 was tight in from the HapMap database samples ($r^2=1.00$). However, the LD structure of rs6295 (functional SNP) and rs878567 (tagging SNP) in our healthy control samples was not tight ($r^2=0.160$). Further, the MAFs in our healthy control samples were similar to those in the HapMap database. The LD of rs6295 and rs878567 in our BP samples was looser than in the healthy controls and schizophrenia samples (r^2 value: healthy controls = 0.160, schizophrenia = 0.101 and BP = 0.00600).

3.1.1. Schizophrenia

Genotype frequencies of all SNPs were in HWE. We detected an association between rs878567 and schizophrenia in the allele-wise analysis (Table 1). However, this significance disappeared after multiple testing (Table 1). We did not detect a significant association between *HTR1A* and schizophrenia in the genotype-wise analysis or haplotype-wise analysis with logistic regression adjusted for age and gender (Table 2 and 3). In the power analysis, we obtained power of more than 80% for the detection of association when we set the genotype relative risk at 1.25–1.33 in schizophrenia for *HTR1A*, under a multiplicative model of inheritance.

3.1.2. Bipolar disorder

Genotype frequencies of all SNPs were in HWE. We detected a significant association between *HTR1A* and BP in the allele/genotype-

Table 1
Association analysis of *HTR1A* with schizophrenia and bipolar disorder.

SNP[a]	Phenotype	MAF$_s$[b]	N	Genotype distribution[c]			P-value[d,e]			Corrected P-value[e,f]	
				M/M	M/m	m/m	HWE	Genotype	Allele	Genotype	Allele
rs6295	Controls	0.247	1810	1024	678	108	0.762				
C>G	Schizophrenia	0.229	857	518	286	53	0.113	0.120	0.146		
	Bipolar disorder	0.283	1028	524	427	77	0.433	**0.0116**	**0.00337**	**0.139**	**0.0404**
rs878567	Controls	0.174	1810	1242	506	62	0.240				
C>T	Schizophrenia	0.149	857	619	220	18	0.764	0.0606	**0.0238**		0.286
	Bipolar disorder	0.225	1028	621	350	57	0.407	**0.00000183**	**0.00000212**	**0.0000220**	**0.0000254**

[a] Major allele>minor allele.
[b] MAFs: minor allele frequencies.
[c] M: major allele, m: mimor allele.
[d] Hardy–Weinberg equilibrium.
[e] Bold numbers represent significant P-value.
[f] Calculated by Bonferroni correction (12 times).