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-

Table 1. Patient characteristics ($n = 52$; mean ± SD)

Age (years)	37.7 ± 11.6
Sex	
Male	29
Female	23
Schizophrenia subtypes	
Paranoid type	32
Disorganized type	6
Residual type	13
Undifferentiated type	1
Outpatients/inpatients	30/22
Onset age (years)	24.9 ± 8.5
Duration of disorder (years)	12.8 ± 10.5
No. admissions	2.1 ± 3.5
Dose of antipsychotics† (mg)	11.2 ± 9.4

†Haloperidol equivalent.

nia, and dose of antipsychotics, were obtained from medical records. A total of 52 patients were enrolled. Table 1 lists the subjects' demographic characteristics. The most common schizophrenia subtype was paranoid type (62%). With regard to the administration of antipsychotic drugs, an atypical antipsychotic drug was prescribed to 41 patients (79%), and more than two kinds of atypical or typical antipsychotic drugs were prescribed to other patients (21%). Average antipsychotic drug dose was 11.2 mg haloperidol-equivalent dose;[29] this was a low-average dose compared with other studies.[24,30] With regard to other psychopharmaceutics, nine patients (17%) received a mood stabilizer and none received antidepressant. The present study was approved by the Ethics Committee of the Nagoya University School of Medicine.

Evaluation

Evaluation of psychopathological symptoms

Evaluation of psychopathological symptoms used the PANSS.[31] The PANSS was administered by trained psychiatrists or psychologists. According to the Lindenmayer *et al.* model, this is classified into the following five areas: (i) negative factors; (ii) excitement factors; (iii) positive factors; (iv) cognitive factors; and (v) depression/anxiety factors,[32,33] and mean scores for each area were calculated.

Subjective QOL evaluation

For subjective QOL evaluation, we used the JSQLS developed by Wilkinson *et al.*[9] and translated by Kaneda *et al.*[10] As proposed by Wilkinson *et al.* the 30 items on the JSQLS were classified into the following three areas: (i) psychosocial conditions; (ii) motivation/energy; and (iii) symptoms/side-effects. Each area scale is transformed to have a range from 0 (the best status as measured on the JSQLS) to 100 (the worst status as measured on the JSQLS), with each scale calculated as follows: the scale score (SS) equals the total of raw scores of each item in the scale (RS_{tot}), divided by the maximum possible raw scores of all the items in the scale (RS_{max}), all multiplied by 100: $SS = (RS_{tot}/RS_{max}) \times 100$. The 'psychosocial conditions' area addresses various emotional conditions such as loneliness, hopelessness, difficulty in social situations, and worries about the future. The 'motivation/energy' area addresses various problems of motivation and activity, such as the lack of will or drive to do things. The 'symptoms/side-effects' area addresses issues such as muscle twitches and dry mouth, which can be caused by medication.

The JSQLS was rated within 2 weeks of the evaluation of psychopathological symptoms.

Examination of cognitive function

Executive functioning was evaluated using the WCST (Keio version),[34] the computerized version of which was developed by Kobayashi.[35] The patient classifies a single card shown at the bottom of a computer screen in terms of color, shape and number and selects one type of card from four basic types of cards shown at the top. Without letting the patient know the correct category, the computer gives feedback as to whether it was a correct or incorrect selection. If the patient makes six continuous correct selections, the categories in the computer are changed, and the patient must select another category to make a correct selection. This test is carried out for up to 48 selections. In the present study Categories Achieved (CA) and Perseverative Errors of Nelson (PEN) were calculated.[34]

Sustained attention was evaluated using the CPT–Identical Pairs.[36] A four-digit number is displayed on a computer screen as a single stimulus, and the patient must click the mouse as quickly as possible while exactly the same stimulus continues. One stimulus is shown for 50 ms, and the interval between stimuli is 950 ms. There are a total of 150 trials, 30 of which involve the target. In the present study d', which is a discrimination index calculated from the number of correct and incorrect answers, was measured.[36]

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

Statistical analysis

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

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

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

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

RESULTS

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

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

Table 2. QOL, psychopathological symptoms and cognitive functioning

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

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

DISCUSSION

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

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

Table 3. JSQLS scores and clinical variables

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

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

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

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

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

Table 4. Multiple regression of psychopathological symptoms and cognitive functioning

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

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

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

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

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

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

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

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

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

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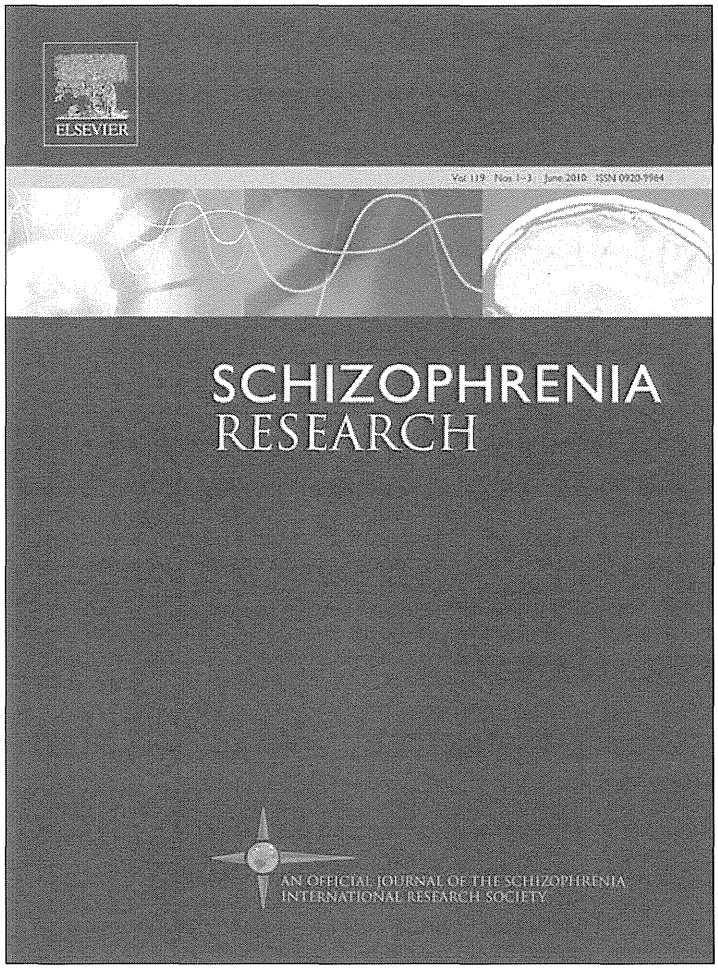

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Diagnostic classification of schizophrenia by neural network analysis of blood-based gene expression signatures

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ABSTRACT

Gene expression profiling with microarray technology suggests that peripheral blood cells might be a surrogate for postmortem brain tissue in studies of schizophrenia. The development of an accessible peripheral biomarker would substantially help in the diagnosis of this disease. We used a bioinformatics approach to examine whether the gene expression signature in whole blood contains enough information to make a specific diagnosis of schizophrenia. Unpaired *t*-tests of gene expression datasets from 52 antipsychotics-free schizophrenia patients and 49 normal controls identified 792 differentially expressed probes. Functional profiling with DAVID revealed that eleven of these genes were previously reported to be associated with schizophrenia, and 73 of them were expressed in the brain tissue. We analyzed the datasets with one of the supervised classifiers, artificial neural networks (ANNs). The samples were subdivided into training and testing sets. Quality filtering and stepwise forward selection identified 14 probes as predictors of the diagnosis. ANNs were then trained with the selected probes as the input and the training set for known diagnosis as the output. The constructed model achieved 91.2% diagnostic accuracy in the training set and 87.9% accuracy in the hold-out testing set. On the other hand, hierarchical clustering, a standard but unsupervised classifier, failed to separate patients and controls. These results suggest analysis of a blood-based gene expression signature with the supervised classifier, ANNs, might be a diagnostic tool for schizophrenia.

1. Introduction

Genome-wide gene expression analysis by DNA microarray and bioinformatics procedures has been conducted to elucidate common gene pathways that underpin the biological mechanisms of schizophrenia (Aston et al., 2004; Hakak et al., 2001; Hemby et al., 2002; Iwamoto et al., 2005, 2004; Mirnics et al., 2000, 2001; Sugai et al., 2004; Tkachev et al., 2003). However, the clinical use of microarray technology is not so widespread in schizophrenia research as compared with cancer research (Rhodes et al., 2004), due to the difficulty in interpreting results obtained from postmortem

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brain tissue that are complicated by agonal factors, anatomical inconsistency, and cellular heterogeneity of the cortical and subcortical regions. Postmortem brain studies use less accessible materials and therefore are limited by small sample size and repeated use of the same cohorts (Iwamoto and Kato, 2006). As a more accessible tissue, several researchers have undertaken expression profiling of peripheral blood cells (Sullivan et al., 2006; Tsuang et al., 2005; Vawter et al., 2004; Zvara et al., 2005). On a transcriptional expression level, peripheral blood cells were reported to share significant similarities with tissues from multiple brain regions (Sullivan et al., 2006). Interestingly, Tsuang et al. (2005) and Middleton et al. (2005) have shown that a set of genes extracted from gene expression signature of isolated peripheral blood cells can discriminate between schizophrenia and control groups.

These studies suggest that analysis of high dimensional data is useful to generate a biomarker of schizophrenia since it can combine data from several molecules, each of which shows small difference but is not exclusively associated with this disease (Schwarz and Bahn, 2008). In cancer research, classification by gene expression signature is widely used to predict tumor classes, drug responses, and prognosis of individual subjects (Khan et al., 2001; Lin et al., 2007; O'Neill and Song, 2003). Development of such classifier will greatly help our diagnosis of schizophrenia that is solely dependent on clinical symptoms so far. There are two approaches in classification: supervised and unsupervised methods. In contrast to unsupervised clustering, supervised classifiers learn a function from training data that consist of pairs of input objects (e.g., gene expression signatures) and desired outputs (e.g., diagnoses) (De Bruyne et al., 2007). The artificial neural network (ANN) is one of those classifiers that works very well, at identifying patterns or trends in a large amount of data with little theory.

Purpose of the present study is to examine whether microarray date obtained from whole blood cells contain enough information to classify schizophrenia. We present here that ANN model can correctly predict the diagnosis with sufficient accuracy.

2. Materials and methods

2.1. Subjects

Samples from 52 patients with schizophrenia and 49 normal controls were analyzed. Patients with schizophrenia or schizophreniform disorder were recruited from outpatients or inpatients of psychiatry unit at 6 centers across Japan. Those who were antipsychotics-free and had no comorbidity were included in the study. Control subjects were recruited from hospital staff and student volunteers who showed no evidence of present or past mental illness. All subjects were evaluated using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I). At least two trained psychiatrists confirmed diagnosis of schizophrenia and specified its subtypes. In patients with schizophreniform disorder, the diagnosis of schizophrenia was reconfirmed 6 months after the onset of the first psychotic episode. The study protocol was approved by the ethics committee of each institution, and written informed consent was obtained from all subjects. If patients were floridly psychotic, informed consent was provided first by their parents or husband/wife, and then the consent was provided again by patients themselves after the psychotic symptoms were ameliorated.

We assessed 611 patients to recruit 52 antipsychotic-free patients without commorbidity. The patients and controls were of similar age (31.8 vs. 31.2 years; $p=0.776$) but significantly different in gender due to our sampling bias (21 males, 31 females vs. 35 males, 14 females; $p=0.0017$) (Table 1). In both patients and controls, there was no abnormal finding in standard laboratory workup including blood chemistries, complete blood count, urinalysis and electrocardiogram. Among 52 patients, 34 patients were drug-naïve, 8 patients were antipsychotics-naïve, and 10 patients were drug-free. All of the patients manifested active-phase symptoms. Current episode was the first psychotic episode for 23 of 34 drug-naïve patients. It was also the first episode for 8 neuroleptic-naïve patients, but they were taking antidepressants, benzodiazepines, or mood stabilizers for prodromal symptoms. Drug-free patients were those who stopped taking medication and relapsed due to non-adherence. They were drug-free for more than 8 weeks.

2.2. RNA isolation and microarray procedures

We extracted total RNA from whole blood because in vitro handling for cell isolation could alter gene expression (Ohmori et al., 2005). A total of 5 ml venous blood was collected in PAXgene Blood RNA Tubes (Qiagen, Valencia, CA) and frozen at −80 °C within 2 h after blood withdrawal. Total RNA was isolated from each of the frozen samples with PAXgene Blood RNA Kit (Qiagen) according to the manufacturer's instructions. Quantity and quality of total RNA were checked by A260/280 readings of spectrophotometry and an

Table 1
Demographic details of patients and controls.

	Control	Schizophrenia	Subtypes			
			Paranoid	Disorganized	Catatonic	Undifferentiated
N	49	52	21	9	1	21
Age	31.2±9.5	31.8±11.4	38.1±13.0	26.1±6.5	30	27.8±8.5
Sex (M/F)	35/14	21/31*	9/12	4/5	1/0	7/14
Age at onset	–	24.5±9.4	30.9±11.5	19.8±3.2	30	20.8±6.0
Medication history						
Drug-naïve	–	34	18	5	1	10
Drug-free	–	10	2	3	0	5
Neuroleptics-naïve	–	8	1	1	0	6

* $p<0.01$ compared to controls by chi-square test.

Agilent BioAnalyzer (Agilent, Santa Clara, CA). Mean ratio of 28S/18S rRNA was 1.8 ± 0.3 and 1.9 ± 0.3 (mean ± SD) for patient and control groups, respectively.

Gene expression profiles were determined using CodeLink Human Whole Genome Bioarray (GE Healthcare Bio-Sciences, Chandler, AZ) according to the manufacturer's protocol. Briefly, cDNA was synthesized with 0.5 μg of total RNA and transcribed into biotinylated cRNA using iExpress Assay Reagent Kit (GE Healthcare Bio-Sciences, Chandler, AZ). Ten micrograms of the biotinylated cRNA was fragmented at 94 °C for 20 min and hybridized to CodeLink Human Whole Genome Bioarray, which contains probes for 54,847 transcripts. The hybridized cRNA probes to oligonucleotide arrays were stained with Cy5-streptavidin and scanned with an Agilent DNA Microarray Scanner (Agilent, Santa Clara, CA).

2.3. Data processing and normalization

The TIFF image from the microarray scanner was quantified using CodeLink Expression Analysis ver4.2 (GE Healthcare Bio-Sciences, Chandler, AZ). The mean intensity was taken for each spot and background corrected by subtracting the surrounding median local background intensity. Raw intensities were global median normalized for each bioarray. Probes were filtered to include only those present in more than two thirds of samples and those with average signal intensities between the 30th and

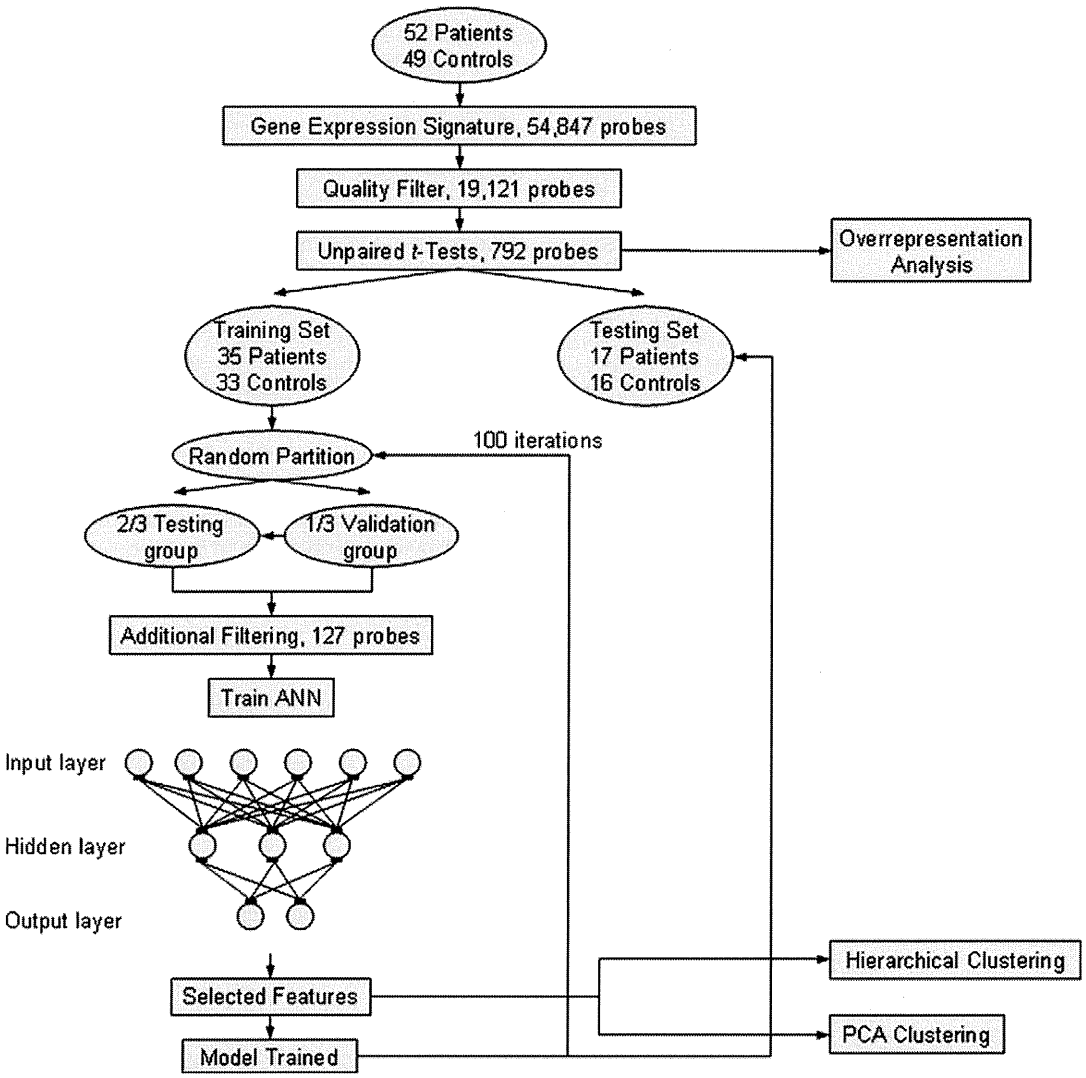

Fig. 1. Workflow for a 3-fold cross-validation artificial neural network (ANN) analysis. A gene expression signature was obtained from whole blood cDNA from 52 patients with schizophrenia and 49 normal controls using CodeLink Human Whole Genome Bioarray, containing about 55,000 probes. After a quality filter, 19,121 probes were used as a dataset for further analysis. To reduce dimensionality and skewness of the data, we selected 127 probes with moderate signal intensity and small coefficient of variation (CV) from 792 differentially expressed probes and subjected them to stepwise forward selection. Subjects were subdivided into training and testing sets to perform hold-out cross-validation. Training sets were randomly partitioned into three groups. One group was selected as a validation set, whereas the remaining two groups were used to train the network. The output produced by the first data passage through the ANN was compared with the ideal output, a known diagnosis, and an error is generated. The error was backpropagated through the ANN, and the weights of various connections between the neural units were adjusted. Then, a different validation set was selected from the same partitioning, and the remaining groups were used for training. The same step was repeated again to use each of the three groups as a validation set. Stepwise forward selection identified the best feature at each round of 3-fold cross-validation cycled with the repartitioning of the groups until an optimal feature size was determined. ANNs were then trained 100 times with selected predictors as the input, and the constructed model was used to classify testing set samples.