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Gene-wide association study between the methylenetetrahydrofolate reductase gene (*MTHFR*) and schizophrenia in the Japanese population, with an updated meta-analysis on currently available data

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ABSTRACT

Methylenetetrahydrofolate reductase (MTHFR) is a critical molecule for single-carbon transfer reactions. Recent evidence suggests that polymorphisms of MTHFR are related to neural tube deficits and the pathogenesis of schizophrenia. While several studies have demonstrated associations between the gene encoding the MTHFR (*MTHFR*) polymorphisms and schizophrenia, these studies lack consistency. Therefore, we conducted a gene-wide association study (patients with schizophrenia = 696, control subjects = 747) and performed imputation analysis. Additionally, we performed meta-analysis on currently available data from 18 studies for two common functional polymorphisms (rs1801131 and rs1801133).

There were no significant associations with schizophrenia in the single marker analysis for the seven tagging SNPs of *MTHFR*. In the haplotypic analysis, a nominally significant association was observed between the haplotypes, which included four SNPs (rs1801133, rs17421511, rs17037396, and rs9651118) and the schizophrenic patients. Additionally, the imputation analysis demonstrated there were several associated markers on the *MTHFR* chromosomal region. However, confirmatory analyses of three tagging SNPs (rs1801133, rs17037396, and rs9651118) and the top SNP (rs17421511) for the imputation results (patients with schizophrenia = 797, control subjects = 1025) failed to replicate the haplotypic analysis and the imputation results. These findings suggest that *MTHFR* polymorphisms are unlikely to be related to the development of schizophrenia in the Japanese population. However, since our meta-analysis results demonstrated strong support for association of rs1801133 with schizophrenia, further replication studies based on a gene-wide approach need to be considered.

1. Introduction

Schizophrenia is a chronic and disabling mental disorder with a lifetime prevalence of approximately 1% in the global population (Freedman, 2003). Accumulating evidence suggests that both genetic and environmental factors contribute to the

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etiology of schizophrenia (Burmeister et al., 2008). Although schizophrenia has a high heritability with rates estimated at 80% (Sullivan et al., 2003), there has been no consistent replication found for the schizophrenia candidate genes (Harrison and Weinberger, 2005). Recent genome-wide association (GWA) studies have demonstrated new promising susceptibility genes for schizophrenia (O'Donovan et al., 2008), as well as for other common diseases (Rioux et al., 2007; The Wellcome Trust Case Control Consortium, 2007; Zeggini et al., 2007). Therefore, use of this methodology can be advantageous when trying to detect potential genetic factors responsible for the development of these disorders. In addition, by focusing on the specific molecular pathway related to the pathophysiology of schizophrenia, this may also be useful when trying to identify susceptibility genes that have a mild contribution to the development of the disease (Kirov et al., 2005).

Dysfunction of homocysteine metabolism has been linked to neurodevelopmental disorders, including neural tube defects (NTDs) (Blom et al., 2006; van der Put et al., 1995), schizophrenia (Allen et al., 2008; Muntjewerff et al., 2006), and depression (Lewis et al., 2006), in addition to other diseases and syndromes (Hobbs et al., 2000; Kluijtmans et al., 1996; Qian et al., 2007). Recent studies have also suggested that elevated plasma homocysteine levels are observed in major psychiatric disorders such as schizophrenia and bipolar disorder (Levine et al., 2005). Plasma homocysteine levels affect the intracellular methylation process of DNA, lipids, proteins, and neurotransmitters (Scott and Weir, 1998). Both elevated homocysteine levels along with physiological levels of its oxidized derivatives, such as homocysteic acid and homocysteine sulfinic acid, have been shown to be toxic for neurons and vascular endothelial cells (Zou and Banerjee, 2005). While levels of homocysteine are affected by various genes involved in the homocysteine metabolic pathway and by environmental factors such as folate or vitamin B_{12} intake (Refsum et al., 2004), methylenetetrahydrofolate reductase (MTHFR) also plays a major role in this pathway. MTHFR converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which serves as a carbon donor for the methylation of homocysteine, leading to the generation of S-adenosylmethionine (SAM) (Andreoli and Maffei, 1975). SAM is a major source of methyl groups in the brain (Godfrey et al., 1990) and is involved in catechol-O-methyltransferase (COMT) reactions such as the catabolism of serotonin and other catecholamines (Anguelova et al., 2003; Chen et al., 2004). Freeman et al. (1975) reported there is direct evidence linking decreased MTHFR activity to schizophrenia (Freeman et al., 1975). These findings have led to multiple genetic analyses examining the link between the MTHFR gene (gene symbol: *MTHFR*, GenBank accession number: NM_005957) and schizophrenia.

MTHFR is composed of twelve exons (Fig. 1) and is localized on chromosome 1p36.3 (Goyette et al., 1994). It has been suggested that this may be a susceptibility locus for schizophrenia, bipolar disorder (Kempisty et al., 2007) and major depressive disorder (McGuffin et al., 2005). Two common functional polymorphisms of *MTHFR*, C677T (rs1801133) and A1298C (rs1801131), are known to cause a decrease of enzyme activity and affect nucleic synthesis and DNA methylation (van der Put et al., 1998). Several studies have confirmed the possible involvement of these SNPs in psychiatric conditions such as schizophrenia (Regland, 2005) and affective disorders (Arinami et al., 1997). Subjects with homozygosity for the 677 T allele have a mild increase in their plasma homocysteine levels, and these subjects have a higher frequency of neural tube deficits and premature cardiovascular disease as compared to other similar genotype carriers (Bakker and Brandjes, 1997; Matsushita et al., 1997). The impact of this polymorphism varies according to environmental factors, such as folate, vitamin B_2 or vitamin B_{12} (Hustad et al., 2000; Refsum et al., 2004; van der Put et al., 1995). Although some studies have reported that carriers of the 677 T allele in *MTHFR* are associated with an increased risk of schizophrenia (Arinami et al., 1997; Muntjewerff et al., 2005; Sazci et al., 2003), others have shown contradictive results (Kunugi et al., 1998; Vilella et al., 2005; Yu et al., 2004). The association of the *MTHFR* C677T variant with schizophrenia may be linked to the excitatory amino acids hypothesis or to decreased plasma concentrations of SAM that have been reported in psychiatric disorders (Andreoli and Maffei, 1975). Another functional polymorphism, A1298C, also has been shown to decrease MTHFR activity, although van der Put et al. (1998) have reported finding no significant effect of this variant on the plasma homocysteine levels.

A recent meta-analysis demonstrated an association between elevated homocysteine levels or carriers of the 677 T allele and an increased risk of developing schizophrenia (Allen et al., 2008; Muntjewerff et al., 2006). It has been suggested that potential associations between genetic variation in folate metabolism and psychiatric disorders could be plausible biological explanations for these disorders (Coppen and Bolander-Gouaille, 2005).

Taken together, *MTHFR* may be related to the development of schizophrenia. Although a number of studies have demonstrated associations between specific polymorphisms of *MTHFR* and schizophrenia, there have been no gene-based analysis studies. Therefore, it is still difficult to interpret these types of studies due to the inconsistent results that have been derived from some of the confounding factors, such as population

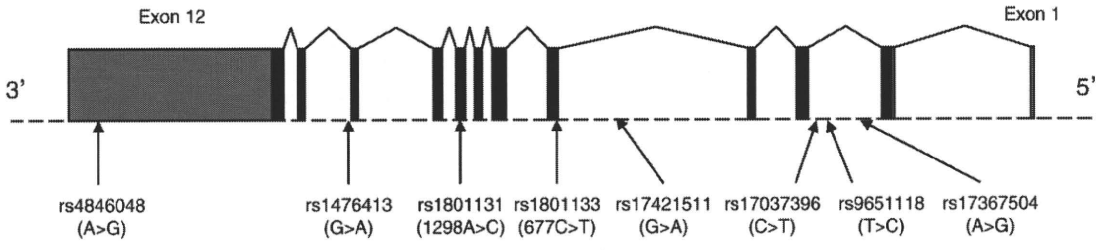

Fig. 1. Genomic structure of *MTHFR*. Black boxes indicate protein-coding regions, while the gray boxes represent the untranslated regions (UTRs). Each box represents *MTHFR* exons. Numbers under the arrows represent the SNP IDs, the tagging SNPs (pairwise tagger: $r^2>0.8$; Haploview 3.32), and the top SNP (rs17421511) of imputation results.

stratifications (ethnic or gender differences) and number of samples. In the present study, we conducted an association study between *MTHFR* and schizophrenia in the Japanese population that was based on the gene-wide approach. In addition, we also performed a meta-analysis on the updated data currently available.

2. Materials and methods

2.1. Subjects

The samples for this association study consisted of 696 patients with schizophrenia and 747 control subjects. The confirmation sample set for four SNPs (rs1801133, rs17421511, rs17037396, and rs9651118), which were positively associated with schizophrenia in the haplotypic analysis and the imputation analysis, consisted of 797 patients with schizophrenia and 1025 control subjects. Detailed demographical data are presented in Supplementary Table 1.

All subjects were unrelated to each other and ethnically Japanese. The schizophrenia diagnosis was made by at least two experienced psychiatrists and based on unstructured patient interviews and reviews of their medical records in accordance with the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR) criteria for schizophrenia. All healthy control subjects were also psychiatrically screened on the basis of unstructured interviews.

This study was approved by the Ethics Committee of the Nagoya University Graduate School of Medicine and Fujita Health University. Written informed consent was obtained from each subject.

2.2. Tagging SNP selection

In order to obtain the SNPs that covered the entire coding region as well as the regulatory elements in the 5′ and 3′ flanking areas for both the 1000 base pairs (bps) upstream and downstream of the coding region, we first examined the *MTHFR* genotyping data from the HapMap database (HapMap Data Rel 21/phase II Jan 06, population: Japanese living in Tokyo). Subsequently, the tagging SNPs were selected using the Haploview software version 4.2 in accordance with the criterion of the Tagger program for pairwise tagging, $r^2>0.8$, with minor allele frequency (MAF)>0.1 (de Bakker et al., 2005) (Supplementary Table 2). We excluded rs13306553 due to the unavailability of a reliable genotyping method (genotype call rate<95%). Therefore, a total of seven SNPs were recruited for these genetic association analyses (Fig. 1).

2.3. SNP genotyping

Venous blood was drawn from each subject and genomic DNA was extracted according to standard phenol/chloroform method. SNP genotyping was carried out using the TaqMan allelic discrimination assay (Applied Biosystems, Foster City, CA, USA). TaqMan probes and Universal PCR Master Mix were purchased from Applied Biosystems. Allelic specific fluorescence was measured on the ABI PRISM 7900HT using the Sequence Detection Systems 2.0 software (Applied Biosystems) for allelic discrimination. To exclude low-quality DNA sample or genotyping probes, data sets were filtered on the basis of tagging SNP genotype call rates (95% completeness). Subjects whose percentage of missing genotypes was >10% or who had evidence of possible DNA contamination were excluded from subsequent analyses. For quality control, we randomly selected 10 samples for each SNP and then genotyped these in duplicate in order to evaluate the genotype error rate.

2.4. Imputation and confirmatory association analysis

To estimate genotypes of untyped SNPs located on the analyzed gene region, we conducted an imputation analysis. This method provides enhanced statistical power for the coverage of common variants within the locus of interest. Specifically, based on directly genotyped SNPs and the haplotypes detected in the hapmap JPT sample, a computational algorithm predicted the genotypes at the SNPs that are not directly genotyped in the study sample (Marchini et al., 2007). We carried out this analysis using the MACH 1.0 program (http://www.sph.umich.edu/csg/abecasis/MACH/) in order to calculate the genotypic prediction for the 11 untyped SNPs. These calculations used information from the screening scan for the seven directly typed SNPs and the HapMap database (HapMap Data Rel 21/phase II Jan 06, population: Japanese/Chinese).

The MACH program has been reported to have imputation accuracy rates similar to IMPUTE and both programs are able to outperform fastPHASE, PLINK, and Beagle (Pei et al., 2008). As previously mentioned, the analyzed region of imputation was limited to the *MTHFR* locus. Associated SNPs were pruned based on the linkage disequilibrium (LD) pattern ($r^2>0.8$; Supplementary Table 2) and minor allele frequency (MAF<0.05), with the SNP showing the smallest allelic p value selected for follow up.

2.5. Statistical analysis

Genotype deviation from the Hardy–Weinberg equilibrium (HWE), and marker–trait associations (allelic, genotypic, and haplotypic analysis) were evaluated by using PLINK v1.06 (Purcell et al., 2007). The significance level for all statistical tests was 0.05. Bonferroni correction was used to control inflation of the type I error rate in the allele-wise, genotype-wise, and haplotype-wise analyses. To reduce the total number of tests, clearly unassociated markers were removed in the first stage (screening sample set) of the present study. Conditional on the first stage findings, which used a less stringent nominal level, we subsequently tested the second stage (confirmation sample set) using the augmented data and the data from the first stage. In this joint sample analysis, p values were generated by the Cochran–Mantel–Haenszel stratified analysis, while the Breslow–Day Test was performed for evaluation of heterogeneous associations as implemented in PLINK. Based on the multiplicative model of inheritance, power calculations were performed using the Genetic Power Calculator (Purcell et al., 2003).

2.6. Meta-analysis

We performed a meta-analysis for rs1801131 and rs1801133, which are the two SNPs that have been previously shown to be associated with schizophrenia (Arinami et al., 1997; Betcheva et al., 2009; Feng et al., 2009; Garcia-Miss et al., 2010; Jonsson et

al., 2008; Joober et al., 2000; Kempisty et al., 2007; Kempisty et al., 2006; Kunugi et al., 1998; Lee et al., 2006; Muntjewerff et al., 2005; Philibert et al., 2006; Sazci et al., 2003; Sazci et al., 2005; Tan et al., 2004; Vilella et al., 2005; Yu et al., 2004). Initially, the Q statistic test was performed to assess the heterogeneity in the combined studies. As substantial amounts of variation have been previously observed, we decided to calculate the cumulative odds ratio (OR) and corresponding p value based on a random effect model (OR was calculated based on minor allele observed in Japanese population). Furthermore, use of this calculation was chosen because many investigators consider the random effects model to be a much more natural choice as compared to the fixed effects approach (Ades et al., 2005; DerSimonian and Laird, 1986; Fleiss and Gross, 1991). The significance of the overall OR was determined by the Z-test. Publication bias was assessed using a linear regression analysis to measure funnel plot asymmetry. A probability level of $p<0.05$ was used as the threshold for statistical significance. Comprehensive Meta-Analysis software (Version 2.2.046, Biostat, Englewood, NJ) was used to perform the analysis.

3. Results

Regarding quality control, the genotype calls of the duplicated samples showed complete concordance (data not shown), and all genotype frequencies of the tagging SNPs were consistent with the HWE. There were no significant differences between the schizophrenic patients and the control subjects in both allele and genotype distributions without imputed (untyped) SNP (rs17421511) (Table 1). In the haplotypic analysis, a nominally significant association was observed between the haplotypes including four SNPs (rs1801133, rs17421511, rs17037396, and rs9651118) and schizophrenic patients (Table 1). Imputation analysis showed several associated markers for schizophrenia on the *MTHFR* chromosomal region (Table 2). These nominally significant associations, however, did not survive after Bonferroni correction. After assessment of the HapMap database, the top SNP (rs17421511) was selected to confirm these nominal significant associations between imputed markers and schizophrenia. The results of the genotyping data in confirmatory analyses and joint analyses for the four SNPs (rs1801133, rs17421511, rs17037396, and rs9651118) after Bonferroni correction showed no significant association signal for either the allele and haplotype frequencies with schizophrenic phenotype (Table 3 and Supplementary Table 3). Assuming a multiplicative model of inheritance, a disease prevalence of 1%, and a high LD between the genotyped SNP and risk variant, we obtained more than 80% power in detecting the gene-wide association with schizophrenia when the genotype relative risk was set at 1.28 to 1.38 (screening sample set) and 1.25 to 1.35 (confirmation sample set) (MAF: 0.11 to 0.40 and 0.10 to 0.40, respectively). In the meta-analysis for the two commonly associated SNPs, we used all available data from 18 studies and data from studies that only focused on Asian populations (seven studies) to calculate the cumulative odds ratio (OR). We observed association only at rs1801133 for schizophrenia ($P_{(\text{random model})}=0.000833$), without any population-wise specific effect (Supplementary Tables 4 and 5).

4. Discussion

Even though we applied the gene-based approach in the present study, we could not confirm any significant associations of the *MTHFR* polymorphisms with schizophrenia. In the association analysis, we examined the SNPs covering the entire gene, including all of the tagging SNPs that had at least ~ 10% MAF listed on the HapMap database. For all of the genoõtyped SNPs, there were no associations noted between the patients with schizophrenia and the controls in any of the allele frequencies after Bonferroni correction (Table 1). To confirm our results, we additionally performed an imputation analysis for the estimated untyped SNPs and genotyped three markers (rs1801133, rs17037396, and rs9651118) and the top SNP

Table 1
Results of association analyses (screening sample set).

	dbSNP		Single marker (allele-wise)					Multi marker (haplotype-wise)[a]	
			SCZ[b]	CON[c]	L95[d]	U95[d]	p value	2 markers	3 markers
Maker 1	rs4846048	A>G	0.104	0.107	0.754	1.231	0.767		
								0.878	
Maker 2	rs1476413	G>A	0.203	0.203	0.833	1.210	0.968		0.681
								0.899	
Maker 3	rs1801131	A>C	0.201	0.208	0.796	1.157	0.667		0.801
								0.711	
Maker 4	rs1801133	C>T	0.395	0.404	0.827	1.125	0.643		0.628
								0.034	
Maker 5[e]	rs17421511	G>A	0.174	0.138	1.070	1.624	0.009		0.078
								0.035	
Maker 6	rs17037396	C>T	0.110	0.110	0.789	1.278	0.972		0.052
								0.972	
Maker 7	rs9651118	T>C	0.355	0.350	0.872	1.195	0.794		0.974
								0.902	
Maker 8	rs17367504	A>G	0.111	0.113	0.774	1.249	0.889		

[a]Log likelihood ratio test p value (sliding window analysis with rare haplotype threshold 10%).
[b]SCZ: Schizophrenia.
[c]CON: Control; minor allele frequency.
[d]95% confidence intervals (odds ratio).
[e]Imputed SNP with lowest p value.

Table 2
Allele-wise analysis of imputed SNPs.

dbSNP		MAF[a]	*p* value	Quality[b]
rs17421511	G>A	0.158	0.014	0.907
rs17421560	G>A	0.129	0.544	0.940
rs11121832	C>T	0.144	0.041	0.901
rs2066471	G>A	0.152	0.016	0.920
rs7533315	C>T	0.151	0.016	0.923
rs17037390	G>A	0.122	0.586	0.967
rs17037397	C>A	0.107	0.503	0.998
rs2066470	C>T	0.108	0.499	0.994
rs3753582	T>G	0.108	0.499	0.988
rs13306561	T>C	0.132	0.499	0.937
rs3737965	C>T	0.108	0.499	0.978

[a]MAF: minor allele frequency.
[b]Quality: the average posterior probability for the most likely genotype.

(rs17421511) of imputation results (rs17421511). The nominally significant associations that were detected in haplotype-wise analysis and also in imputation analysis did not survive in confirmatory association analysis (Table 3). Therefore, as previously reported, it is unlikely that other common variants related to schizophrenia are causal to the development of this disease (Chakravarti, 1999).

Several researchers have reported that two common *MTHFR* variants, C677T (rs1801133) and A1298C (rs1801131), are related to the development of schizophrenia (Allen et al., 2008; Gilbody et al., 2007). Even though other investigators could not reproduce these findings (Kunugi et al., 1998; Vilella et al., 2005; Yu et al., 2004), results of a recent meta-analysis support a relationship between the *MTHFR* C677T polymorphism and the risk for schizophrenia (Muntjewerff et al., 2006; van der Put et al., 1995). The 677TT/1298AA (Virgos et al., 1999) and 677CC/1298CC (Sazci et al., 2005) compound genotypes have been shown to be over-represented in schizophrenia samples. These contradictions might be derived from confounding factors such as age, gender, or ethnicity (population stratifications) (Cardon and Palmer, 2003; Munafo and Flint, 2004). The discrepancy between these results and our current results could be due to the locus heterogeneity of this disease. In fact, since the statistical power to detect an association exceeded 80%, there is a low possibility of a type II error. The GRR value that was calculated using the Genetic Power Calculator appeared to be appropriate when compared to promising candidate genes for schizophrenia (Schwab et al., 2003; Shifman et al., 2002). In findings from a recent whole genome association study that focused on schizophrenia (O'Donovan et al., 2008), results suggested that the effect size of common SNPs might be very low, and therefore, sample sizes used for genetic association studies need to be very large. Our current meta-analysis provides indirect support for such a scenario. In order to evaluate the impact of the SNP that was shown to be associated with schizophrenia in our meta-analysis (rs1801133), we have used the PolyPhen-2 (Adzhubei et al., 2010). The software compares the property of the wild-type (ancestral, normal) allele and the corresponding property of the mutant (derived, disease-causing) allele. The alignment pipeline selects a set of homologous sequences using a clustering algorithm and then constructs and refines the multiple alignments. According to the aforementioned calculation, rs1801133 was shown to have a damaging effect on protein structure while the ancestral allele showed the high level of evolutionary conservation (Supplementary Table 6). This finding is consistent with the meta-analysis results, as these demonstrated the associated allele is the risk allele. However, while we could not detect the association in our sample, it is of note that we have detected a publication bias ($t=2.778$, $df=16$, $p=0.013$), and therefore, the pooled p value might be overestimated.

In order to be able to elucidate the exact role of genetic variants, definitions of phenotypes are vital for a genetic association study. Therefore, sample stratification using endophenotypes, such as being more specific than phenotypes (e.g., prepulse inhibition, event-related potential, and mismatch negativity), clinical symptoms (e.g., response to medication), or environmental factors (e.g., food intake, supplementation) may be required for these clinical investigations (Braff et al., 2007; Craddock et al., 2006; Gottesman and Gould, 2003). Although we did not take advantage of these types of analytical tests for the genetic association in the present study, these might very well be useful in helping to elucidate the role of *MTHFR* in schizophrenia.

In conclusion, the findings of the present study suggest that *MTHFR* is unlikely to be related to the development of schizophrenia in the Japanese population. However, as our meta-analysis results provided strong support for the association of rs1801133 with schizophrenia, further replication studies based on the gene-wide approach using a large cohort

Table 3
Results of association analyses (confirmation sample set).

dbSNP			Single marker (allele-wise)					Multi marker (haplotype-wise)[a]	
			SCZ[b]	CON[c]	L95[d]	U95[d]	*p* value	2 markers	3 markers
Marker 4	rsl801133	C>T	0.409	0.399	0.910	1.195	0.545		
Marker 5	rs17421511	G>A	0.098	0.098	0.800	1.253	0.991	0.527	0.597
Marker 6	rs17037396	T>C	0.104	0.103	0.812	1.258	0.925	0.924	0.073
Marker 7	rs9651118	A>G	0.354	0.358	0.856	1.131	0.824	0.975	

[a]Log likelihood ratio test *p* value (sliding window analysis with rare haplotype threshold 10%).
[b]SCZ: Schizophrenia.
[c]CON: Control; minor allele frequency.
[d]95% confidence intervals (odds ratio).

of subjects need to be undertaken. In addition, by combining such types of studies with endophenotypes or clinical stratifications, this may provide a better understanding of the pathophysiology of schizophrenia.

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Contributors

Authors Akira Yoshimi, Nagahide Takahashi, and Toshiya Inada designed the study and wrote the protocol. Authors Akira Yoshimi and Yukiko Kawamura conducted SNPs genotyping and statistical analyses. Authors Norio Ozaki, Yukihiro Noda, and Kiyofumi Yamada managed the literature searches and analyses. Author Akira Yoshimi wrote the first draft of the manuscript and Branko Aleksic revised. All authors contributed to and have approved the final manuscript.

Conflict of interest

The authors have no conflicts to declare.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.schres.2010.07.011.

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SHORT COMMUNICATION

An association study between the dymeclin gene and schizophrenia in the Japanese population

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Many gene variants are involved in the susceptibility to schizophrenia and some of them are expected to be associated with other human characters. Recently reported meta-analysis of genetic associations revealed nucleotide variants in synaptic vesicular transport/Golgi apparatus genes with schizophrenia. In this study, we selected the dymeclin gene (*DYM*) as a candidate gene for schizophrenia. The *DYM* gene encodes dymeclin that has been identified to be associated with the Golgi apparatus and with transitional vesicles of the reticulum–Golgi interface. A three-step case–control study of total of 2105 Japanese cases of schizophrenia and 2087 Japanese control subjects was carried out for tag single-nucleotide polymorphisms (SNPs) in the *DYM* gene and an association between an SNP, rs833497, and schizophrenia was identified (allelic $P=2\times10^{-5}$, in the total sample). *DYM* is the causal gene for Dyggve–Melchior–Clausen syndrome and this study shows the second neuropsychiatric disorder in which the *DYM* gene is involved. The present data support the involvement of Golgi function and vesicular transport in the presynapse in schizophrenia.
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Keywords: association; Dyggve–Melchior–Clausen syndrome; Golgi; postmortem study; SNP

INTRODUCTION

Schizophrenia is a chronic, severe and disabling brain disorder that affects approximately 1% of the world's population. Family and twin studies indicate a strong genetic factor and recent genome-wide association studies provided molecular genetic evidence for a substantial polygenic component to the risk of schizophrenia involving many common alleles of very small effect.[1,2] Some of the genetic factors influencing susceptibility to schizophrenia may also have roles in other phenotypes.

In a recent study,[3] in which mRNA abundance was determined by sequencing mRNA in postmortem cerebellum, gene ontology annotation of genes with significantly altered expression revealed overrepresentation of membrane-associated genes, genes involved in zinc binding or transport, regulation of transcription, Golgi apparatus and vesicle-mediated transport. The authors mentioned that most striking were 23 genes involved in presynaptic vesicular transport/Golgi apparatus or postsynaptic neurotransmission. Meta-analysis of genetic associations revealed nucleotide variants in synaptic vesicular transport/Golgi apparatus genes with schizophrenia (*DTNBP1*, *DISC1*, *DAOA*, *NRG1*).[4] Reelin accumulated in the Golgi and endoplasmic reticulum in some cell bodies of GABAergic neurons in the cortex and hippocampus,[5]and genetic associations of the reelin gene polymorphism and schizophrenia were reported.[6,7]

Recently, dymeclin has been identified to be associated with the Golgi apparatus and with transitional vesicles of the reticulum–Golgi interface and it seems to be involved in cellular vesicle trafficking.[8,9] The *DYM* gene, located in chromosome 18q21.1 and encoding dymeclin, is a causative gene for Dyggve–Melchior–Clausen syndrome, which shows dwarfism and mental retardation. Furthermore, previous studies have identified a putative gene locus for both schizophrenia and bipolar disorder in the 18q21 region.[10,11] The aim of this study was to evaluate genetic associations of polymorphism(s) in the *DYM* gene with schizophrenia.

MATERIALS AND METHODS

All subjects were unrelated and of Japanese descent and were recruited from the main island of Japan. The first sample set was 576 patients with schizophrenia (mean age ± s.d., 51.6 ± 14.8 years; 322 men and 254 women) and 576 control subjects (mean age ± s.d., 46.8 ± 12.5 years; 268 men and 308 women). The second sample set was 1344 patients with schizophrenia (mean age ± s.d.,

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46.7 ± 14.4 years; 733 men and 611 women) and 1344 control subjects (mean age ± s.d., 47.8 ± 13.8 years; 783 men and 561 women). The third sample set was 212 patients with schizophrenia (mean age ± s.d., 37.3 ± 11.4 years; 107 men and 105 women) and 189 control subjects (mean age ± s.d., 37.6 ± 11.5 years; 92 men and 97 women). Consensual diagnosis of schizophrenia was made according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (American Psychiatric Association, 1994). Control subjects had no history of mental illness and second-degree relatives were free of psychosis in a brief psychiatric interview. This study was approved by the ethics committees of the University of Tsukuba, Niigata University, Fujita Health University, Nagoya University, Okayama University and Seiwa Hospital; and all participants provided written informed consent.

DNA was extracted from blood samples. We genotyped a total of 14 single-nucleotide polymorphisms (SNPs), rs833523, rs357894, rs2044550, rs833497, rs8089472, rs12606288, rs1297381, rs1943000, rs4630621, rs4491603, rs16950465, rs11082743, rs3809924 and rs12606865. The tag SNPs in the gene were selected using the Haploview program (http://www.broad.mit.edu/mpg/haploview/) with the condition of an r^2 threshold of 0.8 and a minor allele frequency of 0.1. SNPs were genotyped by TaqMan genotyping (Applied Biosystems, Foster City, CA, USA). Although the *DYM* gene spans 417 kb, the gene coverage was reached with these 14 SNPs because the gene resided in a large linkage disequilibrium block. Predesigned TaqMan SNP genotyping assays were selected from the Applied Biosystems database (http://www.appliedbiosystems.com). The TaqMan reaction was performed in a final volume of 3 μl consisting of 2.5 ng genomic DNA and Universal Master Mix (Eurogentec, Seraing, Belgium), and genotying was performed with an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems).

In this study, we carried out a three-step case–control association procedure; that is, screening and two independent confirmation studies. SNPs with allelic *P*-values for associations of <0.05 were examined in the second case–control sets, and SNPs with allelic *P*-values <0.05 in the second set were examined in the third case–control sets. Significant association was defined when SNPs survived these three-step procedures.

The Hardy–Weinberg equilibrium and an association between SNP and schizophrenia were determined with the Haploview software program (http://www.broad.mit.edu/mpg/haploview/). Genotype-based association was tested with the Cochran–Armitage test for trend.

RESULTS

In the first screening, we genotyped 14 tag SNPs in 1152 individuals (Figure 1). Genotypic distributions of these SNPs are shown in Table 1. Distributions of all SNPs did not differ significantly from the Hardy–Weinberg equilibrium. Nominally significant association was observed in 4 out of 14 SNPs; rs833523 (P=0.005), rs357894 (P=0.004), rs833497 (P=0.007) and rs3809924 (P=0.005).

In the second sample set, these 4 SNPs were genotyped in 2688 individuals. One SNP (rs833497) was significantly associated with schizophrenia (P=0.006, one sided, Table 2). In the third sample set, rs833497 was again genotyped in 404 individuals and the association was confirmed (P=0.006, one-sided, Table 3). In the combined total samples, the allelic *P*-value for association with schizophrenia was 2×10^{-5} (Table 3). The association was observed in both male and female subjects (data not shown).

DISCUSSION

To our knowledge, this is the first report on the association between *DYM* gene variants and schizophrenia. Four SNPs among 14 tag SNPs we examined showed a trend for association in the screening samples (permutation allelic *P*-values from 0.05 to 0.06). Among the four SNPs, an association of SNP 4 (rs833894) with schizophrenia was confirmed in the second and third case–control samples. Thus, the SNP rs833497 was found to be associated with schizophrenia in this study.

The SNP is not likely to exert an important effect on dymeclin function, because rs833497 is located in the last intron of the *DYM* gene, and therefore, it is assumed that the SNP is in linkage disequilibrium with causal SNP(s) for the association. However, rs833497 was in no complete linkage disequilibrium with other SNPs in this study and in the International HapMap database (http://hapmap.ncbi.nlm.nih.gov/). Therefore, we could not predict where the supposed causal variation(s) is. In addition, because no variant in the exons has been identified by the JSNP project (http://snp.ims.u-tokyo.ac.jp/search_Gene.html), we did not perform resequencing of the DNA of our subjects.

The *DYM* gene is located at chromosome 18q21. Previous linkage and cytogenetic studies reported the 18q21 region for both schizophrenia and bipolar disorders.[10,11] However, genome-wide association studies in other populations have not reported a significant association between variants in the *DYM* gene and schizophrenia or bipolar disorders. In the Wellcome Trust Case Control Consortium

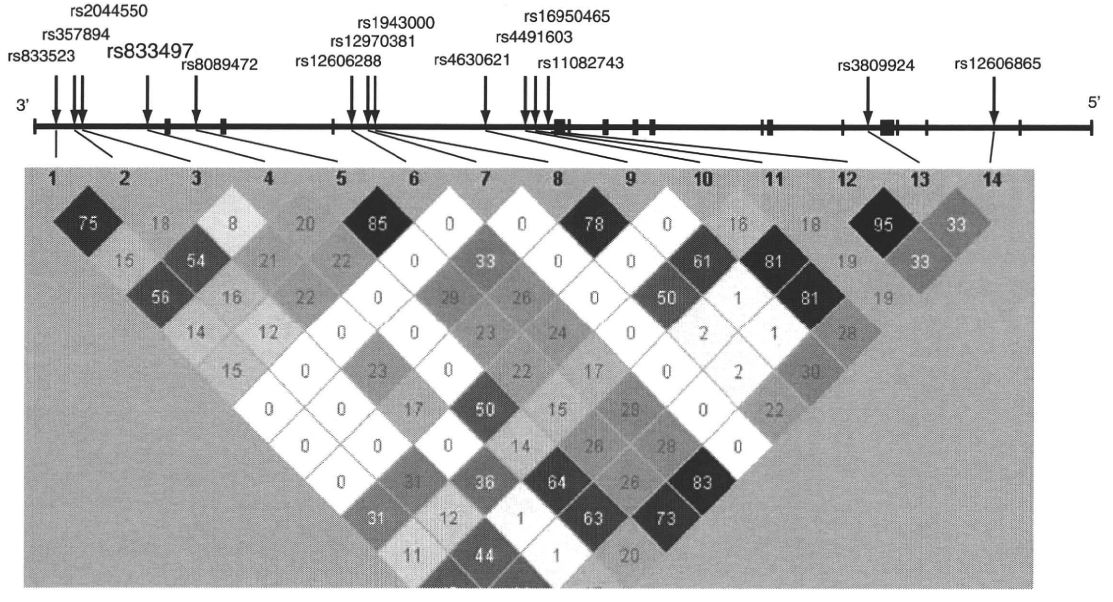

Figure 1 Positions and pairwise linkage disequilibrium (LD) of 14 tag SNPs genotyped in the *DYM* gene.

Table 1 Screening for associations between tag SNPs in the *DYM* gene and schizophrenia

Polymorphism (NCBI ID)	*Subjects*	n	*Genotype count (frequency)*			P[a]	*Allele count (frequency)*		P[b] (P[c])
			AA	GA	GG		A	G	
SNP 1 (rs833523) intron 16	Affected	571	47 (0.08)	227 (0.40)	297 (0.52)		321 (0.28)	821 (0.72)	**0.005 (0.05)**
	Controls	567	35 (0.06)	191 (0.34)	341 (0.60)	0.006	261 (0.23)	873 (0.77)	
			CC	CT	TT		C	T	
SNP 2 (rs357894) intron 16	Affected	572	274 (0.48)	241 (0.42)	57 (0.10)		789 (0.69)	355 (0.31)	**0.004 (0.05)**
	Controls	569	315 (0.55)	216 (0.38)	38 (0.07)	0.02	846 (0.74)	292 (0.26)	
			CC	CT	TT		C	T	
SNP 3 (rs2044550) intron 16	Affected	570	271 (0.48)	237 (0.42)	62 (0.11)		779 (0.68)	361 (0.32)	0.10 (0.55)
	Controls	567	255 (0.45)	228 (0.40)	84 (0.15)	0.14	738 (0.65)	396 (0.35)	
			CC	TC	TT		C	T	
SNP 4 (rs833497) intron 16	Affected	571	72 (0.13)	257 (0.45)	242 (0.42)		401 (0.35)	741 (0.65)	**0.007 (0.06)**
	Controls	570	55 (0.10)	230 (0.40)	285 (0.50)	0.03	340 (0.30)	800 (0.70)	
			GG	GT	TT		G	T	
SNP 5 (rs8089472) intron 15	Affected	563	257 (0.46)	246 (0.44)	60 (0.11)		760 (0.67)	366 (0.33)	0.271 (0.87)
	Controls	543	237(0.44)	235 (0.43)	71 (0.13)	0.44	709 (0.65)	377 (0.35)	
			TT	TC	CC		T	C	
SNP 6 (rs12606288) intron 14	Affected	559	61 (0.11)	251 (0.45)	247 (0.44)		373 (0.33)	745 (0.67)	0.20
	Controls	556	84 (0.15)	232 (0.43)	240 (0.43)	0.11	400 (0.36)	712 (0.64)	0.76
			TT	TG	GG		T	G	
SNP 7 (rs12970381) intron 13	Affected	567	38 (0.07)	233 (0.41)	296 (0.52)		309 (0.27)	825 (0.73)	0.23
	Controls	567	41 (0.07)	202 (0.57)	324 (0.57)	0.17	284 (0.25)	850 (0.75)	0.75
			GG	GA	AA		G	A	
SNP 8 (rs1943000) intron 13	Affected	572	89 (0.16)	276 (0.48)	207 (0.36)		454 (0.40)	690 (0.60)	0.98
	Controls	570	98 (0.17)	257 (0.38)	215 (0.38)	0.53	453 (0.40)	687 (0.60)	1.00
			TT	TC	CC		T	C	
SNP 9 (rs4630621) intron 13	Affected	573	67 (0.12)	266 (0.46)	240 (0.42)		400 (0.35)	746 (0.65)	0.62 (0.99)
	Controls	564	82 (0.15)	241 (0.43)	241 (0.43)	0.26	405 (0.36)	723 (0.64)	
			GG	GC	CC		G	C	
SNP 10 (rs4491603) intron 13	Affected	569	62 (0.11)	268 (0.47)	239 (0.42)		392 (0.34)	746 (0.66)	0.12 (0.68)
	Controls	570	60 (0.11)	238 (0.48)	272 (0.48)	0.14	358 (0.31)	782 (0.69)	
			CC	CT	TT		C	T	
SNP 11 (rs16950465) intron 13	Affected	572	331 (0.58)	210 (0.37)	31 (0.05)		872 (0.76)	272 (0.24)	0.08 (0.44)
	Controls	569	305 (0.54)	221 (0.08)	43 (0.08)	0.19	831 (0.73)	307 (0.27)	
			CC	CT	TT		C	T	
SNP 12 (rs11082743) intron 13	Affected	570	208 (0.36)	280 (0.49)	82 (0.14)		696 (0.61)	444 (0.39)	0.01 (0.11)
	Controls	568	250 (0.44)	251 (0.12)	67 (0.12)	0.03	751 (0.66)	385 (0.34)	
			AA	AG	GG		A	G	
SNP 13 (rs3809924) intron 5	Affected	570	206 (0.36)	282 (0.49)	82 (0.14)		694 (0.61)	446 (0.39)	**0.005 (0.06)**
	Controls	571	257 (0.45)	246 (0.12)	68 (0.12)	0.01	760 (0.67)	382 (0.33)	
			AA	AG	GG		A	G	
SNP 14 (rs12606865) intron 2	Affected	572	221 (0.39)	277 (0.48)	74 (0.13)		719 (0.63)	425 (0.37)	0.38 (0.95)
	Controls	569	229 (0.40)	237 (0.18)	103 (0.18)	0.018	695 (0.61)	443 (0.39)	

[a]The Cochran–Armitage test.
[b]Fisher's exact test (two-sided). *P* values in bold letters indicate nominal $P<0.05$.
[c]Permutation test (10 000 permutations). *P* values in bold letters indicate permutation $P<0.1$.

genome-wide association studies data,[12] the T allele of rs357897 located near rs833497 was more frequent in 2000 bipolar cases than in 3000 controls from the United Kingdom ($P=0.009$). The HapMap data of the Japanese population shows a moderate linkage disequilibrium between the T allele of rs357897 and the risk C allele of rs833497 in this study ($r^2=0.25$, $D'=1$). A significant different expression profile of the *DYM* gene has not been found in the postmortem brain samples between patients with schizophrenia and controls in the Stanley Medical Research Institute Online Genomics Database (https://www.stanleygenomics.org/). Thus, no evidence supporting involvement of the *DYM* gene in schizophrenia has been found in other populations.

The *DYM* gene encodes a protein, dymeclin, which is necessary for normal skeletal development and brain function. Defects in *DYM* gene are the cause of Dyggve–Melchior–Clausen (DMC) syndrome (MIM 223800), a rare autosomal recessive disorder characterized by short limbs, a short trunk, dwarfism, microcephaly and psychomotor retardation.[13–15] DMC syndrome is progressive. Smith–McCort dysplasia (MIM 607326), a rare autosomal recessive osteochondrodysplasia characterized by short limbs and a short trunk with a barrel-shaped chest but without mental retardation, is hypothesized to be allelic with DMC syndrome.[14,16] Most[3] mutations identified in DMC syndrome predict a loss of function, whereas those identified in Smith–McCort dysplasia are mainly missense mutations.[13–15,17] The missense mutation (N469Y) causing DMC syndrome resulted in a mislocation and subsequent protein degradation, whereas the E87K Smith–McCort mutation does not affect the stability and the location of the protein.[8] Dymeclin could not be ascribed to any family of proteins. *DYM* is

Table 2 Replication analyses of SNPs in the *DYM* gene potentially associated with schizophrenia

Polymorphism (NCBI ID)	*Subjects*	n	*Genotype count (frequency)*			P[a]	*Allele count (frequency)*		P[b] (P[c])
			AA	GA	GG		A	G	
SNP 1 (rs833523)	Affected	1332	79 (0.06)	512 (0.38)	741 (0.56)		670 (0.25)	1994 (0.75)	
	Controls	1318	87 (0.07)	501 (0.38)	730 (0.55)	0.77	675 (0.26)	1961 (0.74)	1.00
			CC	CT	TT		C	T	
SNP 2 (rs357894)	Affected	1325	702 (0.53)	529 (0.40)	94 (0.07)		1933 (0.73)	717 (0.27)	
	Controls	1323	689 (0.52)	530 (0.40)	104 (0.08)	0.73	1908 (0.72)	738 (0.28)	1.00
			CC	TC	TT		C	T	
SNP 4 (rs833497)	Affected	1322	142 (0.11)	580 (0.44)	600 (0.45)		864 (0.33)	1780 (0.67)	**0.006 (0.017)**
	Controls	1328	117 (0.09)	548 (0.41)	663 (0.50)	0.01	782 (0.29)	1874 (0.71)	
			AA	AG	GG		A	G	
SNP 13 (rs3809924)	Affected	1326	539 (0.41)	602 (0.45)	185 (0.14)		1680 (0.63)	972 (0.37)	
	Controls	1309	534 (0.41)	594 (0.45)	181 (0.14)	0.99	1662 (0.63)	956 (0.37)	0.47

[a]The Cochran–Armitage test.
[b]Fisher's exact test (one-sided). *P* values in bold letters indicate nominal $P<0.05$.
[c]Permutation test (10 000 permutations). *P* values in bold letters indicate permutation $P<0.05$.

Table 3 The third replication analysis and combined association data of rs833497

Population	*Subjects*	n	*Genotype count (frequency)*			P[a]	*Allele count (frequency)*		P[b]	*Odds ratio (95% CI)*
			CC	TC	TT		C	T		
Third	Affected	212	36 (0.17)	90 (0.42)	86 (0.41)		162 (0.38)	262 (0.62)		
	Controls	189	17 (0.09)	78 (0.41)	94 (0.50)	0.01	112 (0.30)	266 (0.70)	**0.006**	
			CC	TC	TT		C	T		
Combined total	Affected	2105	250 (0.12)	927 (0.44)	928 (0.44)		1427 (0.34)	2783 (0.66)		1.16 (1.06–1.27)
	Controls	2087	189 (0.09)	856 (0.41)	1042 (0.50)	0.00002	1234 (0.30)	2940 (0.70)	**0.00002**	

[a]The Cochran–Armitage tests.
[b]Fisher's exact test (one-sided for the third population and two-sided for the combined total).
P values in bold letters indicated $P<0.05$.

widely expressed in human embryos, especially in the cortex, the hippocampus and the cerebellum. Because dymeclin associates with the Golgi apparatus and with transitional vesicles of the reticulum–Golgi interface, it seems to be involved in cellular vesicle trafficking.[8,9] Differences in the expression of genes involved in Golgi function and vesicular transport in the presynapse have been reported in the postmortem cerebellar cortex of schizophrenia patients.[3]

In conclusion, this case–control study suggests involvement of dymeclin in the susceptibility to schizophrenia.

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Association analysis of *GRM2* and *HTR2A* with methamphetamine-induced psychosis and schizophrenia in the Japanese population

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ABSTRACT

Background: Abnormalities in glutaminergic neural transmission have been suggested to be involved in the pathogenesis of schizophrenia. A recent study reported that alterations in the 5-HT2A–mGluR2 complex may be involved in neural transmission in the schizophrenic cortex. In addition, methamphetamine-induced psychosis is thought to be similar to schizophrenia. Therefore, we conducted a case-control study with Japanese samples (738 schizophrenia patients, 196 methamphetamine-induced psychosis patients, and 802 controls) to evaluate the association and interaction between *GRM2*, *HTR2A* and schizophrenia.
Methods: We selected three 'tagging SNPs' in *GRM2*, and two biologically functional SNPs in *HTR2A* (T102C and A1438G), for the association analysis.
Results: We detected a significant association between methamphetamine-induced psychosis and *GRM2* in a haplotype-wise analysis, but not *HTR2A*. We did not detect an association between *GRM2* or *HTR2A* and schizophrenia. In addition, no interactions of *GRM2* and *HTR2A* were found in methamphetamine-induced psychosis or schizophrenia. We did not detect any novel polymorphisms in *GRM2* when we performed a mutation search using methamphetamine-induced psychosis samples.
Conclusion: Our results suggested that *GRM2* may play a role in the pathophysiology of methamphetamine-induced psychosis but not schizophrenia in the Japanese population. A replication study using larger samples or samples of other populations will be required for conclusive results.

1. Introduction

The glutamate hypothesis for the pathophysiology of schizophrenia is well-known (Weinberger, 2007). A recent clinical study also showed that LY379268, an agonist of the metabotropic glutamate 2/3 receptor (mGluR2/3), which belongs to group II mGluR, regulates glutamate neurotransmission through a presynaptic negative regulatory mechanism (Patil et al., 2007). LY379268 also has been shown to have an effect on psychotic symptoms in schizophrenia that is almost equivalent to the effect with olanzapine (Patil et al., 2007).

Recently, the hyperactivity of mGluR3 knockout mice (induced by amphetamine) was shown to be a reverse abnormal behavior mediated by LY379268 (Woolley et al., 2008). However, LY379268 did not correct the abnormal behavior of these mGluR2 knockout mice (Woolley et al., 2008). This result might show that mGluR2 is a more important therapeutic target than mGluR3 for the antipsychotic effect of LY379268 (Woolley et al., 2008).

Another recent animal study showed that mGluR2 and serotonin 2A receptor (5-HT2A) form complexes that mediate alterations in cellular response in the brain, and that these alterations were reversed by

Abbreviations: mGluR2/3, metabotropic glutamate 2/3 receptor; 5-HT2A, serotonin 2A receptor; LSD, lysergic acid diethylamide; *HTR2A*, 5-HT2A gene; *GRM2*, mGluR2 gene; METH, methamphetamine; SD, standard deviation; JGIDA, Japanese Genetics Initiative for Drug Abuse; LD, linkage disequilibrium; MAFs, minor allele frequencies; dHPLC, denaturing high performance liquid chromatography; HWE, Hardy–Weinberg equilibrium; MDR, multifactor dimensionality reduction; CD–CV hypothesis, common disease–common variants hypothesis; *GRM3*, mGluR3 gene.

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mGluR2 antagonist (Gonzalez-Maeso et al., 2008). This was supported by evidence from a postmortem study using schizophrenia patients untreated by antipsychotics, who showed increased 5-HT2A and decreased mGluR2 in the cortex compared with age and gender match control samples (Gonzalez-Maeso et al., 2008). These findings suggest that abnormality of mGluR2 and 5-HT2A complexes might be involved in the pathophysiology for schizophrenia (Gonzalez-Maeso et al., 2008; Snyder, 2008).

Several genetic studies have reported an association between the 5-HT2A gene (*HTR2A*) and schizophrenia (Abdolmaleky et al., 2004; Baritaki et al., 2004; Golimbet et al., 2007; Inayama et al., 1996). However, other studies showed no association (Basile et al., 2001; Dominguez et al., 2007; Ertugrul et al., 2004; Pae et al., 2005; Sanders et al., 2008; Zhang et al., 2004). Moreover, only one genetic study detected no association between the mGluR2 gene (*GRM2*) and Japanese schizophrenia (Joo et al., 2001). Several genome-wide association studies (GWASs) reported that *HTR2A* and *GRM2* were not associated with schizophrenia (Holmans et al., 2009; Kirov et al., 2009; Moskvina et al., 2009; O'Donovan et al., 2008; O'Donovan et al., 2009; Purcell et al., 2009; Stefansson et al., 2009) or substance dependence (Chen et al., in press). However, since schizophrenia is a complex disease, it seemed to us that evaluation of gene–gene interactions of *HTR2A* and *GRM2* in relation to the pathophysiology of schizophrenia was necessary.

LY379268 significantly inhibited hyperlocomotion in mice induced by methamphetamine (METH) (Satow et al., 2008). This animal model is considered to reflect the positive symptoms of schizophrenia. The symptoms of METH-induced psychosis are similar to those of paranoid type schizophrenia (Sato et al., 1992), which may indicate that METH-induced psychosis and schizophrenia have common susceptibility genes (Bousman et al., 2009). In support of this hypothesis, we reported that the V-act murine thymoma viral oncogene homologue 1 (AKT1) gene was associated with METH-induced psychosis (Ikeda et al., 2006) and schizophrenia (Ikeda et al., 2004) in the Japanese population. Furthermore, we performed an association analysis of these genes with methamphetamine (METH)-induced psychosis, since METH-induced psychosis is similar to schizophrenia (Sato et al., 1983).

GRM2 (OMIM *604099, 5 exons in this genomic region spanning 10.466 kb) and *HTR2A* (OMOM *182135, 3 exons in this genomic region spanning 63.463 kb) are located on 3p and 13q, respectively. The locations of these genomic regions were shown to be in a susceptibility region for schizophrenia (Badner and Gershon, 2002; Hovatta et al., 1998; Lewis et al., 2003; Maziade et al., 2001; Pulver et al., 1995). Therefore, we conducted a case-control study using Japanese schizophrenia and METH-induced psychosis samples.

2. Materials and methods

2.1. Subjects

The subjects were 738 schizophrenia patients (395 males and 343 females; mean age ± standard deviation (SD) 41.2 ± 13.8 years), 196 METH-induced psychosis and METH-dependence patients (163 males and 33 females; mean age ± SD 37.0 ± 10.8 years) and 802 healthy controls (351 males and 451 females; 37.6 ± 14.3 years). All the patients examined in this study suffered not only from METH-induced psychosis but also METH dependence. Consensus diagnoses of methamphetamine psychosis were made by two trained psychiatrists according to the ICD-10-DCR criteria (F15.2 and F15.5) on the basis of interviews and medical records. The patients with methamphetamine psychosis in the present study usually showed predominant positive symptoms such as delusion and hallucination. We excluded cases in which the predominant symptoms were of the negative and/or disorganized type in order to maintain the homogeneity of the patient group. The patients were categorized by prognosis into two types, a transient type and a prolonged type, based on the duration of the psychotic state after METH discontinuance. The transient type of patient was defined as a patient whose symptoms improved within 1 month after METH discontinuance and the start of treatment with antipsychotic, and the prolonged type was defined as a patient whose psychosis continued for more than 1 month after METH discontinuance and the start of treatment with an antipsychotic. In this study, there were 112 patients (56.9%) with the transient type and 85 patients (43.1%) with the prolonged type patients of METH psychosis. Cannabinoids were the most frequency abused drugs (31.4%), followed by cocaine (9.09%), LSD (9.09%), opioids (7.69%), and hypnotics (7.69%). Subjects with METH-use disorder were excluded if they had a clinical diagnosis of psychotic disorder, mood disorder, anxiety disorder, or eating disorder. More detailed characterizations of these subjects have been published elsewhere (Kishi et al., 2008, 2009b).

All healthy controls were also psychiatrically screened based on unstructured interviews including current and past psychiatric history. None had severe medical complications such as cirrhosis, renal failure, heart failure or other Axis-I disorders according to DSM-IV. No structured methods were used to assess psychiatric symptoms in the controls, which included hospital staff and medical students. Written informed consent was obtained from each subject. This study was approved by the ethics committees at Fujita Health University and Nagoya University Graduate School of Medicine, and by each participating member of the Institute of the Japanese Genetics Initiative for Drug Abuse (JGIDA).

2.2. SNP 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 4 SNPs covering *GRM2* (5′-flanking regions including about 6.3 kb from the initial exon and about 1 kb downstream (3′) from the last exon: HapMap database contig number chr17: 51711684.. 51730152). Then three 'tagging SNPs' were 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), an implement of the HAPLOVIEW software program (Barrett et al., 2005), for the following association analysis. *HTR2A* has been reported to have two biologically functional SNPs (T102C: rs6313, A1438G: rs6311) (Myers et al., 2007; Spurlock et al., 1998). According to the HapMap database, LD in these two SNPs in *HTR2A* was $r^2 = 0.770$; therefore, we performed an association analysis for these SNPs in this study.

2.3. SNP genotyping

We used TaqMan assays (ABI: Applied Biosystems, Inc., Foster City, CA,) for all SNPs. One allelic probe was labeled with FAM dye and the other with fluorescent VIC dye. The plates were heated 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, including primer sequences and reaction conditions, can be seen in our previous papers (Kishi et al., 2009b, in press; Tsunoka et al., 2009).

2.4. Mutation screening

We detected significant association between *GRM2* and METH-induced psychosis. Therefore, we performed mutation screening with *GRM2* divided into 17 parts (promoter region, all exons including branch site) using 32 METH-induced psychosis patients (16 males and 16 females) and the primer extension method. Denaturing high performance liquid chromatography (dHPLC) analysis was carried out

to detect mutation. DNA sequencing was then performed using a 3100-Avant Genetic Analyzer (Applied Biosystems, CA). Primers were designed to cover the coding regions, the splice sites and approximately 1.0 kb of the 5′UTR and 500 bp of the 3′UTR of *GRM2*, using the Primer 3 primer design program (http://www.broad.mit.edu/ cgi-bin/ primer/primer3_www.cgi) (Rozen and Skaletsky, 2000). A more detailed description of the methods can be seen in a previous paper (Suzuki et al., 2003). Detailed information, including primer sequence, is available on request.

2.5. Statistical analysis

Genotype deviation from the Hardy–Weinberg equilibrium (HWE) was evaluated by chi-square test (SAS/Genetics, release 8.2, SAS Japan Inc., Tokyo, Japan). Marker-trait association analysis was used to evaluate allele- and genotype-wise association with the chi-square test (SAS/Genetics, release 8.2, SAS Japan Inc., Tokyo, Japan). The distribution of patient characteristics in the schizophrenia group, METH-induced psychosis group and healthy control group was analyzed using a *t* test or a chi-square test. We found significant differences in gender distribution among these groups ($P_{\text{schizophrenia}} \leq 0.001$ and $P_{\text{METH-induced psychosis}} \leq 0.001$), however, there was no difference in age among them ($P_{\text{schizophrenia}} = 0.238$ and $P_{\text{METH-induced psychosis}} = 0.765$). 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 control) was the dependent variable, and gender, age at the time of recruitment and each examined SNP genotype were set as the independent variables. The statistical package JMP for windows was used for logistic regression analysis (JMP 5.0. 1J, SAS Japan Inc., Tokyo, Japan). Haplotype-wise association analysis was evaluated with a likelihood ratio test using the COCAPHASE2.403 program (Dudbridge, 2003). This software uses the 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 controls. In order to avoid misleading results caused by rare haplotypes, all haplotypes with a frequency less than or equal to 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 adjusted for age and gender. To control inflation of the type I error rate, we used Bonferroni's correction. 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 METH-induced psychosis, User-defined: 0.01 (5 SNPs examined in this study. Bonferroni's correction was used to control inflation of the type I error rate).

The significance level for all statistical tests was 0.05.

3. Results

The LD structure in *GRM2* from the HapMap database can be seen in our previous paper (Tsunoka et al., 2009). Genotype frequencies of all SNPs were in HWE (Table 1). In addition, we added twenty-five randomly selected samples that were genotyped again as a measure of genotyping quality control, and the genotype consistency rates for all four SNPs were 100% (Tsunoka et al., 2009). We detected a significant association between *GRM2* and METH-induced psychosis in the allele/genotype-wise analysis with the chi-square test but not with logistic regression adjusted for age and gender (Tables 1 and 2). In addition, we found an association between *GRM2* and METH-induced psychosis in the haplotype-wise analysis adjusting age and gender (Tables 3). However, *HTR2A* was not associated with schizophrenia or METH-induced psychosis (Tables 1–3). Although we performed mutation screening for *GRM2* using METH-induced psychosis samples, we did not detect any novel polymorphisms in *GRM2* in the METH-induced psychosis samples.

To evaluate the interactions with each SNP in these genes, we analyzed the gene–gene interactions with the use of the Multifactor Dimensionality Reduction (MDR) method (Hahn et al., 2003). In this study, each of the genotype variables in one dimension were assessed to determine test accuracy (defined as mean sensitivity and specificity) in terms of predicting delivery type using 10-fold cross-validation for each disorder and control. MDR analysis was performed using MDR software (v 1.0.0; http://www.epistasis.org/). In this analysis, however, no interactions were found in METH-induced psychosis and schizophrenia (data not shown).

In the power analysis, we obtained more than 80% power for the detection of association when we set the genotype relative risk at 1.45–1.90 and 1.32–1.60 in METH-induced psychosis and schizophrenia, respectively, for *GRM2*, and at 1.45–1.47 and 1.27–1.32 in METH-induced psychosis and schizophrenia, respectively, for *HTR2A* under a multiplicative model of inheritance.

Table 1
Association analysis of single markers in *HTR2A* and *GRM2* with schizophrenia and methamphetamine-induced psychosis.

Gene	SNP ID	Phenotype[a]	MAFs[b]	*N*	Genotype distribution[c]				*P*-value[d]		Corrected *P*-value[d,e]	
					M/M	M/m	m/m	HWE[f]	Genotype	Allele	Genotype	Allele
HTR2A	rs6311	Controls	0.440	802	262	374	166	0.128				
	−1438A/G	Schizophrenia	0.409	738	264	344	130	0.328	0.225	0.0828		
		METH-induced psychosis	0.459	196	58	96	42	0.846	0.708	0.497		
	rs6313	Controls	0.485	802	220	386	196	0.301				
	102T/C	Schizophrenia	0.5	738	182	374	182	0.713	0.440	0.407		
		METH-induced psychosis	0.492	196	52	95	49	0.671	0.965	0.795		
GRM2	rs3821829	Controls	0.0468	802	731	67	4	0.0751				
	C>T	Schizophrenia	0.0420	738	676	62	0	0.234	0.158	0.523		
		METH-induced psychosis	0.0408	196	181	14	1	0.219	0.856	0.613		
	rs12487957	Controls	0.333	802	346	378	78	0.0834				
	T>C	Schizophrenia	0.308	738	354	314	70	0.976	0.150	0.132		
		METH-induced psychosis	0.258	196	106	79	11	0.453	**0.0126**	**0.00413**	0.0630	**0.0207**
	rs4687771	Controls	0.376	802	300	401	101	0.0632				
	T>A	Schizophrenia	0.360	738	299	347	92	0.574	0.435	0.352		
		METH-induced psychosis	0.281	196	100	82	14	0.612	**0.00116**	**0.000414**	**0.00580**	**0.00207**

[a] SCZ: schizophrenia METH psychosis: methamphetamine-induced psychosis.
[b] MAFs: minor allele frequencies.
[c] M: major allele, m: minor allele.
[d] Bold numbers represent significant *P*-value.
[e] Calculated by Bonferroni's correction.
[f] Hardy–Weinberg equilibrium.

Table 2
Logistic regression analysis of single markers in *HTR2A* and *GRM2* with schizophrenia and methamphetamine-induced psychosis.

Gene	SNP ID	Genotype	Schizophrenia			METH-induced psychosis[a]		
			P-value	OR[b]	95% CI[c]	*P*-value	OR[b]	95% CI[c]
HTR2A	rs6311	AG	0.836	1.03	0.760–1.40	0.924	0.836	0.760–1.40
	−1438A/G	GG	0.291	1.23	0.839–1.81	0.579	0.291	0.839–1.81
	rs6313	TC	0.816	0.965	0.716–1.30	0.940	0.817	0.716–1.31
	102T/C	CC	0.826	0.961	0.676–1.37	0.801	0.826	0.676–1.37
	rs3821829	CT	0.703	0.952	0.732–1.29	0.702	0.703	0.539–1.22
	C>T	TT	0.709	0.955	0.522–1.22	0.659	0.709	0.557–1.44
GRM2	rs12487957	TC	1.241	1.23	0.869–1.74	0.956	0.241	0.869–1.74
	T>C	CC	0.506	1.19	0.717–1.98	0.0912	0.506	0.717–1.98
	rs4687771	TA	0.797	1.04	0.754–1.45	0.648	0.797	0.754–1.45
	T>A	AA	0.314	1.27	0.802–2.01	0.0986	0.314	0.802–2.01

Reference genotypes are common genotype. Adjustment for age and gender.
[a] METH-induced psychosis: methamphetamine-induced psychosis.
[b] OR: odds ratio.
[c] CI: Confidence interval.

4. Discussion

In the single marker association study, we detected a significant association between *GRM2* and METH-induced psychosis with chi-square test. However, this association may have been due to biased samples, which is unmatched for age. We therefore performed a logistic regression analysis to compare the phenotypes of each of the examined SNPs genotypes, using several clinical factors as other independent variables to adjust for possible confounding. Although we did not detect an association between the three tagging SNP genotypes in *GRM2* and METH-induced psychosis with logistic regression analysis, we found an association between *GRM2* and METH-induced psychosis in the haplotype-wise analysis adjusting for age and gender. Our results therefore suggest that *GRM2* plays a role in the pathophysiology of METH-induced psychosis in the Japanese population. We did not detect novel polymorphisms, although we performed a mutation search for *GRM2* (promoter region, all exons including branch site) using METH-induced psychosis samples.

We designed the study design based on the common disease–common variants hypothesis (CD–CV hypothesis) (Chakravarti, 1999). A recent study has shown associations between common diseases such as schizophrenia and rare variants (Weickert et al., 2008). If the genetic background of METH-induced psychosis is described by the common disease–rare variants hypothesis, further investigation, such as medical resequencing using larger samples, will be required. Moreover, mGluR2/3 agonist has been observed to have certain antipsychotic effects (Patil et al., 2007), and the mGluR3 gene (*GRM3*) has been considered a good candidate gene for the pathogenesis of METH-induced psychosis. Further investigations will be necessary to analyze gene–gene interactions between *GRM2* and *GRM3* in METH-induced psychosis.

It has also been suggested that alterations in mGluR2 and the 5-HT2A complex might be involved in the pathophysiology of schizophrenia. Because 5-HT2A receptors are one of the major pharmacological therapeutic targets of atypical antipsychotics, the pharmacogenomics of psychotic disorders (response to antipsychotics) will also need to be investigated in the future.

In this study, we found an association between *GRM2* and METH psychosis but not schizophrenia in the Japanese population. METH psychosis has long been considered a pharmacologic model of schizophrenia (Snyder, 1973; Ujike, 2002). To date, several genes have been reported to have an association with METH psychosis (Ikeda et al., 2006; Kishi et al., 2009a, 2010; Kishimoto et al., 2008a,b; Kotaka et al., 2009; Morita et al., 2008; Otani et al., 2008; Ujike et al., 2009). However, only a few of these genes have been found to be associated with Japanese schizophrenia (Ikeda et al., 2006; Kishimoto et al., 2008a). One of the reasons for the inconsistent results among these studies is considered to be the difference in sample size among the studies of these disorders. A replication study using larger samples or samples of other populations will be required for conclusive results (Bousman et al., 2009).

Table 3
All markers haplotype-wise analysis of *HTR2A* and *GRM2*.

Gene	Marker	Phenotype[a]	Haplotype frequency	OR[b]	95% CI[c]	Individual haplotype *P*-value[d]	Phenotype[a]	Global *P*-value[d]	Corrected global *P*-value[b,e]
HTR2A	A–T	Control	0.0778						
rs6311–rs6313		Schizophrenia	0.100	1.37	0.908–2.06	0.177			
		METH-induced psychosis	0.0830	1.39	0.750–2.58	0.327			
	G–T	Control	0.467				Schizophrenia	0.298	
		Schizophrenia	0.430	1.00	1.00–1.00	0.212			
		METH-induced psychosis	0.465	1.01	0.698–1.71	0.468			
	G–C	Control	0.455				METH-induced psychosis	0.589	
		Schizophrenia	0.470	1.11	0.825–1.45	0.653			
		METH-induced psychosis	0.452	1.02	0.498–1.89	0.922			
GRM2	C–C–A	Control	0.673						
rs3821829–rs12487957–rs4687771		Schizophrenia	0.659	1.00	1.00–1.00	0.424	Schizophrenia	0.424	
		METH-induced psychosis	0.746	1.00	1.00–1.00	**0.00822**			
	C–T–T	Control	0.327						
		Schizophrenia	0.341	1.07	0.909–1.26	0.424	METH-induced psychosis	**0.00746**	**0.0149**
		METH-induced psychosis	0.254	0.686	0.518–0.908	**0.00822**			

[a] SCZ: schizophrenia METH psychosis: methamphetamine-induced psychosis.
[b] OR: Odds ratio.
[c] CI: Confidence interval.
[d] Bold numbers represent significant *P*-value.
[e] Calculated by Bonferroni correction.

A few points of caution should be mentioned with respect to our results. First, the positive association may be due to biased samples, such as unmatched gender samples, or small sample size. On average, the METH-induced psychosis patients were much younger than the controls. We therefore performed a logistic regression analysis to compare the phenotypes of each of the examined SNPs genotypes, using several clinical factors as other independent variables to adjust for possible confounding. Our control samples for 3SNPs in *GRM2* were within a limit that satisfies HWE. The positive association with METH-induced psychosis could be due to type I error, possibly because of population stratification. However, another recent study confirmed that there is no population stratification in our control samples (Ikeda et al., 2010). In addition, we added twenty-five randomly selected samples that were genotyped again as a measure of genotyping quality control, and the genotype consistency rates for all four SNPs were 100% (Tsunoka et al., 2009). Second, we did not include a mutation scan to detect rare variants with functional effects for schizophrenia. However, Joo et al. reported no association of *GRM2* with Japanese schizophrenia after mutation screening for *GRM2* (Joo et al., 2001). In addition, it is difficult to evaluate the association of rare variants, unless statistical power is obtained. To overcome these limitations, a replication study using larger samples or samples of other populations will be required for conclusive results (Bousman et al., 2009).

5. Conclusion

In conclusion, our results suggest that *GRM2* may play a major role in the pathophysiology of METH-induced psychosis but not schizophrenia in the Japanese population. However, an interaction between mGluR2 and 5-HT2A seen in an animal study was not detected with these genes levels.

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Regular Article

Relationship of psychopathological symptoms and cognitive function to subjective quality of life in patients with chronic schizophrenia

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Aims: The purpose of the present study was to examine the extent of the effects of psychopathological symptoms and cognitive function on quality of life (QOL) in patients with chronic schizophrenia.

Methods: Data were obtained using the Japanese Schizophrenia Quality of Life Scale (JSQLS), Positive and Negative Syndrome Scale (PANSS), Wisconsin Card-Sorting Test (WCST) Keio version, and Continuous Performance Test (CPT) for 52 schizophrenia patients.

Results: Stepwise regression analysis showed that PANSS depression/anxiety factors predicted JSQLS psychosocial conditions and motivation/energy, and that WCST Categories Achieved predicted JSQLS symptoms/side-effects.

Conclusions: Psychopathological symptoms and cognitive function affect subjective QOL in patients with schizophrenia. If the final goal is treatment that improves QOL in a manner that patients themselves are aware of, clinicians probably need to consider a treatment strategy that improves depression/anxiety symptom.

Key words: cognition, positive and negative syndrome scale, quality of life, regression analysis, schizophrenia.

IN ADDITION TO positive and negative symptoms, patients with schizophrenia have reduced cognitive function and are consequently impaired in everyday social functioning. In the past, the first goal of schizophrenia treatment was to reduce psychological symptoms, mainly positive symptoms,[1] rather than recovering social functioning. Recently, as a result of an emphasis on patient needs, the concept of quality of life (QOL) has been brought into the treatment of somatic illness, particularly chronic illness such as chronic heart failure.[2] The goal of treatment has therefore changed from the alleviation of symptoms to improvement of the patient's own satisfaction with social activities. Because of this trend, attempts to evaluate the effects of treatment using QOL as an indicator have occurred in the field of clinical psychiatry, including treatments and rehabilitation for schizophrenia.

Essentially, the basic concept of QOL places importance on subjectivity in terms of patients' self-appraisal of their own satisfaction. Self-evaluations

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by people with schizophrenia were previously thought to lack reliability because of the presence of psychopathological symptoms and poor awareness of the disease.[3] Hence many trials have used objective QOL evaluations, such as the Quality of Life Scale (QLS),[4] which rely on interviews with psychiatrists or other trained interviewers. The importance of evaluating the satisfaction of patients themselves, however, has been recognized in schizophrenia. Reporting that patients with schizophrenia were aware of and could express their social dysfunction, Skantze *et al.* supported the view that QOL could be ascertained only on subjective evaluation.[5] Lehman demonstrated that QOL data from patients with chronic mental illness were reliable and concluded that subjective QOL evaluation was applicable to such patients.[6,7] QOL is considered important in research on treatment outcome for schizophrenia, and researchers have argued strongly for development of a robust QOL scale specific to schizophrenia, based on the subjective judgment of patients.[8]

The Schizophrenia Quality Life of Scale (SQLS), which is a practical and simple self-administered evaluation, was developed for the purpose of measuring patient-specific QOL in patients with schizophrenia. It is primarily intended for use in clinical trials and has been reported to have high levels of reliability and validity.[9] Kaneda *et al.* translated the SQLS into Japanese, and this version also yields high reliability (Japanese Schizophrenia Quality of Life Scale [JSQLS]).[10] With the spread of QOL evaluations for patients with schizophrenia, there has been active research concerning factors related to QOL, which represents the degree to which patients are satisfied with their lives. First of all, in research examining the relationship between psychopathological symptoms and QOL, it has been repeatedly reported that symptoms such as depression and anxiety have a strong effect on subjective QOL,[11–13] but no consistent view on the relationship between QOL and positive symptoms, or that between QOL and negative symptoms has been obtained.[14–17] In addition, QOL evaluation measures used in those studies have been a mixture of subjective and objective ones.

Specific cognitive functions are significantly impaired in patients with schizophrenia when compared to healthy persons.[4,18] Green analyzed the influence of cognitive deficits on the daily lives of patients with schizophrenia, and reported that vigilance (sustained attention) was associated with social skill and that executive functioning was related to community functioning.[19] In the field of schizophrenia research, Heinrichs reported that the Continuous Performance Test (CPT) for sustained attention and Wisconsin Card-Sorting Test (WCST) for executive functioning were powerful and reliable tool, respectively.[20] Relationships between executive functioning and QOL could not be confirmed.[21–23] In addition, only Wegener *et al.* have reported a significant relationship between sustained attention and QOL.[24]

A few studies have examined both aspects of the relationship between psychopathological symptoms and QOL and that between cognitive function and QOL. These studies reported that psychopathological symptoms, particularly negative symptoms,[25,26] have a stronger effect than cognitive function on QOL.[27] In contrast, one report showed that cognitive function and psychopathological symptoms affect each other.[24] Because studies examining the relationship of both psychopathological symptoms and cognitive function to subjective QOL are scarce, and different aspects of cognitive function are measured in each study, a consistent view has not been obtained.

In light of these reports, we verified the relationship between (i) subjective QOL, as measured by the JSQLS, and psychopathological symptoms, as measured by the Positive and Negative Syndrome Scale (PANSS); and (ii) subjective QOL and cognitive function, as measured by the CPT (sustained attention) and the WCST (executive functioning). The ultimate aim of the present study was to identify an objective predictor for treatment that is compatible with the needs of patients and reflects patient satisfaction.

METHODS

Subjects

Subjects were inpatients or outpatients diagnosed with schizophrenia according to DSM-IV.[28] They provided written consent to participate in this research. Diagnosis was performed using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID). Patients fulfilling all of the following three criteria were enrolled in the present study: (i) presence of chronic illness without acute exacerbation; (ii) PANSS total score >50 points; and (iii) absence of other axis I disorders, including major depressive episodes or anxiety disorders. Demographic data, including age, sex, disease subtype, living situation (outpatients/ inpatients), onset age, duration of disorder, number of hospital admissions for schizophre-