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.

Role of funding source

Funding for this study was provided by the Intramural Research Grant for Neurological and Psychiatric Disorders of NCNP, Health and Labour Sciences Research Grants (Comprehensive Research on Disability, Health, and Welfare), JST, CREST, and "Understanding of molecular and environmental bases for brain health" carried out under the Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan (H.K.). They had no further role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgement

The authors would like to thank the participants for taking part in the study.

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Please cite this article as: Sasayama, D., et al., Difference in Temperament and Character Inventory scores between depressed patients with bipolar II and unipolar major depressive disorders, J. Affect. Disord. (2011), doi:10.1016/j.jad.2011.03.009

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Please cite this article as: Sasayama, D., et al., Difference in Temperament and Character Inventory scores between depressed patients with bipolar II and unipolar major depressive disorders, J. Affect. Disord. (2011), doi:10.1016/j.jad.2011.03.009

Psychiatry Research 186 (2011) 183–189

Contents lists available at ScienceDirect

Psychiatry Research

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

Attitudes toward schizophrenia in the general population, psychiatric staff, physicians, and psychiatrists: A web-based survey in Japan

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ARTICLE INFO

Article history:
Received 2 April 2010
Received in revised form 20 July 2010
Accepted 18 August 2010

Keywords:
Schizophrenia
Stigma
Attitude toward mental illness
Web-based survey

ABSTRACT

Little is known about possible differences in the attitudes toward schizophrenia between the general public and various healthcare professionals. After screening for the study enrollment, 197 subjects in the general population, 100 psychiatric staff (other than psychiatrists), 112 physicians (other than psychiatrists) and 36 psychiatrists were enrolled in a web-based survey using an Internet-based questionnaire format. To assess subjects' attitudes toward schizophrenia, we used a 13-item questionnaire created by Uçok et al. (2006), to which five items were added. These 18 items were subjected to exploratory factor analysis, which yielded three factors classified as "stigma," "underestimation of patients' abilities," and "skepticism regarding treatment." These factors were compared between the four groups using analysis of covariance (ANCOVA), controlling for potential confounders. The ANCOVA for the "stigma" factor showed that psychiatrists scored significantly lower than the other three groups. The ANCOVA for the "underestimation of patients' abilities" factor revealed that psychiatric staff scored significantly lower than the general population. The present results indicated that attitudes toward schizophrenia consist of at least three separable factors. Psychiatrists had the least negative attitudes toward schizophrenia, which was followed by the psychiatric staff, and attitudes of the general population and of physicians were equally stigmatizing.

1. Introduction

Negative attitudes toward mental illness are still widely prevalent (Thornicroft et al., 2009) and considered the most significant obstacles impeding improvement in the lives of individuals with such conditions and their families (Kadri and Sartorius, 2005). Stigma toward schizophrenia, among mental illnesses, has been shown to be particularly prominent and especially challenging to people with such a diagnosis. Cross-cultural comparisons on negative attitudes toward schizophrenia have revealed that these attitudes are even greater in Japan than in other countries (Kurihara et al., 2000; Kurumatani et al., 2004; Griffiths et al., 2006).

Studies that compare attitudes toward schizophrenia between the general public and various professional groups have reported that, besides the general public, healthcare professionals such as nurses (Aydin et al., 2003; Nordt et al., 2006), psychologists (Jorm et al., 1999; Nordt et al., 2006), physicians (Jorm et al., 1999; Aydin et al., 2003) and even psychiatrists (Jorm et al., 1999; Ono et al., 1999; Lauber et al., 2004; Uçok et al., 2004; Nordt et al., 2006) hold varying degrees of negative attitudes toward patients with schizophrenia. However, in contrast to the abundance of studies looking at the attitudes toward schizophrenia within the general public, little is known about possible differences in the attitudes between the public and several professional groups. Moreover, findings from studies comparing attitudes toward schizophrenia between the public and healthcare workers are controversial; some studies have found more negative attitudes in the public than in healthcare professionals while others have observed the opposite result. For example, in a study conducted in Turkey (Aydin et al., 2003), attitudes toward schizophrenia and depression were compared between academicians, resident physicians, nurses, and hospital employees (aids and cleaners), with the results showing that the hospital employees had the least negative attitude toward the mentally ill of the four groups. Similarly, a study performed in Australia (Jorm et al., 1999) resulted in more negative ratings for health professionals including general practitioners, psychologists and psychiatrists than the public on long-term outcome and discrimination. A study in Switzerland (Nordt et al., 2006), which compared stereotypes, restrictions of the individual's rights, and social distance between the general public and healthcare professionals (including psychiatrists, psychologists, nurses and other therapists), found that restrictions were greatest in the general public while

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0165-1781/$ – see front matter © 2010 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.psychres.2010.08.019

negative stereotypes were most prominent in psychiatrists. Another study in Switzerland (Lauber et al., 2004) showed that psychiatrists, as compared to the general population, were more willing to accept mental health facilities in the community although they were as socially distant from persons with schizophrenia. On the other hand, a study in Italy (Magliano et al., 2004) showed that the lay public, as compared to mental health professionals, tended to believe that schizophrenia patients were unpredictable and should be admitted to asylums. Similarly, a recent study in Greece (Arvaniti et al., 2009) found that psychiatric staff had more positive attitudes toward mental illness than the other participants. Together, there have been substantial inconsistencies between studies investigating differences in attitudes toward schizophrenia between the general public and healthcare professionals. In addition to demonstrating differing attitudes towards individuals with schizophrenia between lay people and healthcare professionals, studies show significant differences in attitudes concerning therapeutic interventions (i.e. antipsychotics and psychotherapy) between these groups (Furnham et al., 1992; Caldwell and Jorm, 2000). Although the nature of this difference has not been fully elucidated, this finding highlights the importance of comparing attitudes toward schizophrenia between the public, psychiatric staff (other than psychiatrists), physicians (other than psychiatrists) and psychiatrists.

Although it is widely acknowledged that the extent of stigma against mental illness varies across cultures and ethnicities (Pescosolido et al., 2008), these inconsistencies may also stem from the fact that attitudes toward mental illness consist of various categories. Supporting this, a number of questionnaires that examine attitudes toward mental illness contain not only items which are directly related to negative attitudes (e.g., stigma and social distance) but also more neutral items such as realistic views on treatment and prognosis (e.g., Jorm et al., 1999; Uçok et al., 2006). Thus, grouping a large number of the items of a questionnaire into several categories pertaining to attitudes would make the interpretation of data easier and perhaps more pertinent. In this grouping procedure, factor analysis, which assumes the underlying factors in the observed variables, would be useful. Indeed, a few studies have used this technique to examine the underlying structure of attitudes toward mental illness. For example, in cross-cultural comparisons of attitudes, factor analysis was used to identify underlying factors in the questionnaire evaluating people's beliefs about persons with schizophrenia (Furnham and Chan, 2004; Furnham and Wong, 2007). In these studies, the factor analysis facilitated the comparisons of attitudes between two cultural groups. In this context, the advantages of employing the factor analysis in the present study would be twofold; this approach enables us to group a large number of observed items into fewer factors, and has the potential to address the aforementioned mixed findings on the comparison of attitudes toward schizophrenia between the public and healthcare professionals.

Regarding the methods of attitude research, there are two common approaches to survey attitudes: interview and self-reported questionnaire. The questionnaire survey, despite some disadvantages, is more convenient for data collection and enables researchers to collect data from numerous participants. Within the questionnaire survey, there are several ways to distribute self-reported questionnaires (e.g., by hand, by mail, or via email). Apart from these conventional methods, web-based surveys have been gaining increasing attention from researchers as a promising way to conduct questionnaire surveys (Eysenbach and Wyatt, 2002; Rhodes et al., 2003).

In the present study, a web-based survey was administered to investigate the possible differences in attitudes toward schizophrenia, based on the factors extracted from multiple items in a questionnaire between the general population, psychiatric staff (other than psychiatrists), physicians (other than psychiatrists) and psychiatrists. Based on the assumption that increased contact time with schizophrenia patients will lead to less negative attitudes toward such individuals, we predicted that negative attitudes of psychiatrists and of psychiatric staff would be the least, while those of the general population the greatest, and those of physicians in between.

2. Methods

2.1. Subjects and procedure of the web-based survey

The present research was programmed into an Internet-based questionnaire format, using a web-based survey tool. All participants in the present study were members of an online research panel service provided by a major Internet research service in Japan (Yahoo! Japan Research; http://research.yahoo.co.jp). Subjects in the panel were Japanese residents who had agreed to participate in web-based surveys at the time of their service registration. This panel consisted of a variety of subpanels including the general population (e.g., manufacturers, farmers, and construction workers), psychiatric staff as well as physicians. Using this subpanel and by asking specific questions, we were able to identify our target participant groups. For example, psychiatrists were identified by requesting participants in the "physicians" subpanel to specify the medical specialty to which they belonged.

An invitation email to the present study was sent to panel registrants on May 30, 2009. This email contained information about informed consent, points reward and a hyperlink to the online survey. Each participant was rewarded from the internet research company with points corresponding to approximately 100 JPY (about 1 US dollar) for completing our questionnaire. On June 1, 2009, the company provided data for the randomly selected 450 subjects, which was the number of participants we had originally requested. The data for the 450 subjects were sent to us in the format of a Microsoft Excel file without information that could lead to identifying the person (e.g., name, birth date or email address). Of the 450 subjects, one subject who was enrolled as a psychiatrist was removed from our analysis because this person demonstrated subpar knowledge pertaining to schizophrenia (i.e., this person made two errors out of the three basic questions asked about schizophrenia). Three subjects enrolled as the general population and one additional subject enrolled as a psychiatrist were also excluded because they answered "I disagree" to all of the 18 items on the questionnaire of attitudes toward schizophrenia. Consequently, 445 participants (male/female: 285/160) were included in the final analyses; 197 subjects in the general population (male/female: 107/90), 100 psychiatric staff other than psychiatrists (male/female: 44/56), 112 physicians other than psychiatrists (male/female: 100/12) and 36 psychiatrists (male/female: 34/2). This relatively small sample size of psychiatrists can be attributed to the small number of psychiatrists registered in the research panel. Of the 100 participants classified as psychiatric staff, 83 were nurses, 16 were pharmacologists, and one was a community health worker.

2.2. Questionnaires

The questionnaire used in this web-based study comprised three sections, namely demographic information, knowledge about schizophrenia, and the 18-item questionnaire on attitudes toward schizophrenia.

2.2.1. Demographic information

Demographic information of the potential participants included: age, gender, years of education, occupation/qualifications (e.g., manufacturers, farmers, physicians, nurses, and pharmacologists, and so on), specialty in medicine when relevant (e.g., Cardiology, Ophthalmology, Neurology, Psychiatry, etc.), self-reported location of living (i.e., Urban, Suburb, or Rural), household annual income (i.e., 1: Up to 2 000 000 JPY, 2: 2 000 000–3 999 999 JPY, 3: 4 000 000–5 999 999 JPY, 4: 6 000 000–7 999 999 JPY, 5: 8 000 000–9 999 999 JPY, 6: 10 000 000 JPY or more), experience of mental illness via family member or close friend (i.e., "Do you have family or close friends with a past history of psychiatric illness?"), experience of schizophrenia via family member or close friend when relevant (i.e., "Does that include individuals with schizophrenia?"), years of psychiatric education, and number of books on schizophrenia he/she has read. To exclude those who have past or present psychiatric illnesses, we also asked the following question: "Have you ever been prescribed psychiatric medications, such as anxiolytics, hypnotics, antidepressants, antipsychotics, and/or anticonvulsants?" Those who answered "yes" to this question were automatically excluded from the present study.

2.2.2. Knowledge about schizophrenia

Participants' knowledge about schizophrenia was surveyed by three questions, each with five choices (one correct choice and the other four wrong choices): "Please select the approximate prevalence rate of schizophrenia. 1: 1/50, 2: 1/100 (correct answer), 3: 1/300, 4: 1/1000, 5: 'I don't know,'" "What is the typical age of schizophrenia onset? 1: Childhood, 2: Adolescence to early adulthood (correct answer), 3: Late adulthood, 4: Middle age, 5: 'I don't know,'" and "What is the characteristic symptom of schizophrenia? 1: Panic attack, 2: Visual hallucination, 3: Auditory hallucination (correct answer), 4: Obsessive–compulsive behavior, 5: 'I don't know.'" Participants were instructed to answer these questions without referring to any materials including books.

2.2.3. Questionnaire on attitudes toward schizophrenia

An 18-item questionnaire designed to evaluate attitudes toward schizophrenia was administered. This questionnaire was based on the 13-item questionnaire developed by Uçok et al. (2006). First, the 13-item questionnaire of Uçok et al. was translated into Japanese by two research psychiatrists (H.H. and H.K.). Then this Japanese version of the questionnaire was back-translated into English by another Japanese researcher (Y.K.). The three researchers involved in the translation process had adequate English and Japanese reading and writing comprehension. The back-translated English version of the questionnaire was sent to and approved by the original author (Prof. Uçok). Second, we added five items to the original 13-item questionnaire by referring to several prior studies on attitudes (Ruhnke et al., 2000; Hübner-Liebermann et al., 2005; Schulze, 2007; Kuroda et al., 2008), yielding the final 18-item questionnaire used in the present study. The full content of the 18-item questionnaire is presented in Table 2. Participants were asked to answer each question with either "I agree" or "I disagree." For items #1, 10, 11, 12, 15, 16, 17, and 18, those items answered with "I agree" were scored 1 and those answered with "I disagree" were scored 2. For the remaining items (#2, 3, 4, 5, 6, 7, 8, 9, 13, and 14), the scoring was reversed, i.e., "I agree," 2 and "I disagree," 1. Thus, for all items, the higher score indicated negative attitudes and/or skeptical views on treatment/intervention (please see Table 2).

2.3. Statistical analyses

Averages are reported as means ± S.D. for continuous variables and as medians (25–75 percentiles) for ordinal variables. For categorical variables, data are reported as percentages. Means, medians, and categorical variables were compared using the analysis of variance (ANOVA) or *t*-test, Kruskal–Wallis test or Mann–Whitney *U* test, and the χ^2 test, respectively. To examine where in the four occupational groups the difference exists, post-hoc pair-wise comparisons were performed when necessary; ANOVA, Kruskal–Wallis test, and the χ^2 test were followed by the Bonferroni correction, pair-wise Mann–Whitney *U* test, and pair-wise χ^2 test, respectively. The analysis of covariance (ANCOVA) was used to compare the factor scores between the four groups, controlling for confounding variables. Of the demographic characteristics, those variables which were significantly different between the four participant groups or significantly affected the attitudes were considered as confounders. Pearson's *r* or Spearman's ρ was used to examine correlations.

To extract underlying factors from the 18-item questionnaire, the exploratory factor analysis was conducted, using the principal axis factoring method with oblique (promax) rotation. The choice of the number of factors was based on theoretical meaningfulness as well as on the Kaiser criterion where variables with eigenvalues equal to or greater than one are extracted as factors. Items with factor loadings ≥0.40 were deemed meaningful and assigned to the given factor, with only the highest factor loading for each item being considered. We labeled each factor based on what we believed best characterized the group of items that loaded on a particular factor. This factor analysis was performed within the general population group alone because: 1) we cannot pool the whole sample for this purpose since the literature review indicates that the four groups are not homogenous with respect to their attitudes toward schizophrenia and 2) the sample size of the other groups, particularly that of the psychiatrist group, was too small to produce reliable factor analytic results. Since the item #10 in the questionnaire (i.e., "I don't worry about examining a person who is diagnosed with schizophrenia") is considered not suitable for the general population, this item was removed from the factor analysis.

Subsequently, raw scores of the items were averaged within each factor, which yielded a mean score of each factor for each subject. Since the raw score of each item (i.e., "1" or "2") carried no meaning except that the lower score (i.e., "1") and higher score (i.e., "2") indicated positive and negative attitudes, respectively, the mean scores were normalized to the z-score using the general population group data, assuming that this group provides the latent model as well as the normative reference data.

Statistical significance was set at two-tailed $p<0.05$ unless otherwise specified. Conservative $p<0.01$ was adopted as statistically significant and $p<0.05$ as trend-level significance where multiple testings were performed simultaneously (e.g., high number of correlational analyses and post-hoc multiple pair-wise comparisons). Analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 18.0 (SPSS Japan, Tokyo).

3. Results

Demographic characteristics and knowledge about schizophrenia, stratified by the four groups, are shown in Table 1. Since basic demographics, including gender, age, education and location of living, were significantly different between groups, these four variables were controlled for in the ANCOVA model examining the difference of attitudes between the four participant groups.

3.1. Factor analysis

The solution of factor analysis in the general population group ($n=197$) is shown in Table 3. Kaiser–Meyer–Olkin (KMO) measure (which ranges from 0 to1, with values of >0.6 being recommended) was 0.76, indicating a high sampling adequacy for the factor analysis. Bartlett's test of sphericity, which tests whether the correlation matrix is an identity matrix, was significant ($\chi^2=672.3$, $df=136$, $p<0.001$), indicating that the factor model was appropriate. The initial principal axis factoring method yielded five factors with eigenvalues greater than 1.0, explaining 55.1% of the cumulative variance. Although we first retained these five factors according to the Kaiser criterion, we decided not to include the fourth and fifth factors in further analyses as there was only one item that loaded on each of these two factors (see Table 3). Accordingly, we named the first three factors "stigma" (e.g., "Schizophrenia patients are dangerous"), "underestimation of patients' abilities" (e.g., "Patients with schizophrenia cannot comprehend their illness") and "skepticism regarding treatment" (e.g., reverse scoring of "Schizophrenia can be treated") (Table 3).

As presented in Table 4, correlations among the five factors were not small enough to rationalize the orthogonal (e.g., varimax) rotation, which assumes that each factor is totally independent of the other factors. Thus, the oblique method used herein proved to be appropriate. Still, however, taking into account that the orthogonal method has been the most common procedure, we conducted an additional factor analysis with the varimax rotation and obtained virtually the same results as with the promax rotation.

Table 1
Demographic characteristics and knowledge about schizophrenia of the four groups.

Characteristics	General population ($n=197$)	Psychiatric staff ($n=100$)	Physicians ($n=112$)	Psychiatrists ($n=36$)	Analysis	
					Statistics	*p*
Gender, %female	45.7	56.0	10.7	5.6	$\chi^2(3)=71.0$	<0.001
Age, years: mean ± S.D.	39.2 ± 11.2	40.9 ± 9.9	44.2 ± 8.2	42.1 ± 7.9	$F(3,441)=6.22$	<0.001
Education, years: mean ± S.D.	14.5 ± 2.4	16.0 ± 2.4	19.4 ± 2.4	19.4 ± 2.3	$F(3,441)=124.9$	<0.001
Self-reported location of living, %urban/%suburb/%rural	32.0/46.7/21.3	22.0/51.0/27.0	41.1/40.2/18.8	52.8/33.3/13.9	$\chi^2(6)=15.3$	0.018
Household annual income, rank: median (25–75 percentiles)	3.0 (3.0–5.0)	3.0 (3.0–4.0)	6.0 (6.0–6.0)	6.0 (5.8–6.0)	Kruskal–Wallis $\chi^2(3)=191.1$	<0.001
Experience of mental illness via family member or close friend, %positive	21.1	17.0	15.1	32.4	$\chi^2(3)=5.54$	0.14
Experience of schizophrenia via family member or close friend, %positive	4.8	7.5	4.7	6.1	$\chi^2(3)=1.04$	0.79
Psychiatric training, years: mean ± S.D.	0.2 ± 1.2	6.1 ± 3.6	1.6 ± 2.3	7.7 ± 3.1	$F(3,441)=203.8$	<0.001
Number of books on schizophrenia: mean ± S.D.	0.2 ± 0.9	5.4 ± 3.6	2.0 ± 2.9	9.6 ± 1.4	$F(3,441)=230.5$	<0.001
Knowledge about schizophrenia_Prevalence rate, %correct	27.4	59.0	41.1	97.2	$\chi^2(3)=73.0$	<0.001
Knowledge about schizophrenia_Onset age, %correct	41.6	91.0	73.2	97.2	$\chi^2(3)=97.0$	<0.001
Knowledge about schizophrenia_Characteristic symptom, %correct	11.7	95.0	58.9	94.4	$\chi^2(3)=228.7$	<0.001
Number of correct answers: median (25–75 percentiles)	1.0 (0–1.0)	3.0 (2.0–3.0)	2.0 (1.0–3.0)	3.0 (3.0–3.0)	Kruskal–Wallis $\chi^2(3)=208.9$	<0.001

Table 2
Percentage of subjects who answered "I agree" to each of the 18 items concerning attitudes toward schizophrenia, stratified by the group.

	General population ($n=197$)	Psychiatric staff ($n=100$)	Physicians ($n=112$)	Psychiatrists ($n=36$)	Analysis	
					χ^2	p
1. Patients with schizophrenia can work	54.3	87.0	82.1	97.2	59.0	<0.001
2. Would oppose if one of his/her relatives would like to marry someone who has schizophrenia	71.1	86.0	76.8	75.0	8.2	0.04
3. Schizophrenia patients can be recognized by his/her appearance	16.8	47.0	24.1	41.7	34.8	<0.001
4. Schizophrenia patients are dangerous	38.1	18.0	30.4	2.8	26.0	<0.001
5. Would not like to have a neighbor with schizophrenia	41.6	44.0	41.1	11.1	13.5	0.004
6. Schizophrenia patients are untrustworthy	38.1	25.0	36.6	5.6	18.2	<0.001
7. Schizophrenia patients could harm children	55.8	54.0	58.0	22.2	15.5	0.0014
8. Schizophrenia patients should be kept in hospitals	26.4	11.0	17.0	0.0	20.3	<0.001
9. Family members of people with schizophrenia should help with all aspects of care[a]	38.6	14.0	28.6	11.1	25.6	<0.001
10. (Suppose you were a psychiarist) I don't worry about examining a person who is diagnosed with schizophrenia	48.2	72.0	64.3	91.7	33.4	<0.001
11. Would a patient with schizophrenia be treated in the appropriate department of the general hospital	66.0	58.0	61.6	72.2	3.2	0.36
12. Schizophrenia can be treated	80.7	80.0	81.3	88.9	1.5	0.68
13. Patients with schizophrenia cannot comprehend their illness[a]	34.0	16.0	30.4	8.3	18.1	<0.001
14. Patients with schizophrenia cannot comprehend nor apply suggested treatment	11.2	4.0	10.7	0.0	8.4	0.04
15. Schizophrenia has the chance of recovery	89.8	88.0	83.9	97.2	5.4	0.15
16. It is important to always inform a person with schizophrenia of their diagnosis[a]	63.5	69.0	66.1	72.2	1.6	0.66
17. Patients with schizophrenia often benefit from pharmacologic intervention (i.e. antipsychotic medications)[a]	54.3	99.0	90.2	100.0	106.6	<0.001
18. Patients with schizophrenia often benefit from psychotherapy[a]	76.1	78.0	69.6	66.7	3.4	0.33

[a] These five items were newly added to the original questionnaire of Uçok et al. (2006).

Table 3
Promax-rotated pattern matrix for the general population group ($n=197$).

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
1. Patients with schizophrenia can work					
2. Would oppose if one of his/her relatives would like to marry someone who has schizophrenia	0.49				
3. Schizophrenia patients can be recognized by his/her appearance					
4. Schizophrenia patients are dangerous	0.66				
5. Would not like to have a neighbor with schizophrenia	0.78				
6. Schizophrenia patients are untrustworthy	0.76				
7. Schizophrenia patients could harm children	0.63				
8. Schizophrenia patients should be kept in hospitals	0.47				
9. Family members of people with schizophrenia should help with all aspects of care	0.36				
11. Would a patient with schizophrenia be treated in the appropriate department of the general hospital			0.65		
12. Schizophrenia can be treated			0.76		
13. Patients with schizophrenia cannot comprehend their illness		0.82			
14. Patients with schizophrenia cannot comprehend nor apply suggested treatment		0.58			
15. Schizophrenia has the chance of recovery					
16. It is important to always inform a person with schizophrenia of their diagnosis				0.88	
17. Patients with schizophrenia often benefit from pharmacologic intervention (i.e. antipsychotic medications)					0.34
18. Patients with schizophrenia often benefit from psychotherapy					0.60

Extraction method: Principal axis factoring method. Rotation method: Promax with Kaiser normalization.
Only factor loadings ≥0.3 or ≤−0.3 are shown. Factor loadings ≥0.4 or ≤−0.4 are retained for factor contribution (indicated with underline).

3.2. Relationships of attitudes with demographics and knowledge

To investigate the confounding effects of the demographic characteristics and knowledge about schizophrenia on the attitudes toward schizophrenia, relationships of attitudes (as indexed by the three factors) with demographics and knowledge were examined in each of the three participant groups except for the psychiatrists group. Correlation analyses for continuous/ordinal demographics and t-test or ANOVA for categorical demographics were used. There was only one significant (i.e., $p<0.01$) result concerning the relationships between attitudes and demographics/knowledge in the three groups, namely the significant gender difference in the "underestimation of patients' abilities" factor in the general population; males were more likely than females to underestimate the abilities of individuals with schizophrenia (mean z-score: 0.18 vs. −0.21, $t=2.78$, $df=195$, $p=0.006$).

3.3. Comparisons of attitudes toward schizophrenia between the four participant groups

Results of the 18-item questionnaire on the attitudes toward schizophrenia by the four groups are shown in Table 2. Using the χ^2 test, 11 items differed significantly ($p<0.01$) and two items differed at the trend-level ($0.01<p<0.05$) between the four groups. Of note, only one psychiatrist considered schizophrenia patients as dangerous (item # 4) and no psychiatrists believed that schizophrenia patients should be kept in hospitals (item # 8) or that schizophrenia patients cannot comprehend nor apply suggested treatment (item # 14). In contrast, substantial portions of the general population and physicians thought that schizophrenia patients are dangerous and should be kept in hospitals.

Fig. 1 shows the z-score on the three factors contrasting the four participant groups. The ANCOVA on the three factors, controlling for gender, age, education and location of living, demonstrated that the group had a significant main effect on the factors "stigma" [$F(3,421)=4.19$, $p=0.006$] and "underestimation of patients' abilities" [$F(3,421)=4.07$, $p=0.007$], but not on "skepticism regarding treatment" [$F(3,421)=0.21$, $p=0.89$]. Neither gender, age, education, nor location of living had a significant main effect on any of the three factors (all $p>0.1$). None of the interactions between group and the

Table 4
Correlation between factors matrix.

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Factor 1	1	0.497	0.205	−0.008	−0.079
Factor 2		1	0.368	0.009	0.082
Factor 3			1	0.141	0.419
Factor 4				1	0.278
Factor 5					1

Extraction method: Principal axis factoring method.
Rotation method: Promax with Kaiser normalization.

other variables were significant (all $p>0.1$). Post-hoc analysis for the "stigma" factor revealed that psychiatrists scored significantly lower than the other three groups, namely the general population ($p<0.001$), psychiatric staff ($p=0.014$) and physicians ($p=0.015$). Post-hoc analysis for the "underestimation of patients' abilities" factor showed that psychiatric staff scored significantly lower than the general population ($p=0.014$). These results indicated that psychiatrists had the least negative attitudes toward schizophrenia, which was followed by the psychiatric staff, and that the attitudes of the general population and of physicians were the most stigmatizing. On the other hand, with regard to skepticism regarding treatment, the four groups held similar attitudes (see Fig. 1).

We would like to note here that psychiatrists and psychiatric staff, albeit their overall less negative attitudes, showed similar or even greater tendency to oppose if one of their relatives would like to marry someone who has schizophrenia (item #2), as compared to the general population (e.g., psychiatric staff vs. general population: $\chi^2(1)=8.13$, $p=0.004$) (see Table 2). This means that although psychiatric staff and psychiatrists were relatively free of negative attitudes toward schizophrenia, they nevertheless wished to keep their distance from persons suffering from schizophrenia. The extent of such a wish in these two groups was found to be similar to or even greater than that in the general population.

4. Discussion

Using the exploratory factor analysis technique, we identified "stigma," "underestimation of patients' abilities," and "skepticism regarding treatment" as being the three underlying components of the questionnaire. In general, psychiatrists demonstrated the least negative attitudes toward schizophrenia, which was followed by the psychiatric staff (other than psychiatrists). Attitudes of the general population and of physicians were the most negative overall. On the other hand, the four participant groups had the same degree of skepticism regarding treatment of patients with schizophrenia.

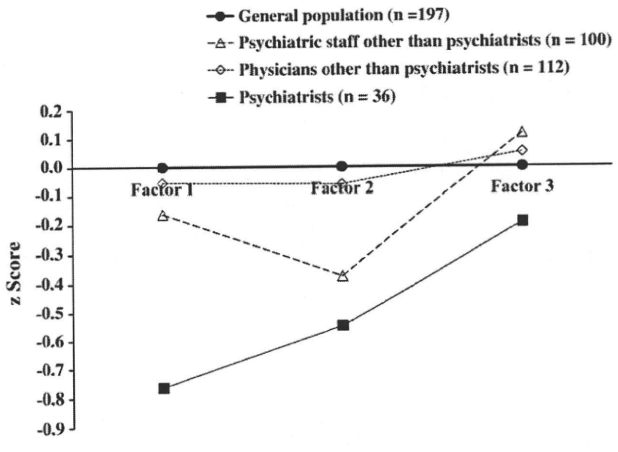

Fig. 1. Z-score of the three factors contrasting the four occupational groups. Bold line with filled circles, broken line with open triangles, dotted line with open diamonds, and solid line with filled squares represent the general population, psychiatric staff, physicians, and psychiatrists, respectively. Factor 1: Stigma; Factor 2: Underestimation of patients' abilities; Factor 3: Skepticism regarding treatment.

4.1. Separable aspects of attitudes toward schizophrenia identified by the factor analysis

The first factor, "stigma," consisted of six items (items #2, 4–8). The three elements of stigma, proposed by Thornicroft et al. (2007), are ignorance, prejudice and discrimination. The six items included in the "stigma" factor are clearly related to ignorance and prejudice (see Table 2). Moreover, such attitudes would result in discriminatory behaviors. Interestingly, Furnham and Chan (2004), using a very different questionnaire from the present one, also identified underlying factors such as "dangerousness of people with schizophrenia" and "abnormality of schizophrenia."

The second factor, "underestimation of patients' abilities," comprised two items (items #13 and 14). Since schizophrenia patients can, if not completely, comprehend their illness and suggested treatment, the "I disagree" answers to these two items are considered to reflect underestimation of their abilities. Given that the tendency to underestimate the abilities' of schizophrenia patients has been relatively understudied compared to stigma, it may be of importance that this factor was separated out from the first stigma factor.

The third factor, "skepticism regarding treatment," included two items (items #11 and 12). The "I disagree" answers to these two items were assigned higher scores. Since in reality schizophrenia patients can, albeit not always, be treated, we named this factor skepticism. Notably, Furnham and Chan (2004) again identified the factor "treatment for people with schizophrenia."

It should be noted, however, that the three factors do not account for all aspects of attitudes toward schizophrenia as there were several items that did not significantly load onto any of the three factors. This suggests that attitudes toward schizophrenia may be complex to investigate, a finding well documented in recent literature (Chee et al., 2005; Schulze, 2007).

4.2. Comparisons of attitudes toward schizophrenia between the four participant groups

The comparisons of the three factors between the four occupational groups revealed that stigma toward schizophrenia was the most common in the general population and physicians and the least common in psychiatrists. Although this result generally confirmed our prediction and supported some previous findings (Magliano et al., 2004; Arvaniti et al., 2009), studies investigating the differences in attitudes toward schizophrenia between various occupational groups have not necessarily yielded the same results. Specifically, several studies reported similar or an even greater extent of negative attitudes in psychiatric staff—including psychiatrists—relative to those in the general public (Jorm et al., 1999; Lauber et al., 2004; Nordt et al., 2006). These controversial findings may be accounted for by the well-known fact that attitudes toward schizophrenia vary across cultures (Pescosolido et al., 2008). Moreover, as described earlier, these precedent studies used different measures to assess their participants' attitudes toward schizophrenia, including participants' opinions about the long-term outcome of a person with schizophrenia described in a vignette (Jorm et al., 1999), and stereotypes, restrictions and social distance (Nordt et al., 2006). Thus, it is possible that these previous findings reflected realistic views of the participants rather than their negative attitudes per se. Indeed, the authors themselves discussed that such negative views held by healthcare professionals—including psychiatrists—could be considered as realistic (Jorm et al., 1999). Supporting this, in the present study the four participant groups held similar attitudes in terms of skepticism regarding treatment.

Besides the items included in the three factors, there were five items where responses of the four participant groups significantly differed. For item #1, most of the participants in the three healthcare professional groups believed that schizophrenia patients can work, whereas only about half of the lay public endorsed this opinion. Since individuals with severe schizophrenia usually recover to the point that they can work in certain environments such as special workplaces for the mentally ill, these high percentages in the professional groups are likely to derive from their expert knowledge. Consistent with the present result, in the study conducted by Uçok et al. (2006), a similar percentage (83%) of general practitioners answered "I agree" to the same question. As for items #17 and 18, while psychiatric staff, physicians, and psychiatrists considered pharmacotherapy as more efficacious than psychotherapy (99.0% vs. 78.0%; 90.2% vs. 69.6%; 100% vs. 66.7%), the beliefs of the general population showed the opposite pattern (i.e., pharmacotherapy: 54.3% vs. psychotherapy 76.1%). In accord with this result, a study in Australia showed that mental health nurses as well as psychiatrists believed antipsychotic medication to be the most helpful in the treatment of schizophrenia (Caldwell and Jorm, 2000). Indeed, by comparing the beliefs about the helpfulness of interventions for mental disorders between general practitioners, psychiatrists and clinical psychologists, Jorm et al. (1997) demonstrated that these health practitioners were more likely to endorse the interventions associated with their own profession. The pattern in the general population was also in line with previous findings that people in the Slovak Republic, Russia, and Germany all favored psychotherapy over psychotropic medication as a recommended treatment (Angermeyer et al., 2005). In contrast, a recent study in Britain showed that lay people thought that drug treatments were more effective than psychotherapy for schizophrenia (Furnham, 2009). This inconsistency may be related to the observation that the British population, as compared to Asian (i.e., Chinese), tended to believe that biological factors are important for the causes and treatments of schizophrenia (Furnham and Wong, 2007). It is also reported that Japanese, compared to Australians, were more likely to consider the causes of schizophrenia as "nervous person" or "weakness of character" (Nakane et al., 2005), which may well be treated with psychotherapy rather than with psychotropics.

At this point we would like to stress that psychiatrists and psychiatric staff, despite their generally less negative attitudes, demonstrated marked tendency to oppose their relatives' marriage to individuals with schizophrenia (#2). In line with this, previous studies (Lauber et al., 2004; Nordt et al., 2006) also showed that the general population and psychiatric staff—including psychiatrists—displayed no overall differences in their social distance toward people with schizophrenia. These findings point to the fact that, among mental health professionals who by definition know the disorder well, the level of negative attitudes toward schizophrenia increases when the situation implies social closeness. According to Lauber et al. (2004), this may be due to realistic assessment of the consequences of schizophrenia based on their professional experiences. This interpretation would be supported by the present finding that the skeptical view of healthcare professionals was as great as that of the general population. Furthermore, this finding could be considered as additional evidence of the complexity of attitudes toward schizophrenia and the resulting difficulty in reducing such attitudes; mental healthcare professionals themselves hold certain strong negative attitude toward schizophrenia.

There is another important but complicated issue; it remains unclear whether the overall less negative attitudes toward schizophrenia of psychiatrists had existed before they became psychiatrists (i.e., individuals who had less negative attitudes tended to become psychiatrists) or they acquired such favorable attitudes during their experience as a psychiatrist. If the former is the case, reducing stigma would be quite difficult, while in the latter case some educational methods could work. From this standpoint, it might be interesting to investigate whether attitudes of medical students who later become psychiatrists find greater resemblance to the attitudes of the general population or to those of psychiatrists. It may be worth noting that Schulze et al. (2003) found that young people's attitudes about schizophrenia are susceptible to change, thereby suggesting that anti-stigma projects at the school level could improve public attitudes and prevent stereotypes.

4.3. Strengths and limitations

Web-based data collection employed in the present study includes both advantages and shortcomings; while this method may be subject to certain sampling biases such as the so-called "digital divide" (Bernhardt, 2000; Rhodes et al., 2003), it is conveniently carried out. Furthermore, this method is likely to provide participants with anonymity (Eysenbach and Wyatt, 2002; Rhodes et al., 2003), leading to less response bias toward social desirability compared to traditional survey methodologies. This point is considered important in collecting sensitive data such as self-focused rumination (Davis, 1999) and sexual behavior (Bailey et al., 2000). Since stigma is also a sensitive issue that renders responders subject to social desirability, this potential merit of reducing such responding bias would work well in our web-based sampling.

A number of limitations should also be acknowledged. First, given that the subjects who participated in this survey had daily internet access, it was possible that they collectively had more information about schizophrenia than average, which may have impacted their attitudes. Second, we cannot fully rule out the possibility that our web-based sampling approach erroneously included some participants who have provided false or misleading information, although we carefully removed from the analyses those participants who made apparently inappropriate responses to the questionnaire, as described above. Third, relatively few psychiatrists were enrolled, which may have resulted in type II errors. Fourth, gender distribution was not balanced in the four participant groups. The fifth limitation relates to the binary-scaled format (i.e., "I agree"/"I disagree") of the 18-item questionnaire. This type of response format does not allow "in between" (or "yes and no") answers, and may be susceptible to the floor and ceiling effect. Finally, given the well-established cross-cultural differences of attitudes toward the mentally ill, the present findings obtained in Japan may not be extrapolated to different countries.

5. Conclusion

The present web-based study found that negative attitudes toward schizophrenia were held strongly by the general population, physicians, and also to some extent by the psychiatric staff other than psychiatrists. While psychiatrists generally showed the least negative attitudes toward schizophrenia patients, it was also revealed that they wish to keep a certain distance from schizophrenia sufferers. These findings suggest that stigma can be reduced through increasing appropriate measures, which can be supported by psychiatrists who understand the disorder best. In doing so, however, psychiatrists should be aware of their own negative attitudes toward schizophrenia.

Acknowledgements

We are grateful to Prof. Alp Uçok for his approval for the back-translated version of the questionnaire. This study was funded by the Fulbright Foundation (M.R.). This study was also supported by Grant-in-Aid for Young Scientists (Start-up) from the Japan Society for the Promotion of Science (JSPS) (H.H.), Health and Labor Sciences Research Grants (Research on Psychiatric and Neurological Diseases and Mental Health) (H.K.), Grant from Japan Foundation for Neuroscience and Mental Health (H.K.), and Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS) (H.K.).

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Journal of Psychiatric Research 45 (2011) 47–53

Contents lists available at ScienceDirect

Journal of Psychiatric Research

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

Possible association of the semaphorin 3D gene (*SEMA3D*) with schizophrenia

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ARTICLE INFO

Article history:
Received 22 January 2010
Received in revised form
1 April 2010
Accepted 6 May 2010

Keywords:
Semaphorin
Plexin
Non-synonymous polymorphism
Schizophrenia
Haplotype

ABSTRACT

Semaphorins are ligands of plexins, and the plexin–semaphorin signaling system is widely involved in many neuronal events including axon guidance, cell migration, axon pruning, and synaptic plasticity. The plexin A2 gene (*PLXNA2*) has been reported to be associated with schizophrenia. This finding prompted us to examine the possible association between the semaphorin 3D gene (*SEMA3D*) and schizophrenia in a Japanese population. We genotyped 9 tagging single nucleotide polymorphisms (SNPs) of *SEMA3D* including a non-synonymous variation, Lys701Gln (rs7800072), in a sample of 506 patients with schizophrenia and 941 healthy control subjects. The Gln701 allele showed a significant protective effect against the development of schizophrenia ($p = 0.0069$, odds ratio = 0.76, 95% confidence interval 0.63 to 0.93). Furthermore, the haplotype-based analyses revealed a significant association. The four-marker analysis (rs2190208–rs1029564–rs17159614–rs12176601), in particular, not including the Lys701Gln, revealed a highly significant association ($p = 0.00001$, global permutation), suggesting that there may be other functional polymorphisms within *SEMA3D*. Our findings provide strong evidence that *SEMA3D* confers susceptibility to schizophrenia, which could contribute to the neurodevelopmental impairments in the disorder.

1. Introduction

The first discovered semaphorin, collapsing-1 (now Sema3A), was originally reported as a repulsive cue in axon guidance (Luo et al., 1993). To date, more than 20 semaphorins of secreted or membrane forms have been identified in various species ranging from nematodes to humans (Luo et al., 1993; Fujii et al., 2002; Yazdani and Terman, 2006). Semaphorins act as ligands for plexins, and the plexin–semaphorin signaling system has been widely investigated in nervous systems (Mann et al., 2007). Class 3 semaphorins (SEMA3A-G) have been well-studied and generally act as secreted ligands for the heterodimerized complex of the plexin A family members and neuropilins (Fujisawa, 2004). For example, Sema3A binds to neuropilin-1 and activates plexin A1 or plexin A2 to transduce a repulsive axon guidance signal (Takahashi and Strittmatter, 2001). Many studies of the plexin–semaphorin signaling system have concentrated on their roles in neuronal development and plasticity (reviewed in (Kruger et al., 2005; Halloran and Wolman, 2006; Waimey and Cheng, 2006; Mann et al., 2007)).

Recently, the relationship between schizophrenia and molecules in the plexin–semaphorin signaling system has begun to receive much attention, for several reasons (Mann et al., 2007). An increase in levels of SEMA3A was noted in the cerebellum in postmortem brains of schizophrenia patients, as measured by immunoreactivity in the inner molecular layer and by the enzyme-linked immunosorbent assay (ELISA) in cerebellar protein extract (Eastwood et al., 2003). A genome-wide association study using 25,494 single nucleotide polymorphisms (SNPs) revealed that an intronic SNP of *PLXNA2* was most consistently associated with schizophrenia in European–American populations (Mah et al., 2006). Our replication study in a Japanese sample failed to confirm such an association (Fujii et al., 2007); however, a meta-analysis combining data from previous studies of *PLXNA2* yielded a positive association with schizophrenia (Allen et al., 2008), in which it was reported that the C allele of the SNP rs752016 of *PLXNA2* showed a nominally significant protective effect (odds ratios (OR) = 0.82, 95%

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doi:10.1016/j.jpsychires.2010.05.004

confidence interval (CI) = 0.69–0.99), and association of the SNP rs841865 approached statistical significance (OR = 0.84, 95% CI = 0.69–1.01) when samples of Mah et al. and Fujii et al. were combined (Mah et al., 2006; Fujii et al., 2007). Furthermore, in the updated online database, "SchizophreniaGene (http://www.schizophreniaforum.org/)," association of the SNP rs1327175 approached statistical significance (OR = 0.76, 95% CI = 0.57–1.00) (Mah et al., 2006; Fujii et al., 2007; Takeshita et al., 2008; Budel et al., 2008). Therefore, genes of the plexin family, the semaphorin family, and neuropilins, are intriguing candidates for schizophrenia susceptibility genes. We then focused on *SEMA3D* as a candidate gene for schizophrenia. *SEMA3D* was mapped to chromosome 7q21 (Clark et al., 2003); interestingly, a previous genome-wide scan suggested that this chromosomal region contains a susceptibility locus for schizophrenia (Ekelund et al., 2000) and recent studies have provided additional support for this possibility (Tastemir et al., 2006; Wedenoja et al., 2008, 2009; Idol et al., 2008).

The aim of the present study was to examine the possible association between *SEMA3D* and schizophrenia. *SEMA3D* has a common variant in the coding region due to an A to C base substitution (rs7800072), which results in an amino acid change (701 Lys to Gln). This SNP has previously been examined with regard to brain morphology (assessed with magnetic resonance imaging) in patients with schizophrenia (Gregorio et al., 2009). Although this study failed to find significant alterations in brain morphology, it is still unclear whether this SNP confers susceptibility to schizophrenia. We examined the possible association of schizophrenia with this non-synonymous SNP, plus 8 tagging SNPs encompassing the entire *SEMA3D* gene.

2. Subjects and methods

2.1. Subjects

Subjects were 506 patients with schizophrenia (278 males [54.9%], mean age 44.3 years [SD 14.1]) and 941 healthy controls (334 males [35.5%], mean age 44.8 years [SD 16.3]). All subjects were Japanese, biologically unrelated, and recruited from the same geographical area (Western part of Tokyo Metropolitan). Consensus diagnosis by at least two psychiatrists was made for each patient according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) criteria (American Psychiatric Association, 1994) on the basis of unstructured interviews and information from medical records. The controls were healthy volunteers recruited from the same geographical area. Control individuals were interviewed and those who had a current or past history of psychiatric treatment were not enrolled in the study. The study protocol was approved by the ethics committee of the National Center of Neurology and Psychiatry, Japan. After description of the study, written informed consent was obtained from every subject.

2.2. SNP selection

The tagging SNPs were selected using the phase III version of HapMap (http://www.hapmap.org/cgi-perl/gbrowse/). SNP genotype data for the JPT (Japanese in Tokyo, Japan) were downloaded for the genomic region of *SEMA3D* plus 2 kb 5′ and 2 kb 3′ of this region (chr7q21.11). The most centromeric and telomeric HapMap markers downloaded were rs6944966 and rs11762367, respectively. HapMap markers were analyzed using the Haploview 4.1 system (http://www.broad.mit.edu/mpg/haploview) with the following criteria of marker selection: Hardy–Weinberg (HW) p value cutoff: 0.05; minimum genotypes: 90%; maximum number of Mendelian errors: 1; minimum minor allele frequency: 0.1; minimum distance between tags: 10 kb. Tagging SNPs were selected using the Tagger function implemented in Haploview with the following criteria: pairwise tagging only and r^2 threshold 0.8. We preselected rs7800072 and rs6966472 as markers and used the Tagger function implemented in Haploview to select other markers. As a result, 9 markers were selected as suitable for analysis for *SEMA3D*. SNP rs7800072 is non-synonymous (2141A > C, Lys701Gln). The numbers of base and amino acid positions were according to NM_152754.2 and NP_689967.2, respectively.

2.3. Genotyping

Venous blood was drawn from the subjects and genomic DNA was extracted from whole blood according to standard procedures. The SNPs were genotyped using the TaqMan 5′-exonuclease allelic discrimination assay; the assay ID (Applied Biosystems, Foster City, CA) of each SNP was C__15937080_10 for rs2190208, C___7585979_10 for rs1029564, C__33462384_10 for rs17159614, C__31373903_10 for rs12176601, C___2635874_10 for rs6966472, C___2635864_10 for rs17559978, C__33462432_10 for rs17159577, C__33462438_10 for rs17159556, and C__25994972_10 for rs7800072. Thermal cycling conditions for polymerase chain reaction (PCR) were 1 cycle at 95 °C for 10 min followed by 50 cycles of 92 °C for 15 s and 60 °C for 1 min. Genotype data were read blind to the case-control status. Ambiguous genotype data were not included in the analysis.

2.4. Haplotype and statistical analysis

Deviations of genotype distributions from the HW equilibrium (HWE) were assessed with the χ^2 test for goodness of fit. Genotype and allele distributions were compared between patients and controls by using the χ^2 test for independence. These tests were performed with SPSS software ver.11 (SPSS Japan, Tokyo, Japan). Haplotype-based association analyses were performed with SNPAlyze software ver.6.5 (http://www.dynacom.co.jp/e/products/package/snpalyze/about.html). The measures of linkage disequilibrium (LD), denoted as D′ and r^2, were calculated from the haplotype frequency using the expectation-maximization (EM) algorithm. Haplotypes with frequencies of less than 1% were considered to be rare and were excluded from the analyses. All p values reported are two-tailed. We performed 100,000 permutations only for some significant haplotypes (e.g., rs2190208–rs1029564–rs17159614–rs121176601) and 10,000 permutations for the other haplotypes. OR and 95% CI were also calculated. To correct the critical p value for multiple testing, we used the spectral decomposition method of SNPSpD software (http://gump.qimr.edu.au/general/daleN/SNPSpD/) (Nyholt, 2004; Li and Ji, 2005), which considers marker linkage disequilibrium information and generates an experiment-wide significance threshold required to keep the type I error rate at 5%.

3. Results

Genotype and allele distributions of the examined SNPs of *SEMA3D* in patients and controls are shown in Table 1. LD estimates of pairwise SNPs, expressed in D′ and r^2, are presented in Fig. 1. The genotype distributions did not significantly deviate from the HWE in patients and controls for any of the examined SNPs. For the non-synonymous polymorphism of *SEMA3D* (rs7800072), there were significant differences in both genotype ($\chi^2 = 8.7$, df = 2, $p = 0.013$) and allele ($\chi^2 = 7.3$, df = 1, $p = 0.0069$, OR = 0.76, 95% CI 0.63–0.93) distributions between patients and controls (Table 1). Furthermore, with respect to the other 8 SNPs (rs2190208, rs1029564,

Table 1
Genotype and Allelic Distribution of the *SEMA3D* SNPs in Japanese Patients with Schizophrenia, and Controls.

dbSNP ID	position[a]	Inter-SNP distance (bp)	Group	N	Genotype distribution (frequency)			Allele distribution (frequency)		Odds ratio (95% CI)	Chi-square test[b]		
											HWE(df = 1)[c]	GF(df = 2)[d]	AF(df = 1)[e]
rs2190208	9986227				GG	GA	AA	G	A				
	5′ promoter	___	Schizophrenia	494	186 (0.38)	231 (0.47)	77 (0.16)	603 (0.61)	385 (0.39)	0.96	$\chi2 = 0.14$, $p = 0.71$	$p = 0.48$	$p = 0.59$
			Control	930	325 (0.35)	466 (0.50)	139 (0.15)	1116 (0.60)	744 (0.40)	(0.82–1.12)	$\chi2 = 1.79$, $p = 0.18$	$\chi2 = 1.48$	$\chi2 = 0.29$
rs1029564	9974131				AA	AC	CC	A	C				
	intron 1	12096	Schizophrenia	492	334(0.68)	140 (0.28)	18 (0.04)	808 (0.82)	176 (0.18)	0.78	$\chi2 = 0.48$, $p = 0.48$	$\mathbf{p = 0.028}$	$\mathbf{p = 0.011}$
			Control	931	565 (0.61)	324 (0.35)	42 (0.05)	1454 (0.78)	408 (0.22)	(0.64–0.94)	$\chi2 = 0.27$, $p = 0.61$	$\chi2 = 7.17$	$\chi2 = 6.40$
rs17159614	9959778				GG	GA	AA	G	A				
	intron 2	14353	Schizophrenia	495	289 (0.58)	181(0.37)	25 (0.05)	759 (0.77)	231 (0.23)	1.00	$\chi2 = 0.24$, $p = 0.62$	$p = 1.00$	$p = 0.96$
			Control	931	545 (0.59)	339 (0.36)	47(0.05)	1429(0.77)	433 (0.23)	(0.83–1.19)	$\chi2 = 0.38$, $p = 0.54$	$\chi2 = 0.0034$	$\chi2 = 0.0023$
rs12176601	9948019				TT	TA	AA	T	A				
	intron 2	11759	Schizophrenia	493	166(0.34)	244 (0.49)	83 (0.17)	576 (0.58)	410 (0.42)	1.21	$\chi2 = 0.17$, $p = 0.68$	$\mathbf{p = 0.029}$	$\mathbf{p = 0.021}$
			Control	917	375 (0.41)	403 (0.44)	139 (0.15)	1153 (0.63)	681 (0.37)	(1.03–1.41)	$\chi2 = 3.16$, $p = 0.08$	$\chi2 = 7.08$	$\chi2 = 5.35$
rs6966472	9933663				AA	AG	GG	A	G				
	intron 4	14356	Schizophrenia	493	381 (0.77)	103 (0.21)	9 (0.02)	865 (0.88)	121 (0.12)	0.73	$\chi2 = 0.43$, $p = 0.51$	$\mathbf{p = 0.023}$	$\mathbf{p = 0.0075}$
			Control	931	656 (0.70)	252 (0.27)	23 (0.02)	1564 (0.84)	298 (0.16)	(0.59–0.92)	$\chi2 = 0.04$, $p = 0.84$	$\chi2 = 7.59$	$\chi2 = 7.16$
rs17559978	9912136				CC	CT	TT	C	T				
	intron 7	21527	Schizophrenia	499	339 (0.68)	138 (0.28)	22 (0.04)	816 (0.82)	182 (0.18)	0.80	$\chi2 = 2.63$, $p = 0.10$	$\mathbf{p = 0.029}$	$\mathbf{p = 0.025}$
			Control	936	571 (0.61)	322 (0.34)	43 (0.05)	1464 (0.78)	408 (0.22)	(0.66–0.97)	$\chi2 = 0.08$, $p = 0.78$	$\chi2 = 7.11$	$\chi2 = 5.05$
rs17159577	9900238				CC	CT	TT	C	T				
	intron 10	11898	Schizophrenia	494	244 (0.49)	195 (0.39)	55 (0.11)	683 (0.69)	305 (0.31)	1.05	$\chi2 = 2.79$, $p = 0.09$	$p = 0.15$	$p = 0.60$
			Control	934	453 (0.49)	403 (0.43)	78 (0.08)	1309 (0.70)	559 (0.30)	(0.88–1.24)	$\chi2 = 0.77$, $p = 0.38$	$\chi2 = 3.78$	$\chi2 = 0.27$
rs17159556	9886562				GG	GT	TT	G	T				
	intron 10	13676	Schizophrenia	496	372 (0.75)	112 (0.23)	12 (0.02)	856(0.86)	136(0.14)	0.76	$\chi2 = 1.03$, $p = 0.31$	$\mathbf{p = 0.024}$	$\mathbf{p = 0.012}$
			Control	932	635 (0.68)	271 (0.29)	26 (0.03)	1541 (0.83)	323(0.17)	(0.61–0.94)	$\chi2 = 0.21$, $p = 0.65$	$\chi2 = 7.43$	$\chi2 = 6.29$
rs7800072	9863265				AA	AC	CC	A	C				
	exon 17	23297	Schizophrenia	502	342 (0.68)	140 (0.28)	20 (0.04)	824 (0.82)	180(0.18)	0.76	$\chi2 = 1.37$, $p = 0.24$	$\mathbf{p = 0.013}$	$\mathbf{p = 0.0069}$
	Lyn701Gln		Control	934	563 (0.60)	327 (0.35)	44 (0.05)	1453(0.78)	415 (0.22)	(0.63–0.93)	$\chi2 = 0.16$, $p = 0.69$	$\chi2 = 8.67$	$\chi2 = 7.31$

[a] Chromosome position was established from the dbSNP database.
[b] Without Bonferroni's correction.
[c] HWE; Hardy–Weinberg equilibrium.
[d] GF; Genotype distribution frequency.
[e] AF; Allele distribution frequency.

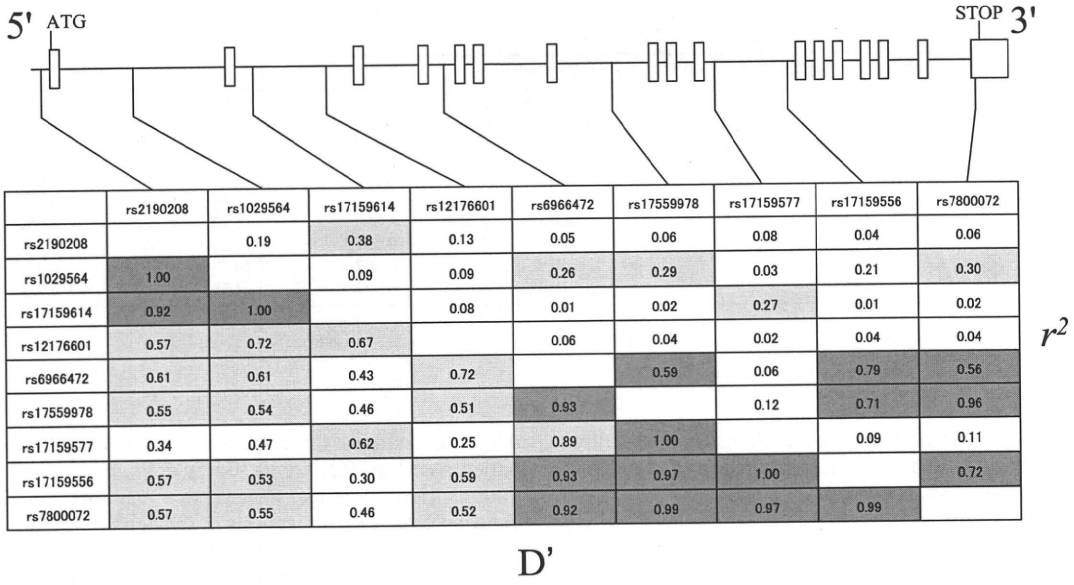

Fig. 1. The genetic structure of *SEMA3D* and location of the examined SNPs. The D′ and r^2 values between paired SNPs are shown in the diagram. The exonic regions are shown as white squares. The intensity of the box color corresponds to the strength of LD or r^2.

rs17159614, rs12176601, rs6966472, rs17559978, rs17159577, and rs17159556), several significant differences in genotype and allele distributions were observed (Table 1). To correct for multiple testing, we calculated the experiment-wide significance threshold required to keep the type I error rate at 5%. As a result, the corrected p value was calculated as 0.0085. The allelic associations with the SNPs rs7800072 (Lys701Gln) and rs6966472 remained significant after the correction (Table 1). Distinguishing between the carriers and the non-carriers with respect to the Gln701 allele for patients and controls, the protective effect became clearer ($p = 0.0033$).

The results of haplotype-based analyses are shown in Table 2. There were significant haplotypic associations of the SNPs in *SEMA3D* when comparing the schizophrenic patients and control subjects. In particular, the four-marker haplotype (rs2190208–rs1029564–rs17159614–rs121176601) showed a statistically significant association with schizophrenia (global permutation $p = 0.00001$). Concerning this haplotype analysis, global p values of 100,000 permutations, which corrected for multiple testing, were also significant. Furthermore, the haplotype frequency of GAGA was significantly higher in schizophrenia patients than in control subjects (0.376 and 0.291, permutation $p = 0.00005$), whereas those of GAGT, AAAA, and GCGA were significantly lower in schizophrenic patients than in controls (0.050 and 0.084, permutation $p = 0.0029$; 0.007 and 0.025, permutation $p = 0.0062$; 0.007 and 0.021, permutation $p = 0.020$, respectively) (Table 3).

When we performed stratified analysis of the data for rs7800072 by sex, a significant association was observed in women ($p = 0.0089$), but not in men ($p = 0.41$) (supplementary Tables 1 and 2). In the haplotype analysis, on the other hand, the four-marker haplotype (rs2190208–rs1029564–rs17159614–rs121176601) showed a statistically significant association in men (global permutation $p = 0.00001$), but was at a trend level in women (global permutation $p = 0.0699$). The haplotype frequency of GAGA

Table 2
Associations with schizophrenia of the 9 SNPs and haplotypes in *SEMA3D*.

SNP No.	dbSNP ID	Allele model	Haplotype p[a]							
		p value	2 Locus	3 Locus	4 Locus	5 Locus	6 Locus	7 Locus	8 Locus	9 Locus
SNP1	rs2190208	0.59								
			0.019							
SNP2	rs1029564	**0.011**		0.10						
			0.029		**0.00001**					
SNP3	rs17159614	0.96		**0.00002**		**0.00005**				
			0.0004		**0.0003**		**0.00007**			
SNP4	rs12176601	**0.021**		**0.0010**		**0.0001**		**0.0003**		
			0.035		**0.0006**		**0.0016**		**0.0007**	
SNP5	rs6966472	**0.0075**		0.053		**0.0004**		**0.0001**		**0.0007**
			0.023		0.098		**0.0001**		**0.0001**	
SNP6	rs17559978	**0.025**		**0.022**		0.061		**0.0004**		
			0.030		**0.025**		0.076			
SNP7	rs17159577	0.60		0.064		**0.024**				
			0.042		0.051					
SNP8	rs17159556	**0.012**		**0.028**						
			0.020							
SNP9	rs7800072	**0.0069**								

[a] global p value.

Table 3
Estimated haplotype frequencies and association significance for *SEMA3D*.

Haplotype	rs2190208	rs1029564	rs17159614	rs12176601	% of individuals					
					Overall	Control	Schizophrenia	χ2	*p value*	Permutation *p* value
1	G	A	G	A	0.321	0.291	0.376	20.40	**0.0000063**	**0.000050**
2	A	A	A	T	0.207	0.201	0.219	1.21	0.27	0.28
3	G	C	G	T	0.190	0.199	0.172	2.98	0.085	0.089
4	A	A	G	T	0.142	0.143	0.139	0.10	0.75	0.76
5	G	A	G	T	0.072	0.084	0.050	11.23	**0.00080**	**0.0029**
6	A	A	G	A	0.034	0.036	0.031	0.37	0.54	0.59
7	A	A	A	A	0.019	0.025	0.007	10.75	**0.0010**	**0.0062**
8	G	C	G	A	0.016	0.021	0.007	7.55	**0.0060**	**0.020**

	χ2	*p value*	Permutation *p* value	Replications
Global	46.07	**0.000000085**	**0.00001**	100000

was significantly higher in schizophrenia patients than in control subjects in both men (0.368 and 0.272, permutation $p = 0.00053$) and women (0.384 and 0.302, permutation $p = 0.003$).

4. Discussion

Our results provide the first evidence for the possible involvement of *SEMA3D* in the pathogenesis of schizophrenia. With respect to the non-synonymous (Lys701Gln) polymorphism, we found a significant preponderance of the Lys/Lys genotype and the Lys701 allele in schizophrenia patients compared with control subjects. In the haplotype-based analyses, we also obtained evidence for an association between *SEMA3D* and schizophrenia. Interestingly, the most significant haplotype, rs2190208–rs1029564–rs17159614–rs121176601, does not include rs7800072 (Lys701Gln) (see Fig. 1). Therefore, it is likely that at least one functional polymorphism other than rs7800072, which is in linkage disequilibrium to the haplotype, could be responsible for susceptibility to schizophrenia. In stratified analysis for rs7800072 by sex, the frequency of the Gln701 allele was significantly lower in schizophrenia patients than in control subjects in women (0.17 and 0.23, $p = 0.0088$) (supplementary Table 2). Likewise, this was also lower in men, but was not statistically significant (0.18 and 0.20, $p = 0.41$) (supplementary Table 1). Regarding analysis of the four-marker haplotype (rs2190208–rs1029564–rs17159614–rs121176601), there remained a statistical significance in men (global permutation $p = 0.00001$) and a tendency in women (global permutation $p = 0.0699$). In addition, the frequency of the most major haplotype (GAGA) was significantly higher in schizophrenia patients than in control subjects in both sexes. These inconsistent results between males and females are likely to have arisen from the lack of statistical power after dividing the sexes.

The neurodevelopmental hypothesis of schizophrenia proposes that abnormalities of brain development are involved in the pathogenesis of schizophrenia (Conrad and Scheibel, 1987; Weinberger, 1987; Murray, 1994; Waddington et al., 1998). In early brain developmental stages, a number of semaphorins play important roles in axonal repulsion, axonal attraction, neuronal cell migration, and axon pruning (reviewed in Kruger et al., 2005; Waimey and Cheng, 2006; Halloran and Wolman, 2006; Mann et al., 2007). Indeed, SEMA3D has been shown to act in axon guidance and cell migration during neuronal development (Wolman et al., 2004, 2007; Liu et al., 2004; Liu and Halloran, 2005; Sakai and Halloran, 2006; Takahashi et al., 2009). With respect to neuronal cell migration, neuronal disarray and abnormal migration in the neocortical white matter were reported in postmortem studies of patients with schizophrenia (Jakob and Beckmann, 1986; Akbarian et al., 1993). Regarding pruning, Feinberg proposed that schizophrenia may arise from excessive synaptic pruning during adolescence (Feinberg, 1982; Keshavan et al., 1994). Indeed, decreased density of dendritic spines was observed in the prefrontal cortex of patients with schizophrenia (Garey et al., 1998; Glantz and Lewis, 2000). These findings suggest that variants of *SEMA3D* may contribute to the pathogenesis of schizophrenia through affecting development of neural networks. The genotypic difference based on the Lys701Gln polymorphism of *SEMA3D* might lead to developmental differences in the brain; the Gln701 carriers would exhibit intrinsically greater protective effects against the development of schizophrenia than the Gln701 non-carriers. Although *SEMA3D* has not yet been well-studied, *SEMA3A* has been investigated in detail. In particular, an increase in the expression of SEMA3A has previously been associated with schizophrenia (Eastwood et al., 2003). Moreover, *PLXNA2*, which encodes one of the receptors for class 3 semaphorins, was identified as a candidate gene for schizophrenia in a genome-wide association study (Mah et al., 2006). Currently, this association is also supported by the meta-analysis of Allen et al. (2008). SEMA3A and SEMA3D belong to the same class and share the most similarity with each other of the class 3 semaphorin genes (Luo et al., 1995). These findings further strengthen the evidence for a possible role of *SEMA3D* in the development of schizophrenia.

It is possible that the amino acid change (Lys701Gln) may affect the function of SEMA3D protein and that this results in susceptibility to schizophrenia. Indeed, this is a substitution from a large and basic amino acid (Lys) to a medium-sized and polar one (Gln). This is likely to lead to functional differences between the two types of SEMA3D. One possibility is that this substitution might result in conformational change of SEMA3D and influence its affinity for its receptors. Another possibility is that the Lys701 and Gln701 variants of SEMA3D have different cellular localization. The basic domain of class 3 semaphorins electrostatically interacts with the proteoglycan components of the extracellular matrix (De Wit et al., 2005) and the granule matrix (de Wit et al., 2009). The substitution from the basic Lys701 to the non-basic Gln701 may affect such interactions between SEMA3D and these matrices. Alteration of the extracellular matrix may modify distribution of SEMA3D in neurons, and that of the granule matrix may affect secretion from secretory vesicles. The class 3 semaphorins not only act as axon guidance cues but also have key roles in synaptic formation and function. Therefore, these modified interactions could impact on the establishment of synaptic contacts and the formation of new synapses. Although the amino acid substitution (Lys701Gln) was predicted to be benign by Polyphen (http://genetics.bwh.harvard.edu/pph/) and SIFT (http://sift.jcvi.org/) programs, its actual effects should be elucidated by cell biological or biochemical approaches.

Accumulating evidence suggests that the semaphorins are regulatory factors of tumor progression and modulators of angiogenesis (reviewed in (Neufeld and Kessler, 2008) and (Capparuccia and Tamagnone, 2009)). Recently, SEMA3D was also reported to

possess anti-tumorigenic and anti-angiogenic properties (Kigel et al., 2008). The hypoactivity of *SEMA3D* could be linked to increased incidence of cancer. Previous studies and reviews have partially supported the idea that the incidence of cancer in patients with schizophrenia is reduced compared with the general population (Grinshpoon et al., 2005; Dalton et al., 2005; Catts et al., 2008). It is possible that semaphorins are related to the development of schizophrenia and also contribute to the associated lower incidence of cancer, and this topic warrants further investigation.

In conclusion, we found a significant association between the Lys701Gln polymorphism of *SEMA3D* and schizophrenia. In addition, the haplotype rs2190208–rs1029564–rs17159614–rs121176601, not including the Lys701Gln variant, was shown to be associated with schizophrenia, which suggests that some other polymorphisms of *SEMA3D* play a role in the pathogenesis of schizophrenia. Taking the previous molecular and developmental findings together with the present genetic findings, *SEMA3D* appears to be a promising candidate gene related to susceptibility to schizophrenia.

Conflict of interest

All authors declare no conflict of interest that could influence their work.

Role of funding source

This study was supported by Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS) (T.F.), Japan Human Sciences Foundation (T.F.), Health and Labor Sciences Research Grants (Research on Psychiatric and Neurological Diseases and Mental Health; Research on Human Genome Tailor made) (H.K.), Japan Health Sciences Foundation (Research on Health Sciences focusing on Drug innovation) (H.K.), the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBIO) (H.K.), JST, CREST (H.K.), and Grant-in-Aid for Scientific Research on Priority Areas of Applied Genomics from the Ministry of Education, Culture, Sports, Science and Technology of Japan (H.K.). These agencies had no role in study design, acquisition and interpretation of data or writing the report.

Contributors

T.F. designed the study, performed genotyping of *SEMA3D*, made statistical analysis, managed literature search, interpreted the data, and wrote the manuscript. H.U. and N.Y. took part in genotyping. H.H., M.T., M.I., K.A., and T.H. collected samples and gave comments to the manuscript. H.K. organized recruitment and genotyping of schizophrenic patients and control subjects, and took part in analyzing the data and writing the manuscript.

Acknowledgements

We thank the patients and the healthy volunteers for their participation.

Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.jpsychires.2010.05.004.

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Psychiatry Research 185 (2011) 20–26

Contents lists available at ScienceDirect

Psychiatry Research

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

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

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ARTICLE INFO

Article history:
Received 21 September 2009
Received in revised form 11 May 2010
Accepted 3 June 2010

Keywords:
Serotonin 1A receptor gene (*HTR1A*)
Functional SNP
Tagging SNP
Bipolar disorder
Schizophrenia

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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0165-1781/$ – see front matter.
doi:10.1016/j.psychres.2010.06.003

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 (3×2×2) 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).

Table 2
Logistic regression analysis of single markers in *HTR1A* with schizophrenia and bipolar disorder.

SNP[a]	Genotype	Schizophrenia			Bipolar disorder		
		OR[b]	95% CI[c]	*P*-value	OR[b]	95% CI[c]	*P*-value
rs6295	CC (reference)						
C>G	CG	2.09	0.932–4.59	0.0682	1.03	0.706–1.50	0.886
	GG	1.34	0.534–3.18	0.518	1.08	0.742–1.57	0.702
rs878567	CC (reference)						
C>T	CT	1.98	0.105–1.20	0.0667	1.00	0.648–1.54	0.993
	TT	2.91	0.589–16.3	0.201	1.90	0.930–3.89	0.0782

Reference genotypes are common genotype. Adjustment for age and gender.
[a] Major allele>minor allele.
[b] OR: odds ratio.
[c] CI: confidence interval.

wise analysis with the chi-square test (Table 1), but not with logistic regression adjusted for age and gender (Table 2). In addition, we found an association between *HTR1A* and BP in the haplotype-wise analysis adjusted for age and gender (Table 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 1.20–1.26 in BP for *HTR1A*, under a multiplicative model of inheritance.

3.2. Meta-analysis

3.2.1. Schizophrenia

In the meta-analysis, two association studies, including our study, met our criteria for rs6295 (Table 4). We found significant heterogeneity among ORs ($P(Q) = 0.000142$). The pooled OR derived from all studies comprising 965 patients and 1964 healthy control subjects did not indicate a significant association (random model: pooled OR = 0.793, 95% CI = 0.387–1.623, $P(Z) = 0.526$) (Fig. 1).

3.2.2. Bipolar disorder

In the meta-analysis, three association studies, including our study, met our criteria for rs6295 (Table 4).We did not find significant heterogeneity among ORs ($P(Q) = 0.789$). The pooled OR derived from all studies comprising 1148 patients and 1964 healthy control subjects indicated a significant association (fixed model: pooled OR = 0.794, 95% CI = 0.641–0.983, $P(Z) = 0.0344$) (Fig. 1). No publication bias was found ($t = 0.656$, $p = 0.536$).

4. Discussion

Although we detected an association between rs878567 and schizophrenia in the allele-wise analysis, this significance disappeared after multiple testing. 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 (Tables 2 and 3). Therefore, our results suggest that *HTR1A* does not play a role in the pathophysiology of schizophrenia in the Japanese population. On the other hand, in the single-marker association study, we detected a significant association between *HTR1A* and BP with the chi-square test. However, this association may have been due to biased samples, which were unmatched for gender. We therefore performed a logistic regression analysis to compare the phenotypes of each of the examined SNP genotypes, using several clinical factors as other independent variables to adjust for possible confounding. Although we did not detect an association between the two SNP genotypes in *HTR1A* and BP with logistic regression analysis, we found an association between *HTR1A* and BP in the haplotype-wise analysis adjusted for age and gender. Our results, therefore, suggest that *HTR1A* plays a role in the pathophysiology of BP in the Japanese population.

We detected an association between *HTR1A* and BP, but not schizophrenia. Ivleva and colleagues suggested that genes, which are associated with schizophrenia but not BP, may play a major role in the pathophysiology of psychosis. Genes associated with BP, but not schizophrenia, may also play a major role in the pathophysiology of mood dysregulation (Ivleva et al., 2010). Considering the above, *HTR1A* was considered to have an influence in mood regulation. However, we reported that *HTR1A* was associated with methamphetamine-induced psychosis in the Japanese population (Kishi et al., 2009c). We also detected a marginal association between *HTR1A* and schizophrenia in the Japanese population. Considering the neurodevelopmental model of the pathophysiology of both disorders, *HTR1A* may relate to neurodevelopment (Ivleva et al., 2010). It will be necessary to conduct further studies, including environmental factors.

The LD of rs6295 and rs878567 in our BP samples was looser than in controls and schizophrenia samples (r^2 value: controls = 0.160, schizophrenia = 0.101 and BP = 0.00600). Although we detected no association between *HTR1A* and BP in the single-marker association analysis, it may be that the difference in LD reflects the haplotype-wise analysis.

We detected an association between rs6295 and BP in the meta-analysis. The studies of Huang and colleagues and Sullivan and colleagues found no association between rs6295 in *HTR1A* and BP (Huang et al., 2004; Sullivan et al., 2009). However, in our meta-analysis, we detected an association between *HTR1A* and BP. The following may be causes for these different results: First, because the samples in the two original studies were small, there is a possibility of type II errors in their studies. Second, although we did not detect significant heterogeneity among ORs ($P(Q) = 0.789$), the MAFs of the studies included in the meta-analysis were each different. Third, because there are few samples to use in meta-analysis, the significant associations between *HTR1A* and BP in the case-control study and the meta-analysis also may have been due to type I errors. Further, different screening methods were used in each study for the samples included in this meta-analysis. Rs 6295 is associated with disorders, including major depressive disorder (Lemonde et al., 2003; Parsey et al., 2006; Anttila et al., 2007; Kraus et al., 2007; Neff et al., 2009),

Table 3
Haplotype-wise analysis of *HTR1A*.

Haplotype rs6295–rs878567	Phenotype	Number of subjects	Individual haplotype frequency	OR[a]	95% CI[b]	Individual *P*-value[c]	Phenotype	Global *P*-value[c]	Corrected Global *P*-value[c,d]
C-C (reference)	Controls	1372	0.814						
	Schizophrenia	635	0.788	1.20	0.971–1.47	0.0921			
	Bipolar disorder	736	0.761	1.38	1.19–1.59	**0.0000176**	Schizophrenia	0.0935	
G-C	Controls	314	0.186				Bipolar disorder	**0.0000203**	**0.000244**
	Schizophrenia	171	0.212	1.20	0.971–1.47	0.0921			
	Bipolar disorder	231	0.239	1.38	1.19–1.59	**0.0000176**			

Adjustment for age and gender.
[a] OR: odds ratio.
[b] CI: confidence interval.
[c] Bold numbers represent significant *P*-value.
[d] Calculated by Bonferroni correction (12 times).