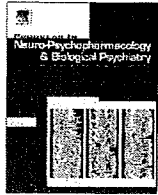




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Genetic association analysis of *NRG1* with methamphetamine-induced psychosis in a Japanese population

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ABSTRACT

The neuregulin 1 gene (*NRG1*) has been identified as a candidate gene for schizophrenia in a linkage study in the Icelandic population. Recent evidence also suggested that it might be related to the neurodevelopmental hypothesis and glutamate hypothesis for schizophrenia. Because the symptomatology of methamphetamine (METH) use disorder with accompanying psychosis is similar to that of patients with schizophrenia, *NRG1* is an appropriate candidate gene for METH-induced psychosis. We conducted a case-control association study between *NRG1* and METH-induced psychosis in a Japanese population (184 subjects with METH-induced psychosis and 534 controls). Written informed consent was obtained from each subject. We selected four SNPs (SNP8NRG221533, SNP8NRG241930, SNP8NRG243177, and rs3924999) in *NRG1* from previous reports. No significant association was found between *NRG1* and METH-induced psychosis in the allele/genotype-wise or haplotype-wise analyses. In conclusion, *NRG1* might not contribute to the risk of METH-induced psychosis in the Japanese population.

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1. Introduction

Drug dependency is a major social problem in countries all over the world, and there is a great need to understand the mechanisms of dependency and study treatments. Because genetic factors strongly influence drug dependency (Agrawal and Lynskey, 2006), it is important to identify genes related to drug dependency. Methamphetamine (METH) is an illegal drug used widely in the world, and it is known to cause psychiatric symptoms such as hallucination and delusion. Since the symptomatologic character of METH-induced

psychosis is similar to that of schizophrenia, it would be of interest to examine the association between schizophrenia candidate genes and METH-induced psychosis.

The neuregulin 1 gene (*NRG1*) was first reported as a candidate gene for schizophrenia in the Icelandic population. *NRG1* (OMIM *142445) is located on chromosome 8p12–21, which is a susceptibility region for schizophrenia identified by recent meta-analyses of genome wide linkage studies (Badner and Gershon, 2002; Lewis et al., 2003). Recent reports suggest that *NRG1* is a neuronal axial filament growth factor that acts through receptors of the erbB family and affects modulation of neuronal migration, expression and plasticity of specific N-methyl D-aspartate (NMDA), gamma-aminobutyric acid (GABA) and acetylcholine (ACh) receptor subunits, and myelination (Corfas et al., 2004). Therefore, *NRG1* may be related to the neurodevelopmental hypothesis and Glutamate/GABA hypothesis of schizophrenia. In this study, we conducted a case-control association study between *NRG1* and METH-induced psychosis in a Japanese population.

Abbreviations: *NRG1*, The neuregulin 1 gene; METH, methamphetamine; NMDA, N-methyl D-aspartate; GABA, gamma-aminobutyric acid; ACh, acetylcholine; JGIDA, Japanese Genetics Initiative for Drug Abuse; HWE, Hardy–Weinberg equilibrium; SNP, Single Nucleotide Polymorphism.

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2. Materials and methods

2.1. Subjects

The subjects in the association analysis were 184 patients (all patients were diagnosed as having METH dependence; 152 males and 32 females: mean age \pm SD 36.7 \pm 11.6 years) and 534 healthy controls (243 males and 291 females: mean age \pm SD 37.5 \pm 14.4 years). All subjects were unrelated to each other, ethnically Japanese, and lived in Japan. Among the subjects with METH use disorder, all subjects had a comorbid diagnosis of METH-induced psychosis. 149 subjects with METH use disorder abused or had dependence on drugs other than METH. Cannabinoids were the most frequently 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. The patients were diagnosed according to DSM-IV or ICD-10 criteria with consensus of at least two experienced psychiatrists on the basis of unstructured interviews and a review of medical records. All healthy controls were also psychiatrically screened through unstructured interviews, and those with past individual or family history of drug dependence or axis 1 disorder such as psychotic or mood disorders were excluded. More detailed characterizations of these subjects have been published elsewhere (Kishi et al., 2008). After description of the study, written informed consent was obtained from each subject. This study was approved by the Ethics Committee at Fujita Health University and each participating institute of the Japanese Genetics Initiative for Drug Abuse (JGIDA).

2.2. SNP selection and genotyping

We selected four SNPs (SNP8NRG221533, SNP8NRG241930, SNP8NRG243177, and rs3924999) in *NRG1* from previous reports (Stefansson et al., 2002; Yang et al., 2003). We used TaqMan assays (Applied Biosystems) for all SNPs. Detailed information on TaqMan assays, including primer sequences and reaction conditions, can be seen in our previous paper (Ikeda et al., 2008).

2.3. Statistical analysis

Genotype deviation from the Hardy–Weinberg equilibrium (HWE) was evaluated by the chi-square test (SAS/Genetics, release 8.2, SAS Japan INC, Tokyo, Japan). Marker–trait association was also evaluated by the chi-square test in allele- and genotype-wise analyses. Haplotype frequencies were estimated in a two- to four-marker sliding window fashion and log likelihood ratio tests were performed for global *P*-values with COCAPHASE program version 3.0.6 (Dudbridge, 2003). In these haplotype-wise analyses, rare haplotypes (less than 0.05) either of cases and controls were excluded from the association analysis. Power calculation was performed using a statistical program prepared by a Genetic Power Calculator (<http://pngu.mgh.harvard>).

Table 1
Results of association study between *NRG1* and METH-induced psychosis.

SNP ID	Phenotype	N	MAF ^a	Genotype distribution ^b			P-value		HWE ^c
				M/M	M/m	m/m	Allele	Genotype	
SNP8NRG221533	METH	184	0.445	50	104	30	0.328	0.161	0.0508
	CON	524	0.475	144	262	118			
SNP8NRG241930	METH	183	0.122	139	43	1	0.431	0.475	0.225
	CON	510	0.107	405	100	5			
SNP8NRG243177	METH	180	0.437	48	102	30	0.658	0.530	0.052
	CON	533	0.463	146	280	107			
rs3924999	METH	171	0.228	103	58	10	0.363	0.652	0.631
	CON	534	0.205	339	171	24			

^a MAF = minor allele frequency.

^b N = number, METH = METH-induced with psychosis, CON = control. M = major allele, m = minor allele.

^c HWE = Hardy–Weinberg equilibrium.

Table 2
Results of haplotype analysis between *NRG1* and METH-induced psychosis.

SNP ID	Global P-value		
	2 SNP	3 SNP	4 SNP
SNP8NRG221533	0.0683		0.213
SNP8NRG241930	0.221	0.162 ^a	
SNP8NRG243177	0.522	0.235	
rs3924999			

^a Stefansson et al. (2002) reported.

edu/~purecell//gpc/) (Purcell et al., 2003). The level of significance for all statistical tests was 0.05.

3. Results

Genotype frequencies of subjects and controls did not deviate significantly from HWE. No significant association was found between *NRG1* and METH-induced psychosis in the allele/genotype-wise analysis (Table 1), or in the haplotype analysis (Table 2).

Our METH samples had the characteristic of being unmatched for gender. Consequently, no common results between gender samples were obtained. Therefore, we performed an explorative analysis of gender effects, and no association was detected between any of the SNPs and either sex in allele/genotype-wise analysis (Table 3), or in the haplotype analysis (data not shown).

In a power analysis, we obtained more than 80% power for the detection of association when we set the genotype relative risk at 1.43–1.87, under a multiplicative model of inheritance.

4. Discussion

The positive symptoms of schizophrenia, such as hallucinations, delusions and METH-induced psychosis, show similar clinical backgrounds, which is thought to be causally related to shared mechanisms. It was reported from recent animal pharmacological studies that repeating METH-induced caused glutamate overflow in the brain and decreased NMDA function (Simoes et al., 2008). This mechanism might contribute to behavioral problems, such as a clinical phenotype of schizophrenia (Abekawa et al., 2008). Moreover, such abnormal behavior improved with the use antipsychotics such as risperidone (Abekawa et al., 2008). On the other hand, a number of animal studies suggested that *NRG1* affects glutamatergic transmission (Talmage, 2008). Based on these studies, we conducted the first genetic association study between polymorphisms in *NRG1* and METH-induced psychosis.

Significant associations between *NRG1* and schizophrenia have been reported in two different populations. Stefansson et al. (2002) found a significant association with schizophrenia of haplotypes constructed by three SNPs (SNP8NRG221533, SNP8NRG241930, and SNP8NRG243177) in the HAPice region, which is harbored by the 5'-region of the GGF2 isoform in Caucasians. Yang et al. (2003) found

Table 3
Results of explorative analysis between *NRG1* and METH-induced psychosis.

SNP ID	Gender	Phenotype	N	MAF ^a	Genotype distribution ^b			P-value		
					M/M	M/m	m/m	Allele	Genotype	HWE ^c
SNP8NRG221533	Male	METH	152	0.434	43	86	23	0.379	0.0717	0.0617
		CON	275	0.465	83	128	64			
	Female	METH	32	0.484	6	21	5	0.981	0.447	0.075
		CON	249	0.485	61	134	54			
SNP8NRG241930	Male	METH	151	0.119	116	34	1	0.500	0.403	0.374
		CON	269	0.104	217	48	4			
	Female	METH	32	0.140	23	9	0	0.501	0.666	0.354
		CON	241	0.112	188	52	1			
SNP8NRG243177	Male	METH	145	0.444	39	83	23	0.808	0.209	0.0550
		CON	280	0.453	85	136	59			
	Female	METH	31	0.435	10	15	6	0.525	0.605	0.929
		CON	252	0.474	61	141	50			
rs3924999	Male	METH	140	0.242	82	48	10	0.183	0.364	0.423
		CON	281	0.202	179	90	12			
	Female	METH	31	0.161	21	10	0	0.392	0.458	0.284
		CON	253	0.207	160	81	12			

^a MAF = minor allele frequency.

^b N = number, METH = METH-induced with psychotic, CON = control. M = major allele, m = minor allele.

^c HWE = Hardy–Weinberg equilibrium.

a significant association with schizophrenia of rs392499 in the Chinese population. However, in our allele/genotype-wise analysis in the Japanese population, we did not find that either the haplotype (SNP8NRG221533–SNP8NRG241930–SNP8NRG243177) (global *P*-value = 0.162) or rs392499 was associated with METH-induced psychosis. Moreover, our previous study reported no association between *NRG1* and Japanese schizophrenia using large samples (Ikeda et al., 2008). Therefore, our results suggest that it is unlikely that the genetic factor of *NRG1* is involved in the pathogenesis of METH-induced psychosis in the Japanese population.

The genetic contribution of substance-related disorder is differentially heritable by gender (Jang et al., 1997). In addition, our sample had an unmatched gender ratio of METH-induced psychosis (152 male, 32 female). Therefore, the results in this study might have been influenced by gender. We conducted an explorative analysis divide by sex, and found no evidence of a gender effect.

Because the METH-induced psychosis sample in this study was small, our result could be a type II error due to inadequate sample size. Therefore, an increased sample size will be required to rule out type II error. In a recent systematic review, Talmage (2008) showed that the majority of genetic association studies of methamphetamine use disorders had small sample sizes, and a proposed meta-analysis to solve the limitation of sample correction. It will be necessary to replicate our association with the same phenotype and other, larger population samples.

A few points of caution must be mentioned with regard to our findings. (1) It is important to evaluate associations between METH use disorder with and without psychosis. However, there were a small number of subjects without psychosis, so we did not evaluate this association to avoid type I error due to our small sample size. This was due to the limitations of sample collection, since we collected cases of METH use disorder in psychiatric hospitals. (2) We could not adopt an LD-based strategy and perform a mutation scan, because *NRG1* has a massive gene structure. Therefore, in future studies it will be necessary to evaluate associations between other common variants or rare variants with functional effects and *NRG1* in METH-induced psychosis.

5. Conclusion

Our results suggest that *NRG1* does not play a major role in METH-induced psychosis in the Japanese population. However, the number of METH samples used in this study was small, and even though it is difficult to find samples of METH use disorder, it will be necessary

to validate or replicate our association in other, larger population samples.

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1 *BDNF* is not associated with schizophrenia: Data from a Japanese population 2 study and meta-analysis

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17 ABSTRACT

18 A variety of evidence suggests brain-derived neurotrophic factor (*BDNF*) as a candidate gene for
19 schizophrenia, and several genetic studies have shown a significant association between the
20 disease and certain SNPs within *BDNF* (specifically, Val66Met and C270T). According to a recent
21 study, the functional microsatellite marker *BDNF*-LCPR (*BDNF*-linked complex polymorphic
22 region), which affects the expression level of *BDNF*, is associated with bipolar disorder. The
23 goals of our current study were to 1) evaluate the quality of HapMap-based linkage
24 disequilibrium (LD) tagging of *BDNF*-LCPR, 2) examine whether these tagging SNPs are
25 associated with schizophrenia in a Japanese population, and 3) conduct a meta-analysis of the
26 two most extensively studied polymorphisms: Val66Met and C270T. We genotyped eight
27 tagging SNPs, including Val66Met and C270T. Our LD evaluation showed that *BDNF*-LCPR could
28 be represented by these tagging SNPs in controls (with 73.5% allelic coverage). However, the
29 functional A1 allele was not captured due to its low minor allele frequency (2.2%). In a case-
30 control study (1117 schizophrenics and 1102 controls), no association was found in single-
31 marker or multimarker analysis. Moreover, in a meta-analysis, the Val66Met polymorphism
32 was not associated with schizophrenia, whereas C270T showed a trend for association in a fixed
33 model ($p=0.036$), but not in a random model ($p=0.053$). From these findings, we conclude
34 that if *BDNF* is indeed associated with schizophrenia, the A1 allele in *BDNF*-LCPR would be the
35 most promising candidate. Further LD evaluation, as well as an association study in which
36 *BDNF*-LCPR is genotyped directly, would be required for a more conclusive result.

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51 1. Introduction

52 Brain-derived neurotrophic factor (*BDNF*) plays a key role
53 in the central nervous system as a mediator of neuronal

54 survival and plasticity of dopaminergic, cholinergic, and 54
55 serotonergic neurons (Angelucci et al., 2005). There is a 55
56 growing body of evidence supporting an association between 56
57 *BDNF* and schizophrenia: 1) postmortem studies show 57
58 reduced expression levels of *BDNF* in the anterior cingulate 58
59 cortex (Iritani et al., 2003) and hippocampus of schizophrenia 59
60 patients (Durany et al., 2001), 2) a reduced level of *BDNF* was 60
61 confirmed in the blood serum of schizophrenic patients 61
62 (Toyooka et al., 2002), and 3) mice in which the *BDNF* 62

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receptor *trkB* was knocked out show behavioral changes (hyper-locomotion, stereotyped behaviors, and cognitive impairments) related to the symptomatology of schizophrenia (Zorner et al., 2003). In addition, *BDNF* maps to chromosome 11p13, a region with potentially significant linkages to schizophrenia (Suarez et al., 2006).

A number of genetic association studies have shown that SNPs in *BDNF* are associated with schizophrenia (Nanko et al., 2003; Szekeres et al., 2003), and a meta-analysis study also showed a weak association between C270T and schizophrenia (Zintzaras, 2007), but not between Val66Met and schizophrenia (Kanazawa et al., 2007; Naoe et al., 2007; Xu et al., 2007; Zintzaras, 2007).

A recent study focused on the complex microsatellite polymorphism *BDNF*-LCPR located ~1.0 kbp upstream of the translation initiation site of *BDNF* (Okada et al., 2006). This polymorphism contained 23 novel allelic variants, including four major alleles (A1–A4). A luciferase assay showed a significantly lower expression level of the A1 allele than the other three alleles of *BDNF*-LCPR. Furthermore, the A1 allele frequency was significantly higher in bipolar disorder patients than in controls. Therefore, *BDNF*-LCPR can be seen as an important schizophrenia-susceptibility factor, but determination of genotype distributions of this polymorphism is difficult due to technical limitations.

Two main goals of the present study were to examine 1) whether tagging SNPs from the HapMap database can represent *BDNF*-LPCR through linkage disequilibrium (LD) evaluation, and 2) whether these tagging SNPs are associated with schizophrenia in a Japanese population. We also performed meta-analysis regarding two polymorphisms (Val66Met and C270T), which have been intensively examined in relation to *BDNF*.

2. Materials and methods

2.1. Subjects

The subjects in the LD evaluation of *BDNF*-LPCR were 66 healthy controls (35 males and 31 females; age 50.33 ± 11.03 (mean \pm SD) years) who had participated in a previous study (Okada et al., 2006). The sample used in the association analysis comprised 1,117 schizophrenia patients (628 males and 489 females; 47.4 ± 15.3 years) and 1,102 healthy controls

(504 males and 598 females; 37.1 ± 14.4 years). All participants were unrelated Japanese people living in central areas of Japan. The patients were diagnosed according to DSM-IV criteria with consensus from at least two experienced psychiatrists on the basis of an unstructured interview and review of medical records. Patients with any other axis-I disorder were excluded.

All healthy controls were also psychiatrically screened based on unstructured interviews to exclude subjects with brain/psychotic disorders, or those with first-degree relatives with a psychotic disorder. A trained psychiatrist interviewed each participant with respect to current or past mental states (psychotic, mood, anxiety, obsessive-compulsive symptoms) and family history (Ikeda et al., 2005). Healthy controls were mainly recruited from the staff of participating hospitals.

Written informed consent was obtained from each subject. This study was approved by the Ethics Committee of each institution involved.

2.2. Tagging SNP selection and LD evaluation

First we consulted the HapMap database (release#16.c.1, Jun 2005 www.hapmap.org, population: Japanese Tokyo: minor allele frequencies (MAFs) of more than 0.05) and selected 38 SNPs covering the *BDNF* gene (5'-flanking regions ranging from 9467 bp away from the initial exon to 4454 bp downstream (3') from the last exon: HapMap database contig number chr11: 27709339..27628562). Then, seven 'tagging SNPs' (rs1491851, rs11030121, rs7934165, rs12291063, rs11030101, rs6265 [Val66-Met], rs1519480: Table 1) were selected with the criterion of an r^2 threshold greater than 0.8 in 'pair-wise tagging only' mode using the 'Tagger' program (de Bakker et al., 2005) (<http://www.broad.mit.edu/mpg/tagger>), a tool within the HAPLOVIEW software (Barrett et al., 2005). In addition to these tagging SNPs, we included the C270T polymorphism, which has been intensively examined in other papers. A total of eight SNPs were selected for the following LD evaluation and case-control association analysis.

2.3. Genotyping

Information on genotypic distributions of *BDNF*-LPCR, which had been determined previously (Okada et al., 2006), was used for LD evaluation (66 healthy controls). Genotyping

Table 1
Association analysis of tagging SNPs in *BDNF*.

Gene	Marker IDs	Distance to next SNP (bp)	N ^a		MAF ^b		M/M ^c		M/m ^d		m/m ^e		p-values		
			SCZ	CON	SCZ	CON	SCZ	CON	SCZ	CON	SCZ	CON	SCZ	CON	Genotype
<i>BDNF</i> (minus strand)	M1	rs1491851	0	1114	1100	0.234	0.247	647	622	413	413	54	65	0.491	0.312
	M2	rs11030121	16556	1111	1097	0.047	0.045	1011	999	96	97	4	1	0.409	0.79
	M3	rs7934165	4224	1109	1099	0.46	0.468	332	320	534	529	243	250	0.861	0.581
	M4	C270T	10185	1115	1102	0.032	0.03	1046	1036	67	66	2	0	0.372	0.716
	M5	rs12291063	27697	1117	1095	0.206	0.202	698	695	378	358	41	42	0.842	0.736
	M6	rs11030101	13357	1110	1099	0.344	0.34	484	485	489	480	137	134	0.969	0.813
	M7	rs6265	828	1111	1100	0.413	0.428	394	365	516	529	201	206	0.528	0.326
	M8	rs1519480	4204	1112	1100	0.246	0.235	631	640	414	403	67	57	0.621	0.375

^a N = number, SCZ = schizophrenia, CON = control.

^b MAF = minor allele frequency.

^c M/M = major allele/major allele.

^d M/m = major allele/minor allele.

^e m/m = minor allele/minor allele.

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160 of remaining SNPs was performed by allelic discrimination
162 assay (Applied Biosystems, CA).

163 2.4. Statistical analysis

164 2.4.1. LD evaluation and gene-based case-control association 165 study

166 Genotype deviation from the Hardy–Weinberg equili-
167 brium (HWE) was evaluated by χ^2 goodness-of-fit test.

168 For LD evaluation, multiallelic D' was calculated by the
169 COCAPHASE 2.403 program (Dudbridge, 2003). Haplotype
170 frequencies of tagging SNPs (M6, M7 and M8) and *BDNF*-LCPR
171 were then estimated using PHASE software (<http://www.stat.washington.edu/stephens/software.html>) (Stephens and Don-
172 nelly, 2003). Because this region shows high LD, a block-like
173 structure was conserved, and the sum of haplotype frequencies
174 was calculated to examine whether these haplotypes could
175 capture the specific allele of *BDNF*-LCPR in accordance with the
176 haplotype-tagging method (Kamatani et al., 2004).

178 Marker-trait association was evaluated with the use of a
179 likelihood ratio test (allele-wise and haplotype-wise analyses)
180 and χ^2 test (genotype-wise analysis). For exhaustive screening,
181 we examined eight-marker haplotype analysis in sliding-
182 window fashion using the COCAPHASE 2.403 program (Dud-
183 bridge, 2003). A power calculation was performed using a sta-
184 tistical algorithm implemented in the Genetic Power Calculator
185 program (<http://pngu.mgh.harvard.edu/~purcell/gpc/>).

186 We also carried out imputation of SNPs that are not
187 directly genotyped but are present in the HapMap database
188 (JPT + CHB founders, release 23, filtered by MAF greater than
189 0.01 and genotyping rate greater than 0.95) by PLINK
190 software (version 1.04) (Purcell et al., 2007). A total of 108
191 SNPs were included for this analysis, from rs12574598
192 (chr11.27531152) to rs11030149 (chr11.27798413; the loca-
193 tion is based on Human, Mar, 2006, hg18 assembly). We used
194 the “-proxy -drop” option and picked up only imputed SNPs
195 with an INFO value greater than 0.8 in accordance with the
196 authors' recommendation.

197 The level of significance for all statistical tests was set at 0.05.

198 2.4.2. Meta-analysis

199 To identify studies eligible for the meta-analysis, we
200 searched PubMed citations through December 2008 using
201 the terms “*BDNF*,” “brain-derived neurotrophic factor,” and
202 “schizophrenia” as key words.

203 Regarding selection of studies, we included the case-
204 control genetic association studies of the Val66Met and/or
205 C270T polymorphism. Studies with data for only schizophre-
206 nic patients or only healthy controls were excluded, as were
207 family-based studies.

208 We also carried out quality assessment using the New-
209 castle–Ottawa Scale (NOS) for case-control studies. The
210 Newcastle–Ottawa Scale uses a “star” rating system to judge
211 the quality based on three aspects of the study: selection of
212 study groups, comparability of study groups and ascertain-
213 ment of either the exposure or outcome of interest. The
214 maximum number of stars a study may receive in each of these
215 categories is 4, 2 and 3, respectively, for a total of 9 possible
216 stars. The validity of these tools has been previously established
217 (http://www.ohri.ca/programs/clinical_epidemiology/oxford.htm).

219 We then assessed the HWE in the patients and control
220 subjects of each study using the χ^2 goodness-of-fit test, and
221 we explored whether heterogeneity was present using *Q*
222 statistics. Finally, we performed a fixed-effects as well as
223 random-effects model meta-analysis within groups of homo-
224 geneous odds ratios (ORs). The fixed effects model assumes
225 that all existing studies are included in the meta-analysis, and
226 therefore weights each study only by the inverse of the
227 variance of that study. A random-effect model, in contrast,
228 assumes that between-study variation is due to chance and/
229 or random variation and an individual study effect. Random-
230 effect models are more conservative than fixed effects models
231 and generate a wider confidence interval (CI). The signifi-
232 cance of the pooled OR was determined using a Z-test. Fourth,
233 publication bias was assessed using a linear regression
234 analysis to measure funnel plot asymmetry. A probability
235 level of $p < 0.05$ was used as a threshold for statistical sig-
236 nificance. Data were analyzed using the “Comprehensive
237 Meta Analysis” (Version 2.2.046) statistical software package.

3. Results

3.1. LD evaluation of *BDNF* in the Japanese population

240 Sixteen variants of *BDNF*-LCPR were found in our sample
241 ($N_{\text{allele}} = 132$): three samples for the A1 allele [(CG)_{del}(CA)₁₂
242 (GA)₃], 21 samples for the A2 allele [(CG)₄(CA)₁₂(GA)₃], 39
243 samples for the A3 allele [(CG)₅(CA)₁₂(GA)₂], 42 samples for
244 the A4 allele [(CG)₅(CA)₁₃(GA)₃], and 27 samples for the A5
245 allele [combination of remaining rare alleles]. The results for
246 LD between *BDNF*-LCPR and our eight tagging SNPs are

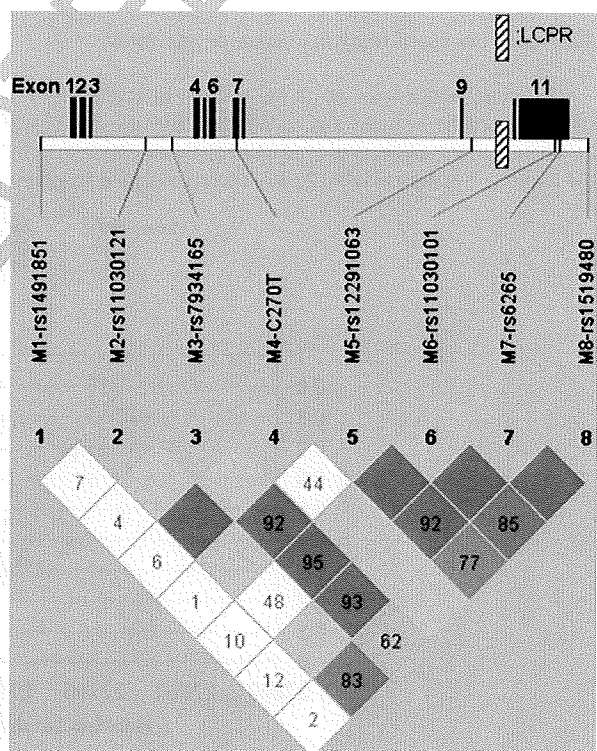


Fig. 1. LD structure in the *BDNF* gene.

t2.1 **Table 2**
t2.2 Sliding-window analysis of tagging SNPs in *BDNF*.

Gene	Marker IDs	p-values						
		2-window	3-window	4-window	5-window	6-window	7-window	8-window
BDNF	M1							
	M2	0.4111	0.7189					
	M3	0.8163	0.9294	0.7133				
	M4	0.6334	0.8741	0.9513	0.9801	0.9883		
	M5	0.7537	0.9088	0.9594	0.9962	0.9697	0.9851	
	M6	0.9304	0.8204	0.8325	0.9827	0.9167	0.8971	0.975
	M7	0.5905	0.4048	0.6972	0.7188			
	M8	0.4796						

247 shown in Supplementary Table 1. The LD analysis of *BDNF*-
248 LCPR and M6–M8 polymorphisms showed D' values ranging
249 from 0.955 to 0.975, indicating tight linkage between M6, M7
250 and M8 and *BDNF*-LCPR (Supplementary Table 1).

251 On the other hand, single marker tests may be inefficient
252 when each single marker carries a small to moderate amount
253 of association information about the trait. In this situation, the
254 association might not be detected when markers are analyzed
255 individually, whereas combining genotypes from neighboring
256 markers may provide a more powerful test. Therefore, in
257 order to perform exploratory multimarker association testing,
258 we estimated the haplotype frequencies and summed the
259 haplotype frequencies to examine whether the haplotypes
260 could capture the specific alleles of *BDNF*-LCPR in accordance
261 with the haplotype-tagging method (Kamatani et al., 2004)
262 (Supplementary Table 2).

263 3.2. Population-based study in a Japanese population

264 Genotype frequencies of all SNPs were in HWE. The LD
265 structure can be seen in Fig. 1. We detected no significant
266 association in the allele/genotype-wise analyses (Table 1) or in
267 the haplotype analysis (Table 2). We then performed stratified

287 analysis by gender, since a recent paper reported the effect of
288 gender on *BDNF* in major depressive disorder (Verhagen et al.,
289 2008). Again, we could not find any association in this subgroup
290 analysis (Supplementary Tables 3 and 4).

291 The A1 allele of *BDNF*-LCPR is a low-frequency allele (around
292 2.3%). Therefore, considering both the low frequency of A1 and
293 the number of alleles in the LCPR polymorphism, we included
294 imputation analysis around *BDNF*, because additional SNPs
295 might provide better coverage of this very polymorphic repeat.
296 35 SNPs passed our quality control, but no evidence for asso-
297 ciation could be detected between imputed SNPs and schizo-
298 phrenia in the Japanese population (Fig. 2).

299 We obtained more than 80% power for the detection of an
300 association when we set the genotype relative risk at 1.19–1.57
301 in schizophrenia under a multiplicative model of inheritance.

302 3.3. Meta-analysis

303 3.3.1. Val66Met polymorphism (rs6265, high-resolution plot is 304 shown in Supplementary Figure 1)

305 An updated meta-analysis (total of 22 population-based
306 association studies), in which three studies (Donohoe et al.,
307 2007; Han et al., 2008; Takahashi et al., 2008) and our current
308

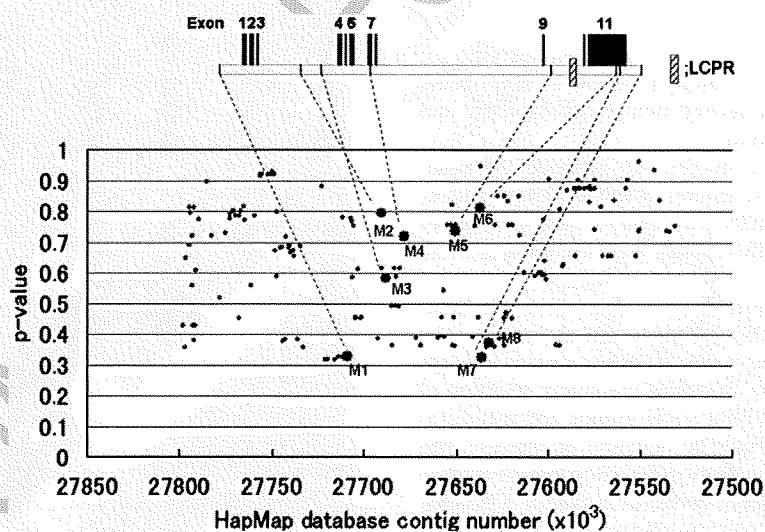


Fig. 2. Results of imputing SNP in the *BDNF* gene. The weights of evidence were calculated using imputed genotypes (small circles) and observed genotypes (big circles). The SNP position from the HapMap database is plotted on the X axis.

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308 results were added to the last meta-analysis (Kanazawa et al.,
326 2007), can be seen in Table 4 (Egan et al., 2003; Hong et al.,
327 2003; Nanko et al., 2003; Skibinska et al., 2004; Anttila et al.,
328 2005; de Krom et al., 2005; Neves-Pereira et al., 2005;
329 Schumacher et al., 2005; Tan et al., 2005; Chen et al., 2006;
330 Jonsson et al., 2006; Numata et al., 2006; Tochigi et al., 2006;
331 Watanabe et al., 2006; Zhang et al., 2006; Donohoe et al.,
332 2007; Naoe et al., 2007; Qian et al., 2007; Xu et al., 2007; Han
333 et al., 2008; Takahashi et al., 2008). All studies were

334 independent and in HWE. We did not observe significant
335 heterogeneity among ORs ($Q=18.99$, $df=21$, $p=0.586$). The
336 pooled OR derived from all studies comprising 6568 patients
337 and 8824 control subjects was not significant in each model
338 (fixed model: pooled OR=0.976, 95% CI=0.928–1.026,
339 $p=0.345$, random model: OR=0.976, 95% CI=0.928–1.026,
340 $p=0.345$; Table 3). Next, in order to limit the ethnic hetero-
341 geneity, we analyzed the Caucasian and Asian samples
342 separately. These subgroup analyses also showed no significant

3.1 **Table 3**
3.2 Meta-analysis of case-control studies between the rs6265 and schizophrenia.

3.3	First author	Year	Ethnicity	No.		No. of G(Val) major allele		No. of A(Met) minor allele		Diagnostic system	OR	95% CI	p-value	NOS
				SCZ	CON	SCZ	CON	SCZ	CON					
3.4														
3.5	Egan	2003	American	203	133	332	218	74	48	DSM-IV	1.012	0.68–1.51	0.952	7 (selection 3/4 comparability 2/2 exposure 2/3)
3.6	Skibinska	2004	Polish	336	375	565	613	107	137	DSM-IV	0.847	0.64–1.12	0.242	7 (selection 3/4 comparability 2/2 exposure 2/3)
3.7	Anttila	2005	Finnish	94	98	156	166	32	30	DSM-IV	1.135	0.66–1.96	0.648	7 (selection 3/4 comparability 2/2 exposure 2/3)
3.8	Neves-Pereira	2005	Scottish	321	350	541	547	101	153	DSM-IV	0.667	0.51–0.88	0.004	8 (selection 3/4 comparability 2/2 exposure 3/3)
3.9	Shumacher	2005	German	533	1097	842	1777	224	417	DSM-IV	1.134	0.95–1.36	0.176	8 (selection 3/4 comparability 2/2 exposure 3/3)
3.10	de Krom	2005	Dutch	273	580	437	928	109	232	DSM-IV	0.998	0.77–1.29	0.986	7 (selection 3/4 comparability 2/2 exposure 2/3)
3.11	Jonsson	2006	Swedish	187	275	312	452	60	98	DSM-III-R	0.887	0.62–1.26	0.504	7 (selection 3/4 comparability 2/2 exposure 2/3)
3.12	Zhang	2006	American	84	250	135	406	33	94	DSM-IV	1.056	0.68–1.64	0.810	8 (selection 3/4 comparability 2/2 exposure 3/3)
3.13	Donohoe	2007	Irish	359	745	598	1241	120	249	DSM-IV	1.000	0.79–1.27	0.999	7 (selection 3/4 comparability 2/2 exposure 2/3)
3.14	Hong	2003	Han Chinese	93	198	85	189	101	207	DSM-IV	1.085	0.76–1.54	0.648	7 (selection 3/4 comparability 2/2 exposure 2/3)
3.15	Nanko	2003	Japanese	178	332	209	382	147	282	DSM-IV	0.953	0.73–1.24	0.716	7 (selection 3/4 comparability 2/2 exposure 2/3)
3.16	Tan	2005	Han Chinese	108	145	117	165	99	125	DSM-IV	1.117	0.78–1.59	0.541	8 (selection 3/4 comparability 2/2 exposure 3/3)
3.17	Chen	2006	Han Chinese	560	576	573	607	547	545	DSM-IV	1.063	0.90–1.25	0.465	7 (selection 3/4 comparability 2/2 exposure 2/3)
3.18	Numata	2006	Japanese	159	318	198	364	120	272	DSM-IV	0.811	0.62–1.07	0.137	7 (selection 3/4 comparability 2/2 exposure 2/3)
3.19	Tochigi	2006	Japanese	401	569	487	675	315	463	DSM-IV	0.943	0.78–1.13	0.533	7 (selection 3/4 comparability 2/2 exposure 2/3)
3.20	Watanabe	2006	Japanese	349	423	407	491	291	355	DSM-IV	0.989	0.81–1.21	0.914	7 (selection 3/4 comparability 2/2 exposure 2/3)
3.21	Naoe	2007	Japanese	211	205	249	258	173	152	DSM-IV	1.179	0.89–1.56	0.247	7 (selection 3/4 comparability 2/2 exposure 2/3)
3.22	Qian	2007	Han Chinese	604	650	616	657	592	643	DSM-IV	0.982	0.84–1.15	0.820	7 (selection 3/4 comparability 2/2 exposure 2/3)
3.23	Xu	2007	Han Chinese	275	297	292	297	258	297	DSM-IV	0.884	0.70–1.11	0.296	7 (selection 3/4 comparability 2/2 exposure 2/3)
3.24	Han	2008	Korean	96	79	97	84	95	74	DSM-IV	1.112	0.11–0.22	0.622	7 (selection 3/4 comparability 2/2 exposure 2/3)
3.25	Takahashi	2008	Japanese	33	29	39	37	27	21	ICD-10	1.220	0.20–0.37	0.592	7 (selection 3/4 comparability 2/2 exposure 2/3)
3.26	Current study	2007	Japanese	1111	1100	1304	1259	918	941	DSM-IV	0.942	0.86–1.06	0.326	7 (selection 3/4 comparability 2/2 exposure 2/3)
3.27	Pooled (22) ^a fixed		All	6568	8824	8591	11813	4543	5835		0.973	0.93–1.02	0.293	
3.28	Pooled (22) ^a random		All								0.973	0.93–1.02	0.293	
3.29	Pooled (9) ^b fixed		Caucasian	2390	3903	3918	6348	860	1458		0.967	0.88–1.06	0.480	
3.30	Pooled (9) ^b random		Caucasian								0.956	0.85–1.08	0.444	
3.31	Pooled (13) ^c fixed		Asian	4178	4921	4673	5465	3683	4377		0.980	0.92–1.04	0.501	
3.32	Pooled (13) ^c random		Asian								0.980	0.92–1.04	0.501	

SCZ, schizophrenia patients; CON, control.

3.33 OR, odd ratio; CI, confidence interval; NOS, The Newcastle–Ottawa Scale.

3.34 ^a $Q=18.99$, $df=21$, $p=0.586$ for heterogeneity.

3.35 ^b $Q=11.54$, $df=8$, $p=0.173$ for heterogeneity.

3.36 ^c $Q=7.39$, $df=12$, $p=0.831$ for heterogeneity.

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343 association in either ethnic sample (Table 3). No publication
344 bias was found ($t = 0.369, p = 0.716$).

345 3.3.2. C270T polymorphism (high-resolution plot is shown in
346 Supplementary Figure 2)

347 For C270T polymorphism, nine population-based associa-
348 tion studies and our current study met our criteria for the
349 updated meta-analysis (Watanabe et al., 2007). (Table 4)
350 (Nanko et al., 2003; Szekeres et al., 2003; Anttila et al., 2005;
351 Galderisi et al., 2005; Szczepankiewicz et al., 2005; Jonsson
352 et al., 2006; Watanabe et al., 2006; Zhang et al., 2006; Xu et al.,
353 2007). Total sample sizes for patients and control subjects were
354 2887 and 3336, respectively. All studies were independent and
355 in HWE. We did not observe significant heterogeneity among
356 ORs ($Q = 13.57, df = 9, p = 0.139$). The ORs and 95% CIs for the
357 10 population-based studies are shown in Table 3. When the
358 analysis was run within a fixed model, the T allele was sig-
359 nificantly associated with schizophrenia (pooled OR = 1.219,
360 95% CI = 1.013–1.468, $Z = 2.100, p = 0.036$). However, in a
361 random effects model, there was no evidence of a significant
362 association (pooled OR = 1.268, 95% CI = 0.997–1.614,
363 $Z = 1.934, p = 0.053$). When the study that showed the most
364 significant results (Szekeres et al., 2003) was removed, the
365 association in the fixed model did not remain significant
366 (pooled OR = 1.165, 95% CI = 0.966–1.407, $Z = 1.596, p = 0.110$).

367 The subgroup analyses based on the ethnic differences
368 were then analyzed separately. The pooled OR derived from
369 the six Caucasian studies comprising 970 patients and 1182

control subjects was not significant (fixed model: pooled 370
OR = 1.210, 95% CI = 0.922–1.589, $p = 0.169$, random model: 371
OR = 1.273, 95% CI = 0.884–1.834, $p = 0.195$), nor was the 372
pooled OR derived from the four Asian studies comprising 373
1917 patients and 2154 control subjects (fixed model: pooled 374
OR = 1.227, 95% CI = 0.953–1.580, $p = 0.112$, random model: 375
pooled OR = 1.293, 95% CI = 0.894–1.870, $p = 0.173$). No 376
publication bias was found ($t = 1.89, p = 0.101$). 377

4. Discussion 378

379 In this study, we carried out a detailed LD evaluation of the
380 region harboring BDNF-LCPR, a gene-based association study
381 in the Japanese population, and we updated the meta-
382 analysis of two functional SNPs, Val66Met and C270T. From
383 these results, we conclude that the commonly identified
384 variants in BDNF are not associated with schizophrenia.

385 Regarding single marker based LD tagging, our data
386 showed that BDNF-LCPR could be represented well. On the
387 other hand, as for multimarker tagging, our data showed that
388 BDNF-LCPR could be represented moderately well by the
389 haplotypes constructed with our tagging SNPs (73.5% cover-
390 age) since the most important functional allele in BDNF-LCPR,
391 the A1 allele [(CG)_{del}(CA)₁₂(GA)₃], could not be captured
392 adequately by our tagging SNPs. These included C270T,
393 which showed a trend for significance in the meta-analysis
394 ($p_{fix model} = 0.036, r^2 = 0.000945$). In other words, we could not
395 evaluate the tagging SNPs haplotype coverage (M6–M7–M8)

t4.1 **Table 4**
t4.2 Meta-analysis of case-control studies between the C270T polymorphism and schizophrenia.

t4.3	First author	Year	Ethnicity	No.		No. of major (C) allele		No. of minor (T) allele		Diagnostic system	OR	95% CI	p-value	NOS
				SCZ	CON	SCZ	CON	SCZ	CON					
t4.5	Szekeres	2003	Finnish	101	68	174	132	28	4	DSM-IV	5.310	1.82–15.51	0.002	7 (selection 3/4 comparability 2/2 exposure 2/3)
t4.6	Anttila	2005	Finnish	94	98	171	182	17	14	DSM-IV	1.292	0.62–2.70	0.495	8 (selection 3/4 comparability 2/2 exposure 3/3)
t4.7	Galderisi	2005	Italian	107	111	201	211	13	11	DSM-IV	1.241	0.54–2.83	0.609	8 (selection 3/4 comparability 2/2 exposure 3/3)
t4.8	Szczepankiewicz	2005	Polish	397	380	755	725	39	35	DSM-IV	1.070	0.67–1.71	0.777	8 (selection 3/4 comparability 2/2 exposure 3/3)
t4.9	Jonsson	2006	Swedish	187	275	354	521	20	29	DSM-IV	1.015	0.57–1.82	0.96	7 (selection 3/4 comparability 2/2 exposure 2/3)
t4.10	Zhang	2006	American	84	250	158	470	10	30	DSM-IV	0.992	0.47–2.07	0.982	8 (selection 3/4 comparability 2/2 exposure 3/3)
t4.11	Nanko	2003	Japanese	178	332	339	649	17	15	DSM-IV	2.170	1.07–2.13	0.032	8 (selection 3/4 comparability 2/2 exposure 3/3)
t4.12	Watanabe	2006	Japanese	349	423	672	827	26	19	DSM-IV	1.684	0.92–3.07	0.089	7 (selection 3/4 comparability 2/2 exposure 2/3)
t4.13	Xu	2007	Han Chinese	275	297	534	574	16	20	DSM-IV	0.860	0.44–1.68	0.658	7 (selection 3/4 comparability 2/2 exposure 2/3)
t4.14	Current study	2007	Japanese	1115	1102	2159	2138	71	66	DSM-IV	1.065	0.76–1.50	0.716	7 (selection 3/4 comparability 2/2 exposure 2/3)
t4.15	Pooled (10) ^a fixed	All		2887	3336						1.219	1.01–1.47	0.036	
t4.16	Pooled (10) ^a random	All									1.268	0.997–1.614	0.053	
t4.17	Pooled (6) ^b fixed	Caucasian		970	1182						1.210	0.92–1.59	0.169	
t4.18	Pooled (6) ^b random	Caucasian									1.273	0.88–1.83	0.195	
t4.19	Pooled (4) ^c fixed	Asian		1917	2154						1.227	0.95–1.58	0.112	
t4.20	Pooled (4) ^c random	Asian									1.293	0.89–1.87	0.173	

SCZ, schizophrenia patients; CON, control.

t4.21 OR, odd ratio; CI, confidence interval; NOS, The Newcastle–Ottawa Scale.

t4.22 ^a $Q = 13.57, df = 9, p = 0.139$ for heterogeneity.

t4.24 ^b $Q = 8.24, df = 5, p = 0.143$ for heterogeneity.

t4.25 ^c $Q = 5.32, df = 3, p = 0.150$ for heterogeneity.

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for the A1 allele appropriately, due to the small size of the LD evaluation sample and the low population frequency of A1 (only 3 subjects had the A1 allele). Therefore, we included the imputation analysis of SNPs around *BDNF* to examine whether unobserved SNPs would be associated with schizophrenia. This is because we expected to increase the possibility of capturing the A1 allele in *BDNF*-LCPR by multi-marker information in the HapMap database. Nevertheless, we could find no evidence for association from imputed SNPs with schizophrenia. Thus, taking these findings together, we speculate that: 1) Variants in *BDNF* including *BDNF*-LCPR may not be associated with schizophrenia, and 2) we merely overlooked the association of the A1 allele in *BDNF*-LCPR in our analysis. The aforementioned scenario also implies that the LD structure between the A1 allele and common SNPs examined in the LD evaluation (or the imputation) may be unique. Therefore, further LD evaluation and a mutation scan will be needed to obtain conclusive results and in order to capture the A1 allele, considering the technical difficulty regarding direct genotyping of this complex polymorphic region.

Our gene-based association study and meta-analysis of Val66Met did not show an association with schizophrenia. Our sample size in these analyses was large enough to rule out type II error. Meanwhile, meta-analysis of C270T showed a trend for significance in the fixed model. In general, the meta-analytic strategy has the advantage of increasing the statistical power; however the interpretation of positive results from meta-analysis in genetic association studies is difficult. Speaking conservatively, the pooling of results can be truly reasonable only when the causal variants have been identified (i.e. biological evidence for such variants has been established clearly) (Sand, 2007). Of course we cannot safely say in every case, therefore we have to try to reduce the possible confounding effects, such as LD differences among populations; it is well known that LD structures are often unstable and cannot be generalized across populations of different origin (Ingles et al., 1997). In relation to this, the author proposed that the MAFs among populations must be examined for meta-analysis (Sand, 2007). In our case, a two-fold difference in C270T allele frequency was found between Caucasian samples (5.2%) and Asian samples (2.7%), suggesting that there is genetic heterogeneity among populations. Thus, we assume from the viewpoint of this heterogeneity or just from multiple testing that this trend for association may be derived from false-positives. To correct the *p*-value using the Benjamini–Hochberg (BH) method, which is a type of false discovery rate (FDR) controlling procedure, the *Q*-value in the C270T meta-analysis ($Q = 0.0136$) is higher than the *p*-value in the fixed model ($p = 0.036$). This gap suggests no significance in the C270T meta-analysis. Therefore, our case-control analysis based on LD and meta-analyses does not provide evidence for an association of the *BDNF* gene with schizophrenia.

Several caveats should be noted. Firstly, we did not include a systematic mutation scan in the 5' flanking region or exon regions. Secondly, our phenotypic diagnosis is not based on structured interviews and control samples are significantly younger than case samples.

To conclude, our results indicate that common SNPs in the *BDNF* gene do not play a major role in patients with schizophrenia. However, if there is a possibility for an association of

BDNF with schizophrenia, the A1 allele in *BDNF*-LCPR is the most attractive candidate variant. Further LD evaluation or an association study in which *BDNF*-LCPR is genotyped directly will be required for a conclusive result.

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Contributors

KK and TK performed laboratory assays and the data-analysis. KK, TK and MI drafted the manuscript. MI, TK, YY, YK, NT, SS, KO, YY, RH, BA, MT and TI advised on data-analysis, NO and NI participated in the design of the study, interpretation of the data, and drafting of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

None.

Acknowledgements

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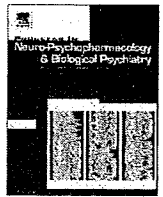
Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.schres.2009.03.040.

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Association analysis of Group II metabotropic glutamate receptor genes (*GRM2* and *GRM3*) with mood disorders and fluvoxamine response in a Japanese population

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ABSTRACT

Background: Several lines of evidence implicate abnormalities in glutamate neural transmission in the pathophysiology of mood disorders, including major depressive disorder (MDD) and bipolar disorder (BP). Preclinical antidepressant effects were also reported for group II metabotropic glutamate receptor (Group II mGluRs) antagonists show dose-dependent antidepressant-like effects in murine models of depression. Also, it has been suggested that abnormalities in the hypothalamic–pituitary–adrenal axis and serotonergic neural transmission are important mechanisms in the pathophysiology of mood disorders. Group II mGluRs play an important role in regulating the function of these mechanisms. From these results, it has been suggested that abnormalities in Group II mGluRs might be involved in the pathophysiology of mood disorders, including MDD and BP, and may influence the clinical response to treatment with SSRIs in MDD. Therefore, we studied the association between Group II mGluR genes (*GRM2* and *GRM3*) and mood disorders and the efficacy of fluvoxamine treatment in Japanese MDD patients. **Materials and methods:** Using three tagging SNPs in *GRM2* and an SNP (rs6465084) reported functional variant in *GRM3*, we conducted a genetic association analysis of case-control samples (325 MDD patients, 155 BP patients and 802 controls) in the Japanese population. In addition, we performed an association analysis of *GRM2* and *GRM3* and the efficacy of fluvoxamine treatment in 117 Japanese patients with MDD. The MDD patients in this study had scores of 12 or higher on the 17 items of the Structured Interview Guide for Hamilton Rating Scale for Depression (SIGH-D). We defined a clinical response as a decrease of more than 50% in baseline SIGH-D within 8 weeks, and clinical remission as an SIGH-D score of less than 7 at 8 weeks.

Results: We found an association between rs6465084 in *GRM3* and MDD in the allele-wise analysis after Bonferroni's correction (P -value = 0.0371). However, we did not find any association between *GRM3* and BP or the fluvoxamine therapeutic response in MDD in the allele/genotype-wise analysis. We also did not detect any association between *GRM2* and MDD, BP or the fluvoxamine therapeutic response in MDD in the allele/genotype-wise or haplotype-wise analysis.

Discussion: We detected an association between only one marker (rs6465084) in *GRM3* and Japanese MDD patients. However, because we did not perform an association analysis based on LD and a mutation scan of *GRM3*, a replication study using a larger sample and based on LD may be required for conclusive results.

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Abbreviations: Group II mGluRs, Group II metabotropic glutamate receptors; MDD, major depressive disorder; BP, bipolar disorder; SIGH-D, Structured Interview Guide for Hamilton Rating Scale for Depression; LD, linkage disequilibrium; NMDA, *N*-methyl-*D*-aspartate; mGluRs, metabotropic glutamate receptors; AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazole propionate; MAFs, minor allele frequencies; HWE, Hardy-Weinberg equilibrium; MDR, Multifactor Dimensionality Reduction; TSNAX-DISC1 gene, Translin-associated factor X-Disrupted-in-Schizophrenia-1 gene; GWAS, whole genome association study.

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1. Introduction

Several lines of evidence implicate abnormalities in glutamate neural transmission in the pathophysiology of mood disorders, including major depressive disorder (MDD) (Hashimoto et al., 2007; Paul and Skolnick, 2003) and bipolar disorder (BP) (Hashimoto et al., 2007; Witkin et al., 2007), and in the mechanisms of therapeutic actions of antidepressants (Palucha and Pilc, 2002). Glutamate exerts its actions through activation of receptors such as *N*-methyl-*D*-aspartate (NMDA) receptor, metabotropic

glutamate receptors (mGluRs) and α -amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA). NMDA receptor antagonists such as dizocilpine and memantine show antidepressant-like actions in mice exhibiting depressive behavior, such as in the forced swim test (Poleszak et al., 2007). Preclinical antidepressant effects were also reported for group II metabotropic glutamate receptor (mGluR2 and mGluR3) antagonists, MGS0039 and LY341495 show dose-dependent antidepressant-like effects in murine models of depression, such as the forced swim and tail suspension tests (Bespalov et al., 2008).

The mGluRs are classified into three groups (group I mGluRs: mGluR1 and mGluR5, group II mGluRs: mGluR2 and mGluR3, and group III mGluRs: mGluR4 and mGluR6–8). Postmortem study has shown decreased mGluR3 in the perirhinal cortex in MDD patients compared with control subjects (Beneyto et al., 2007). Group II mGluRs are highly expressed in brain structures apparently related to emotional states, including the forebrain and limbic areas (Tamaru et al., 2001; Wright et al., 2001). It has been suggested that abnormalities in the hypothalamic-pituitary-adrenal axis (HPA axis) (Buckley and Schatzberg, 2005) and serotonergic neural transmission (Levinson, 2006; Serretti and Mandelli, 2008) are important mechanisms in the pathophysiology of mood disorders. Group II mGluRs have been shown to regulate the function of HPA axis activity (Holsboer and Barden, 1996; Scaccianoce et al., 2003). In animal studies, MGS0039 elevated serotonin levels in the rat medial prefrontal cortex in an *in vivo* microdialysis study (Karasawa et al., 2005) and MGS0039 and LY341495 increased the activity of serotonin neurons in the rat dorsal raphe nucleus (Kawashima et al., 2005). In addition, Matriciano and colleagues reported that reported that both the expression and function of group II mGluRs are amplified in rat hippocampus when rat was administered imipramine chronically (Matriciano et al., 2002). From these results, group II mGluRs appear to be good candidates both for involvement in the pathophysiology of, and as therapeutic targets in, MDD.

The mGluR2 gene (*GRM2*: OMIM *604099, 7 exons in a genomic region spanning 10.466 Kb) is located on 3p21. The mGluR3 gene (*GRM3*: OMIM *601115, 6 exons in a genomic region spanning 221.763 Kb) is 7q21. This genomic region has been shown to be closely related to susceptibility for BP (Detera-Wadleigh et al., 1997). Therefore, we studied the association between *GRM2* or *GRM3* and mood disorders and the efficacy of fluvoxamine treatment in Japanese MDD patients.

2. Materials and methods

2.1. Subjects

The subjects in the association analysis were 325 MDD patients (159 males and 166 females; mean age \pm standard deviation 47.3 \pm 14.9 years), 155 BP patients (80 males and 75 females; 96 patients

with bipolar I disorder and 59 patients with bipolar II disorder; 47.9 \pm 14.2 years) and 802 healthy controls (351 males and 451 females; 37.2 \pm 15.9 years). Of the 325 MDD patients, 117 (58 males and 59 females; 44.8 \pm 16.7 years) were treated with fluvoxamine and diagnosed according to DSM-IV criteria with the consensus of at least two experienced psychiatrists on the basis of a review of medical records and assessments with the Structured Interview Guide for Hamilton Rating Scale for Depression (SIGH-D). The remaining MDD patients were diagnosed according to DSM-IV criteria with the consensus of at least two experienced psychiatrists on the basis of unstructured interviews and a review of medical records. All subjects were unrelated to each other, ethnically Japanese, and lived in the central area of Japan. All healthy controls were also psychiatrically screened based on unstructured interviews. None had severe medical complications such as liver cirrhosis, renal failure, heart failure, or other Axis-I disorders according to DSM-IV.

2.2. Data collection

The 117 MDD patients in this study had scores of 12 or higher on the 17 items of the SIGH-D (Peveler and Kendrick, 2005). We defined a clinical response as a decrease of more than 50% in baseline SIGH-D within 8 weeks, and clinical remission as a SIGH-D score of less than 7 at 8 weeks. Detailed information on data collection was described in a previous paper (Saito et al., 2006). The clinical characteristics of patients in this study, classified according to these definitions, can be seen in Table 1.

2.3. SNP selection

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 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 (Barrett et al., 2005), for the following association analysis. In addition, we selected rs6465084 in *GRM3*, which is reported to be associated with prefrontal brain functioning, for use in the later association analysis.

2.4. SNP genotyping

We used TaqMan assays (Applied Biosystems, Foster City, CA, U.S.A.) for all SNPs. Detailed information is available on request.

Table 1
Clinical characteristics of the patients in both definition groups.

	N			Age (mean \pm SD)	Baseline SIGH-D (avg \pm SD)	Fluvoxamine dose at 8 weeks (mg/day) (avg \pm SD)	Number of previous episode (avg \pm SD)
	Total	Male	Female				
Overall	117	58	59	44.8 \pm 16.7	20.1 \pm 5.84	122 \pm 41.0	1.38 \pm 0.656
Clinical response group ^a							
Responders	60	29	31	45.1 \pm 16.5	21.4 \pm 6.19	119 \pm 41.0	1.36 \pm 0.574
Nonresponders	57	29	28	44.4 \pm 17.2	18.7 \pm 5.14	125 \pm 41.2	1.41 \pm 0.780
P-value	0.783			0.849	0.0102	0.468	0.750
Clinical remission group ^b							
Remitters	46	22	24	44.3 \pm 16.2	19.5 \pm 5.05	114 \pm 43.7	1.37 \pm 0.598
Nonremitters	71	37	34	45.2 \pm 17.3	20.5 \pm 6.30	127 \pm 38.6	1.39 \pm 0.718
P-value	0.650			0.809	0.350	0.118	0.879

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

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

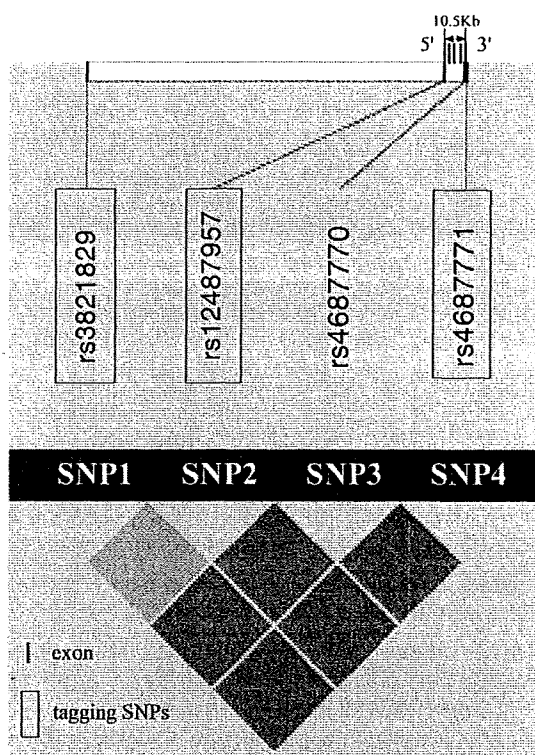


Fig. 1. LD evaluation and tagging SNPs in *GRM2*. Black bars represent exons of *GRM2*. Tagging SNPs selected from HapMap database are represented by black boxes. The color scheme is based on D' values. Other information can be seen at the HAPLOVIEW website.

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 associations with the chi-square test (SAS/Genetics, release 8.2, SAS Japan Inc, Tokyo, Japan), and haplotype-wise association analysis was done with a likelihood ratio test using the COCAPHASE 2.403 program (Dudbridge, 2003). Bonferroni's correc-

tion was used to control inflation of the type I error rate. Power calculation was performed using a statistical program prepared by Purcell et al. (2003).

3. Results

The linkage disequilibrium (LD) structure of *GRM2* can be seen in Fig. 1. Genotype frequencies were in HWE for all SNPs. In addition, regarding genotyping quality control measures, 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%. We found an association between rs6465084 in *GRM3* and MDD in the allele-wise analysis after Bonferroni's correction (P -value = 0.0371). However, we did not find any association between *GRM3* and BP in the allele/genotype-wise (Table 2). We also did not detect any association between *GRM2* and MDD or BP in the allele/genotype-wise or haplotype-wise analysis (MDD: P -value = 0.2537 and BP: P -value = 0.1349)(Table 2).

With regard to the clinical characteristics of patients, only one difference was detected between responders and nonresponders in baseline SIGH-D scores (P -value = 0.0102) (Table 1). One patient each was prescribed alprazolam, loflazepate and etizolam. Two patients each were prescribed lorazepam, brotizolm, flunitrazepam and zopiclone. We did not find any association between *GRM2* or *GRM3* and the fluvoxamine therapeutic response in MDD patients in allele/genotype-wise (Table 3) or haplotype-wise analysis (response: P -value = 0.2459 and remission: P -value = 0.3181).

Moreover, 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 analysis, however, no interactions were obtained with MDD and BP (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 1.31–1.63 and 1.55–2.18 in MDD and BP, respectively, for *GRM2* and at 1.51 and 2.02 in MDD and BP, respectively, for *GRM3* under a multiplicative model of inheritance.

4. Discussion

We performed the first association study of *GRM2* and *GRM3* with mood disorders and fluvoxamine treatment outcome in MDD in the

Table 2
Association analysis of *GRM2* and *GRM3* with mood disorders.

Gene	SNP ID ^a	Phenotype ^b	MAF ^c	N	Genotype distribution ^d			P-value ^f			Corrected P-value ^{g,h}		
					M/M	M/m	m/m	HWE ^e	Genotype	Allele	Genotype	Allele	
<i>GRM2</i>	rs3821829	Controls	0.0468	802	731 (91.1)	67 (8.35)	4 (0.500)	0.0751					
		BP	0.0516	155	139 (89.7)	16 (10.3)	0 (0.00)	0.498	0.501	0.713			
	rs12487957	MDD	0.0477	325	295 (90.8)	29 (8.92)	1 (0.308)	0.750	0.869	0.924			
		Controls	0.333	802	346 (43.1)	378 (47.1)	78 (9.73)	0.0834					
	rs4687771	BP	0.281	155	80 (51.6)	63 (40.6)	12 (7.74)	0.934	0.148	0.0719			
		MDD	0.335	325	144 (44.3)	144 (44.3)	37 (11.4)	0.912	0.579	0.910			
<i>GRM3</i> A	rs6465084	Controls	0.0842	802	673 (83.9)	123 (15.3)	6 (0.748)	0.884					
		BP	0.0903	155	128 (82.6)	26 (16.8)	1 (0.645)	0.796	0.896	0.722			
	A>G	MDD	0.0523	325	292 (89.8)	32 (9.85)	1 (0.308)	0.901	0.0344	0.00928	0.138	0.0371	
		Controls	0.376	802	300 (37.4)	401 (50.0)	101 (12.6)	0.0632					
	T>A	BP	0.332	155	66 (42.6)	75 (48.4)	14 (9.03)	0.260	0.309	0.145			
		MDD	0.378	325	121 (37.2)	162 (49.8)	42 (12.9)	0.283	0.989	0.911			

^a Major allele > minor allele.

^b BP: bipolar disorder MDD: major depressive disorder.

^c MAF: minor allele frequency.

^d M: major allele, m: minor allele, The number in the parenthesis showed the percentage (%).

^e Hardy–Weinberg equilibrium.

^f Bold numbers represent significant P-value.

^g Calculated by Bonferroni correction.

Table 3
Genotype and allele distributions of *GRM2* and *GRM3* in both definition groups.

Gene	SNP ID ^a	Clinical groups	MAF ^b	N	Genotype distribution ^c			P-value ^d		
					M/M	M/m	m/m	HWE	Genotype	Allele
<i>GRM2</i>	rs3821829 C>T	Responders	0.0667	60	52 (86.7)	8 (13.3)	0 (0.0)	0.580		
		Nonresponders	0.0263	57	54 (94.7)	3 (5.26)	0 (0.0)	0.838	N.A.	0.145
		Remission	0.0435	46	42 (91.3)	4 (8.70)	0 (0.0)	0.758		
		Nonremission	0.0493	71	64 (90.1)	7 (9.86)	0 (0.0)	0.662	N.A.	0.837
	rs12487957 T>C	Responders	0.350	60	25 (41.7)	28 (46.7)	7 (11.7)	0.843		
		Nonresponders	0.298	57	26 (45.6)	28 (49.1)	3 (5.26)	0.190	0.462	0.398
		Remission	0.370	46	19 (41.3)	20 (43.5)	7 (15.2)	0.650		
		Nonremission	0.296	71	32 (45.1)	36 (50.7)	3 (4.23)	0.0673	0.114	0.239
	rs4687771 T>A	Responders	0.383	60	22 (36.7)	30 (50.0)	8 (13.4)	0.656		
		Nonresponders	0.333	57	23 (40.4)	30 (52.6)	4 (7.02)	0.164	0.527	0.425
		Remission	0.391	46	17 (37.0)	22 (47.3)	7 (15.2)	0.979		
		Nonremission	0.338	71	28 (39.4)	38 (53.5)	5 (7.04)	0.0989	0.361	0.407
<i>GRM3</i>	rs6465084 A>G	Responders	0.0667	60	52 (86.7)	8 (13.4)	0 (0.0)	0.580		
		Nonresponders	0.0614	57	50 (87.7)	7 (12.3)	0 (0.0)	0.621	N.A.	0.869
		Remission	0.0652	46	40 (87.0)	6 (13.0)	0 (0.0)	0.636		
		Nonremission	0.0634	71	62 (87.3)	9 (12.7)	0 (0.0)	0.569	N.A.	0.955

^a Major allele>minor allele.

^b MAF: minor allele frequency.

^c M: major allele, m: minor allele, The number in the parenthesis showed the percentage (%).

^d Hardy–Weinberg equilibrium.

Japanese population. We detected a significant association between *GRM3* and MDD. In this study, we selected rs6465084 in *GRM3* as the SNP. Rs6465084 has been found to be associated with decreased verbal list learning and verbal fluency (Egan et al., 2004). In addition, Egan and colleagues reported that the rs6465084 A allele predicted decreased levels of *N*-acetylaspartate in the prefrontal cortex in an *in vivo* study, and suggested that the rs6465084 A allele reduced tissue glutamate levels and synaptic abundance (Egan et al., 2004). This influence of *GRM3* on prefrontal cortex and cognitive function suggests that abnormalities in glutamate neurotransmission may be involved in the pathophysiology of MDD by altering glutamate neurotransmission. Rs6465084 was shown to have MAFs_{controls}: 0.0842 and MAFs_{MDD}: 0.0523. Tochigi and colleagues reported that with this SNP, the MAFs in the Japanese population appear to be smaller than in Caucasians (Tochigi et al., 2006). Also, the MAFs in MDD patients were smaller than in controls. Our result is similar to the results of several studies in schizophrenia (Egan et al., 2004; Mossner et al., 2008). It might be that mood disorders and schizophrenia have common susceptibility genes. In support of this hypothesis, we show recent evidence (TSNAX-DISC1 gene)(Schosser et al., in press). Two studies have also suggested that rs6465084 was associated with the cognitive function in schizophrenic patients (Egan et al., 2004; Mossner et al., 2008). Therefore, further study will be required to investigate the relationship between rs6465084 and cognitive function in MDD patients. Recently, a whole genome association study (GWAS) reported an association between rs2237554 in *GRM3* and bipolar disorder (Sklar et al., 2008). Another GWAS reported that rs2189813, which was in LD with rs2237554 according to the HapMap database ($D' = 1.00$ and $r^2 = 0.831$), was not associated with Japanese bipolar disorder patients (Hattori et al., in press)(release#23.a.phase2, Mar 2008, www.hapmap.org, population: Japanese Tokyo). Although we did not perform an association analysis of rs2237554, a replication study using larger samples than in the original studies will need to be carried out in the future. Also, Sartorius and colleagues reported that rs2228595 in *GRM3* predicted increased expression of the *GRM3*Delta4 splice variant (Sartorius et al., 2008). This SNP was shown to have “minor allele frequencies: 0.0330%” in the HapMap database (release#23.a.phase2, Mar 2008, www.hapmap.org, population: Japanese Tokyo). However, because our study had a small sample size, we considered it to be difficult to evaluate the association of rare variants from the viewpoint of power. Therefore, we did not include an association analysis of rs2228595.

A few points of caution should be noted in interpreting our results. First, the lack of association may be due to biased samples, such as small sample sizes, especially for BP and the fluvoxamine therapeutic response in MDD samples, or unmatched age or gender samples. Because our samples for BP and the fluvoxamine therapeutic response in MDD were small, type II errors are possible in the results of these statistical association analyses. On average, the controls were much younger than the patients. This means that a number of young controls may go on to develop one of these disorders, most likely MDD, since the incidence of major depression is as high as 5% or more. Our subjects did not undergo structured interviews. MDD patients who are not diagnosed by structured interview may develop bipolar disorder in the future (Bowden, 2001; Kishi et al., 2009; Kishi et al., 2008; Stensland et al., 2008). However, in this study patients were carefully diagnosed according to DSM-IV criteria with consensus of at least two experienced psychiatrists on the basis of a review of medical records. In addition, when we found a patient who had been misdiagnosed, we promptly excluded the misdiagnosed case to maintain the precision of our sample. Second, we could not use an LD based strategy and perform a mutation search because *GRM3* has a massive gene structure. Therefore, in future studies it will be necessary to evaluate associations between other variants and mood disorders. It will be important to replicate and confirm these findings in other independent studies using larger samples.

5. Conclusion

In conclusion, we detected an association between only one marker (rs6465084) in *GRM3* and Japanese MDD patients. However, because we did not perform an association analysis based on LD and a mutation scan of *GRM3*, a replication study using a larger sample and based on LD may be required for conclusive results.

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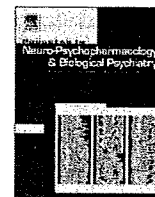
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A functional polymorphism in estrogen receptor alpha gene is associated with Japanese methamphetamine induced psychosis

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ABSTRACT

Background: A recent study reported an association between rs2234693, which influences enhancer activity levels in estrogen receptor alpha gene (*ESR1*), and schizophrenia. This study reported that schizophrenic patients with the CC genotype have significantly lower *ESR1* mRNA levels in the prefrontal cortex than patients with other genotypes. The symptoms of methamphetamine induced psychosis are similar to those of paranoid type schizophrenia. Therefore, we conducted an association analysis of rs2234693 with Japanese methamphetamine induced psychosis patients. **Method:** Using rs2234693, we conducted a genetic association analysis of case-control samples (197 methamphetamine induced psychosis patients and 197 healthy controls). The age and sex of the control subjects did not differ from those of the methamphetamine induced psychosis patients. **Results:** We detected a significant association between *ESR1* and methamphetamine induced psychosis patients in allele/genotype-wise analysis. For further interpretation of these associations, we performed single marker analysis of subjects divided by sex. Rs2234693 was associated with male methamphetamine induced psychosis. **Discussion:** Our results suggest that rs2234693 in *ESR1* may play a role in the pathophysiology of Japanese methamphetamine induced psychosis patients.

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1. Introduction

Methamphetamine (METH) use disorder is a common psychiatric disorder and a serious problem worldwide. METH stimulates the release of dopamine in the mesolimbic system (Munzar et al., 2004), and dopamine is in turn involved in the reinforcing action of many addictive drugs such as METH (Vocci et al., 2005). In addition,

increased dopamine in the mesolimbic system is considered to produce psychotic symptoms such as hallucination and delusion (Laviolette, 2007). The symptoms of METH-induced psychosis are similar to those of paranoid type schizophrenia (Sato et al., 1992). Therefore, it may be that METH-induced psychosis and schizophrenia have common susceptibility genes. 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.

A recent study reported an association between rs2234693, which influences enhancer activity levels of estrogen receptor alpha (*ERalpha*) in the *ERalpha* gene (*ESR1*) (Maruyama et al., 2000), and schizophrenia (Weickert et al., 2008). This study reported that schizophrenic patients with the CC genotype have significantly lower *ESR1* mRNA levels in the prefrontal cortex than patients with other genotypes (Weickert et al.,

Abbreviations: METH, methamphetamine; *AKT1*, V-act murine thymoma viral oncogene homologue 1; *ERalpha*, estrogen receptor alpha; *ESR1*, estrogen receptor alpha gene; SD, standard deviation; JGIDA, Japanese Genetics Initiative for Drug Abuse; HWE, Hardy-Weinberg equilibrium; SNP, single nucleotide polymorphism; mRNA, messenger ribonucleic acid; CD-CV hypothesis, common disease-common variants hypothesis.

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2008). Additionally, allele C of the *ESR1* SNP in intron 1 was thought to eliminate the transcription factor binding sites for activating enhancer binding protein 4 (transcriptional activator) in the sense strand and zinc finger protein (transcriptional repressor) in the antisense strand (Aoki et al., 1998; Fuks et al., 2001; Weickert et al., 2008). Therefore, Weickert and colleagues suggested that rs2234693 might be a functional SNP influencing *ESR1* expression regulation (Weickert et al., 2008). Estrogen has been shown to play an important role in protection from dopamine-induced toxicity caused by METH (Dluzen and McDermott, 2002; Gajjar et al., 2003; Liu and Dluzen, 2006).

From the above results, we suspected that estrogen receptor alpha might be related to the pathophysiology of METH-induced psychosis, and therefore conducted an association analysis of a functional SNP (rs2234693) in *ESR1* and METH-induced psychosis in the Japanese population.

2. Materials and methods

2.1. Subjects

The subjects in the association analysis were 197 METH-induced psychosis patients (164 males and 33 females; mean age \pm standard deviation (SD) 37.6 ± 12.2 years) and 197 healthy controls (164 males and 33 females; 37.5 ± 12.2 years). The age and sex of the control subjects did not differ from those of the METH-induced psychosis patients. All subjects were unrelated to each other, ethnically Japanese, and lived in the central area of Japan. The patients were diagnosed according to DSM-IV criteria with consensus of at least two experienced psychiatrists on the basis of unstructured interviews and a review of medical records. One hundred thirty-seven subjects with METH-induced psychosis also had dependence on drugs other than METH. Subjects with METH-induced psychosis 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). All healthy controls, which included hospital staff, their families and medical students, were also psychiatrically screened based on unstructured interviews including current and past psychiatric history. None had severe medical complications such as liver cirrhosis, renal failure, heart failure or other Axis-I disorders according to DSM-IV. Written informed consent was obtained from each subject. This study was approved by the ethics committees at Fujita Health University, Nagoya University Graduate School of Medicine and each participating member of the Institute of the Japanese Genetics Initiative for Drug Abuse (JGIDA).

2.2. SNP genotyping

We used a TaqMan assay (Applied Biosystems, Foster City, CA, U.S.A.) for the SNP. 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 for 10 min at 95 °C, followed by 45 cycles at 95 °C for 15 s and 58 °C for 1 min. The primers used were forward 5'-TGCCTCATATCAAAGTGTCAAAT-3' and reverse 5'-CCTGCTGCCTCATGTCTATTGGCTAC-3'. Detailed information is available on request.

2.3. 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 chi-square test (SAS/Genetics, release 8.2, SAS Japan Inc, Tokyo, Japan). Bonferroni's correction was used to control inflation of the type I error rate.

The significance level for all statistical tests was 0.05.

We performed an explorative analysis of gender effects for the following reasons: (1) our subjects were unmatched for sex, especially those with METH-induced psychosis (164 males and 33 females); and (2) there is a possible sex difference in the pathophysiology of METH use disorder, since a sex difference has been reported for not only the use of METH but also the response to it (Brecht et al., 2004; Russell et al., 2008).

3. Results

Genotype frequencies of the SNP were in HWE (Table 1). We detected a significant association between rs2234693 and METH-induced psychosis in the allele/genotype-wise analyses (Table 1). For further interpretation of these associations, we performed single marker analysis of subjects divided by sex. Rs2234693 was associated with male METH-induced psychosis patients in the allele/genotype-wise analyses (Table 1). These results remained positive after correction for type I error due to multiple testing using Bonferroni's correction (Table 1).

4. Discussion

Our results showed an association between rs2234693 in *ESR1* and METH-induced psychosis in the Japanese population. Therefore, we reasoned that *ESR1* may play a role in the pathophysiology of METH-induced psychosis in the Japanese population. In addition, we performed an explorative analysis of gender effects. In this study, *ESR1* was associated with male METH-induced patients, who were the majority of our METH-induced psychosis samples. Similar to the study of Weickert and colleagues, there was a significantly higher percentage of the CC genotype among patients (percentage of CC genotype in total METH-induced psychosis and male METH-induced psychosis: 27.9% and 31.7%, respectively) than among controls (percentage of CC genotype in controls and male controls: 19.3% and 18.9%, respectively) (Weickert et al., 2008). This would seem to

Table 1
Association analysis of rs2234693 with methamphetamine induced psychosis and explorative analysis of gender subgroups.

Phenotype ^a	MAF ^b	N	Genotype distribution			P-value ^c			Corrected P-value ^{c,e}	
			TT	TC	CC	HWE ^d	Genotype	Allele	Genotype	Allele
Controls	0.414	197	72	87	38	0.208				
METH-induced psychosis	0.505	197	53	89	55	0.176	0.0493	0.0101		
Controls in males	0.421	164	57	76	31	0.528				
METH-induced psychosis in males	0.530	164	42	70	52	0.0668	0.0199	0.00489	0.0398	0.00978
Controls in females	0.379	33	15	11	7	0.0938				
METH-induced psychosis in females	0.379	33	11	19	3	0.199	0.114	1.00		

^a METH-induced psychosis: methamphetamine induced psychosis.

^b MAF: minor allele frequency.

^c Bold represents significant P-value.

^d HWE: Hardy–Weinberg equilibrium.

^e Calculated using Bonferroni's correction.

support the hypothesis of common susceptibility genes for METH-induced psychosis and schizophrenia. It has also been reported that the CC genotype for rs2234693 has significantly lower expression of *ESR1* mRNA than the other genotype groups (T/C and T/T) in the prefrontal cortex of schizophrenics (Weickert et al., 2008). Two animal studies involving ovariectomized rats reported that long-term estradiol treatment may preserve striatal dopamine concentrations by decreasing the affinity of the dopamine transporter (Disshon et al., 1998; Ohtani et al., 2001). However, no human studies have been performed to date. We hypothesize that in these psychotic disorders decreased *ESR1* mRNA in the brain may weaken the estrogen response, resulting in increased dopamine-induced toxicity and psychotic symptoms. Several studies have suggested that treatment with estrogen can be an effective adjunctive therapy in schizophrenia. Our results suggest that estrogen therapy may be effective for not only schizophrenia but also for other psychotic disorders such as METH-induced psychosis (Kulkarni et al., 2008).

ESR1 was associated with male METH-induced patients in this study. It is known that there are sex differences in not only the response to METH but also in striatal dopamine release (Russell et al., 2008). It has also been shown that response to and use of METH differ with sex (Dluzen and Liu, 2008), and that estrogen plays an important role in protection from dopamine-induced toxicity caused by METH (Dluzen and McDermott, 2002; Gajjar et al., 2003; Liu and Dluzen, 2006). Other investigations have found an association of rs2234693 with risk of developing cognitive impairment in older women and major depressive disorder in females in the Chinese population (Yaffe et al., 2002). However, because our study included few female subjects, there is a possibility of type II errors in the results of our association analysis for these phenotypes. To overcome this limitation, a replication study using larger samples or samples from other populations will be required for conclusive results.

Because testing for HWE is commonly used for quality control in large-scale genotyping and is one of the few ways to identify systematic genotyping errors in unrelated individuals (Wittke-Thompson et al., 2005), we estimated HWE and confirmed the genotyping quality in this study. Genotype frequencies were in HWE for this SNP.

Other studies have reported associations of rs2234693 with endometrial cancer, breast cancer (van Duijnhoven et al., 2005), osteoporosis (Albagha et al., 2005; Albagha and Ralston, 2003; Ferrari and Rizzoli, 2005), stroke (Shearman et al., 2005), adiposity (Fox et al., 2005), atherosclerosis (Lehtimäki et al., 2002) and Alzheimer's disease (Mattila et al., 2000).

A few points of caution should be mentioned with respect to our results. First, the positive association we found may have been due to our small sample size. Ideal samples for this study are METH use disorder subjects with and without psychosis. Because only a few of our subjects had METH use disorder without psychosis, and we wanted to avoid statistical error, we did not perform association analysis in this group. Second, we did not include a mutation scan to detect rare variants. We designed the study based on the common disease–common variants hypothesis (CD–CV hypothesis) (Chakravarti, 1999). However, Weickert and colleagues have shown associations between schizophrenia, a common disease, and rare variants in *ESR1* (Weickert et al., 2008). To describe the genetic background of METH-induced psychosis by the common disease–rare variants hypothesis, further investigation will be required, such as medical resequencing using larger samples. However, statistical power is needed to evaluate the association of rare variants. To overcome these limitations, a replication study using larger samples or samples from other populations will be required for conclusive results.

5. Conclusion

Our results suggest that rs2234693 in *ESR1* may play a role in the pathophysiology of Japanese METH-induced psychosis.

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