TABLE III. Pairwise Linkage Disequilibrium Estimations Between Single Nucleotide Polymorphisms (SNPs) in the *GLYT1* Gene

	SNP1	SNP2
SNP2	$D' = 0.76$ $r^2 = 0.064$	
SNP3	$D' = 0.92$ $r^2 = 0.087$	$D' = 0.87$ $r^2 = 0.70$

TABLE V. Estimated Haplotype Frequencies for Patients With Methamphetamine-Use Disorder and Controls

SNP	Haplotype	METH	CON	*P* value
SNP1–2	C-G	0.48	0.54	0.080
	C-A	0.24	0.27	0.30
	T-G	0.28	0.16	0.000039
	T-A	0.00	0.027	—

DISCUSSION

We found that the T allele of SNP1 (IVS3 + 411C > T, rs2486001) and the T-G haplotype consisting of SNP1 and SNP2 (1056G > A, rs2248829) of the *GLYT1* gene showed a substantially significant association with methamphetamine-use disorder (allele $P = 0.0018$, haplotype $P = 0.000039$). The T-G haplotype of the gene approximately doubles the risk of predisposition to methamphetamine-use disorder.

GlyTs strictly maintain glycine concentrations in the vicinity of NMDA receptors. Glycine binds to glycine sites on the NR1 subunit of NMDA receptors and activates NMDA receptor signaling. Because the glycine concentration is set low by GlyTs, glycine sites are subsaturated in the physiological condition [Sato et al., 1995]. An increase of glycine concentration due to a glycine diet or administration of a glycine transport inhibitor, NFPS, enhanced the NMDA receptor function in vitro [Bergeron et al., 1998; Martina et al., 2004] and in vivo [Chen et al., 2003]. Heterozygous *GLYT1* gene knockout mice showed enhancement of NMDA receptor function [Gabernet et al., 2005]. Therefore, increases and decreases in glycine induce stronger and weaker NMDA receptor neurotransmission. Because many lines of experimental evidence have shown that the glutamatergic system and NMDA receptor signaling in the brain play pivotal roles in the development of substance dependence on psychostimulants including amphetamine, methamphetamine, and cocaine [Cervo and Samanin, 1995; Bespalov, 1996; Kim and Jang, 1997; Wolf, 1998], it is possible that modulation of glycine sites or GlyTs also affects substance dependence. Induction of amphetamine-induced CPP in rodents was prevented by a glycine site antagonist L-701,324 (7-chloro-4-hydroxy-3-(2-phenoxy)phenyl-2(1H)-quinolone) [Mead and Stephens, 1999], and a glycine site partial agonist ACPC (1-aminocyclopropanecarboxylic acid), which disturbs the effects of endogenous glycine [Papp et al., 2002]. Combined with our findings, variants of the *GLYT1* gene may affect susceptibility to methamphetamine dependence by modulating NMDA receptor function.

NMDA receptor signaling is also considered to be involved in psychotic disorders. Phencyclidine and ketamine, noncompetitive antagonists of NMDA receptors, produce a psychotic state in healthy subjects and exacerbate symptoms in schizophrenics [Javitt and Zukin, 1991; Breier et al., 1997], and hypofunction of NMDA receptors is assumed to be a possible pathophysiology of schizophrenia. Mice with reduced expression of NR1 and the ε1 subunit of NMDA receptors due to genetic manipulation showed abnormal phenotypes similar to those observed in animal models of schizophrenia [Mohn et al., 1999; Miyamoto et al., 2001]. Recent human genetic studies also revealed a significant association of the *GRIN1* and *GRIN2A* genes encoding the NR1 and NR2A subunits of NMDA receptors, respectively, with susceptibility to schizophrenia [Itokawa et al., 2003; Zhao et al., 2006]. In addition, the GlyT inhibitors SSR504734 (2-chloro-*N*-[(*S*)-phenyl[(2*S*)-piperidin-2-yl]methyl]-3-trifluoromethyl benzamide) and NFPS produced antipsychotic profiles in experimental paradigms [Javitt et al., 2004; Depoortere et al., 2005], and heterozygous *GLYT1* knockout mice had reversed amphetamine-induced disruption of prepulse inhibition, one of the physiological phenotypes of psychosis [Tsai et al., 2004]. Because the majority of patients with methamphetamine-use disorder examined in the present study had a comorbid diagnosis of methamphetamine psychosis, it is possible that the *GLYT1* gene may be involved in liability to psychotic symptoms. We re-analyzed the data and found that methamphetamine psychosis is also significantly associated with SNP1 of the *GLYT1* gene (data not shown). Hence, the present findings may indicate that genetic variants of the *GLYT1* gene could predict a risk of comorbidity of psychosis after repeated methamphetamine abuse.

The coding sequence of the human *GLYT1* gene is divided into 14 exons (based on NM 1024845) distributed over a region of 133 kb, most of which (exons 2–14) cluster within 7 kb [Adams et al., 1995]. SNP1 (IVS3 + 411C > T) is located in intron 3, and SNP2 (1056G > A) is located in exon 7, but is synonymous. Usually, these SNPs are considered nonfunctional. However, recent advances in molecular genetics show intronic RNAs can actually be processed to smaller RNAs, snoRNAs, or miRNAs, which control various levels of gene expression in physiology and development, including transcription, RNA splicing, editing, translation, and turnover [Mattick and Makunin, 2006]. It is possible that SNP1 affects the *GLYT1* gene expression and results in susceptibility to methamphetamine-use disorders. Alternatively, other functional mutations in the *GLYT1* gene with LD with SNP1 or locating on the T-G haplotype of SNP1–SNP2 may be involved in an increased genetic risk for methamphetamine-use disorder because SNP1 was shown to be in an LD block extending at least from intron 3 to intron 11 in our Japanese samples. HapMap project data of a Japanese population (Rel 21a) showed a larger LD block from intron 1 to intorn 12. *GLYT1* is expressed as three splice forms by alternative splicing in 5′-regions, GLYT1a, GLYT1b, and GLYT1c [Borowsky et al., 1993; Zafra et al., 1995]. It is possible that unexamined polymorphisms in intron 1 in LD with SNP1 may affect alternative splicing of the *GLYT1* mRNA and result in susceptibility to methamphetamine-use disorders.

TABLE IV. Multi-Loci Association Analyses of the *GLYT1* Gene

SNP ID	1SNP	2SNP	3SNP
SNP1 (C > T)	0.0019		
		0.000011	
SNP2 (G > A)	0.043		0.000050
		0.0040	
SNP3 (G > A)	0.20		

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Genetic variants of D2 but not D3 or D4 dopamine receptor gene are associated with rapid onset and poor prognosis of methamphetamine psychosis

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ABSTRACT

D2-like receptors are key targets for methamphetamine in the CNS, and their activation is an initial and indispensable effect in the induction of dependence and psychosis. It is possible that genetic variants of D2-like receptors may affect individual susceptibility to methamphetamine dependence and psychosis. To test this hypothesis, 6 putatively functional polymorphisms of D2-like receptors, −141C Ins/Del, Ser311Cys and *TaqI*A of the *DRD2* gene, Ser9Gly of the *DRD3* gene, and −521C>T and a variable number of tandem repeats in exon 3 of the *DRD4* gene, were analyzed in 202 patients with methamphetamine dependence and/or psychosis and 243 healthy controls in a Japanese population. No polymorphism examined showed significant association with methamphetamine dependence, but two polymorphisms of *DRD2* were associated with the clinical course and prognosis of methamphetamine psychosis. The A1/A1 homozygote of *DRD2* was a negative risk factor for a poorer prognosis of psychosis that continues for more than 1 month after the discontinuance of methamphetamine abuse and the beginning of treatment with neuroleptics ($p=0.04$, odds ratio (OR) = 0.42, 95% CI; 0.27–0.65) and the complication of spontaneous relapse of methamphetamine psychosis after remission ($p=0.014$, OR = 0.34, 95% CI; 0.22–0.54). The genotype of −141C Del positive (Del/Del and Del/Ins) was at risk for rapid onset of methamphetamine psychosis that develops into a psychotic state within 3 years after initiation of methamphetamine abuse ($p=0.00037$, OR = 3.62, 95% CI 2.48–5.28). These findings revealed that genetic variants of *DRD2*, but not *DRD3* or *DRD4*, confer individual risks for rapid onset, prolonged duration, and spontaneous relapse of methamphetamine psychosis.

1. Introduction

Human and animal studies have indicated that the D2-like dopamine receptors play a central role in development of substance dependence and substance-induced psychotic disorders due to consumption of diverse classes of drugs, e.g., alcohol, nicotine, opioids, cannabinoids, cocaine, and amphetamines (Drago et al., 1998; Self and Nestler, 1998; Volkow et al., 2004). In particular, the primary central effects of the psychostimulants amphetamines and cocaine are enhancement of the dopamine concentration in the synaptic cleft and activation of D2-like dopamine receptors in the nucleus accumbens, which is one of the key mechanisms of induction of addiction and reward behaviors. Neuroleptics, which are D2-like receptor antagonists, reduced self-administration, place preference, and behavioral sensitization to amphetamines and cocaine in animal models of substance dependence and substance-induced psychosis (Amit and Smith, 1992; Beninger et al., 1989; Green and Schenk, 2002; Ujike et al., 1989). Mice deficient in dopamine D2 receptors showed insensitivity to the motor-activating effects of methamphetamine and no behavioral sensitization, and did not self-administer cocaine (Caine et al., 2002). In contrast, mice deficient in other dopamine D2-like

Abbreviations: *DRD2*, dopamine D2 receptor gene; *DRD3*, dopamine D3 receptor gene; *DRD4*, dopamine D4 receptor gene; VNTR, variable number of tandem repeats; OR, odds ratio; CI, confidential interval; PCR-RFLP, polymerase chain reaction-restriction enzyme length polymorphism; PET, positron emission tomography.

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receptor families, the D3 and D4 receptors, showed supersensitivity to amphetamines and cocaine (Kruzich et al., 2004; Rubinstein et al., 1997; Xu et al., 1997). These findings indicate that genetic variants of the dopamine D2, D3, and D4 receptors genes (*DRD2*, *DRD3*, and *DRD4*) may affect individual response to the central effects of psychostimulants and susceptibility to development of substance dependence. In fact, many previous genetic studies showed a possible association between D2-like receptor genes and substance dependence and related disorders. For example, associations of *DRD2* with polysubstance abuse (Comings et al., 1994; Smith et al., 1992), psychostimulant abuse (Noble et al., 1993; Persico et al., 1996), nicotine dependence (Noble et al., 1994), opioid dependence (Lawford et al., 2000) and alcoholism (Noble, 2000), *DRD3* with nicotine dependence (Huang et al., 2009), and *DRD4* with opioid dependence (Kotler et al., 1997; Shao et al., 2006) and nicotine dependence (Hutchison et al., 2002) have been demonstrated, although the findings are still inconclusive. As to methamphetamine dependence and/or psychosis, there were only two previous studies examined *DRD2* and/or *DRD4*, and they examined only one or two polymorphisms of the genes and showed inconsistent results (Tsai et al., 2002; Li et al., 2004). Therefore, we examined the genetic association of the dopamine D2, D3, and D4 genes at 6 putatively functional polymorphisms in Japanese patients with methamphetamine dependence and psychosis.

2. Methods

2.1. Subjects

The subjects consisted of 202 patients (167 male, 35 female; mean age ± SD, 38.1 ± 12.6) with methamphetamine dependence, 192 of whom also suffered from methamphetamine psychosis, and 243 age-, sex-, and geographical origin-matched healthy controls (193 male, 50 female; mean age ± SD, 37.2 ± 12.0) who had no individual or family history of drug dependence or major psychotic disorders such as schizophrenia and bipolar disorders. All subjects were unrelated Japanese born and living in relatively restricted areas of Japan. All patients were out- or inpatients of psychiatric hospitals of the Japanese Genetics Initiative for Drug Abuse (JGIDA). Consensus diagnoses of methamphetamine psychosis were made by two trained psychiatrists according to the ICD-10 criteria on the basis of unstructured interviews and medical records. All healthy controls were also psychiatrically screened by unstructured interviews. The study protocol and purpose were explained to all subjects participating in the study, and written informed consent was obtained from all subjects. This study was approved by the Ethics Committee of each participating institute of JGIDA.

The patients with methamphetamine psychosis were divided into the following subgroups according to three clinical phenotypes that may indicate indirectly the severity of and liability to psychosis. 1) Latency to onset of psychotic state after initial methamphetamine consumption: median of latency was 3 years; 95 (55.2%) of patients developed psychotic symptoms within 3 years of the first methamphetamine abuse, and 77 (44.8%) patients did so after 3 or more years. 2) Duration of the psychotic state after therapy: usually methamphetamine-induced psychosis will subside within 10 days, or one month at the longest, after the discontinuance of methamphetamine consumption and beginning of pharmacological therapy with antipsychotics, such as haloperidol or risperidone (transient type). However, some patients show sustained (longer than 1 month) psychotic symptoms regardless of detoxification from methamphetamine and adequate antipsychotic therapy (prolonged type) (Ujike et al., 2003; Ujike and Sato, 2004). One-hundred and six patients (57.9%) showed the transient type of psychosis, and 77 patients (42.1%) showed the prolonged type of psychosis. 3) Complication of spontaneous psychosis after remission of methamphetamine-induced psychosis: it has been documented that once methamphetamine psychosis has developed, spontaneous relapse can occur in some remitted patients due to nonspecific stresses, e.g., severe fatigue or life problems, without re-consumption of methamphetamine. The observation period of the presence or absence of spontaneous relapse was 1 year at the shortest, 20 years at the longest, with an average of 12.3 ± 11.1 years. Seventy-nine patients (41.8%) experienced spontaneous relapse, and 110 (58.2%) did not.

2.2. DNA analysis

Six polymorphisms, −141C insertion/deletion, Ser311Cys and *TaqIA* of *DRD2*, Ser9Gly of *DRD3*, −521C>T, and a variable number of tandem repeats (VNTR) of 48 base pairs in exon 3 of *DRD4*, were selected for genetic testing because they may have physiological potential. Thus, the two nonsynonymous single-nucleotide polymorphisms, Ser311Cys of *DRD2* and Ser9Gly of *DRD3*, produce amino acid substitutions, the two polymorphisms located in the promoter regions, −141C insertion/deletion of *DRD2* and −521C>T of *DRD4*, were demonstrated to change promoter activity by *in vitro* luciferase assays, and the VNTR of *DRD4* may associate with a novelty-seeking character in humans. The genomic DNA was extracted from peripheral leukocytes using a standard method. Genotyping of the three single-nucleotide polymorphisms and the Ins/Del polymorphism were performed by the PCR–RFLP method as previously reported (Arinami et al., 1994; Mant et al., 1994; Okuyama et al., 2000) with minor modification. The VNTR of the *DRD4* gene was examined by the length polymorphism method of Van Tol et al. (1992) using an SQ5500 DNA sequencer (Hitachi Co., Japan) and Fragyls 2 (Hitachi Co.) computer software. All genotyping was performed in a blinded fashion, with the control and case samples mixed randomly.

2.3. Statistical analysis

Statistical analysis of association was performed using SNPAlyze software (Dynacom Co., Japan). Deviation from Hardy–Weinberg equilibrium and case-control studies were tested using the χ^2 test or likelihood ratio test. Repeat number polymorphisms of the VNTR of the *DRD4* gene were analyzed by a MonteCarlo simulation as implemented in the CLUMP program (Sham and Curtis, 1995).

3. Results

The genotypic distribution of any polymorphism of the genes of the controls and patients examined in the present study did not deviate from Hardy–Weinberg equilibrium. No significant difference in allelic or genotypic distribution at any polymorphism of the *DRD2*, *DRD3*, or *DRD4* gene between the controls and patients with

Table 1
Association of *DRD2*, *DRD3* and *DRD4* with methamphetamine dependence.

Locus	Genotype			p	Allele		p
DRD2 Taq IA	A1/A1	A1/A2	A2/A2		A1	A2	
Control	15.4%	46.7%	37.9%		38.8%	61.2%	
Patient	17.3%	41.6%	41.1%	0.57	38.1%	61.9%	0.89
DRD2 −141 Ins/Del	Ins/Ins	Ins/Del	Del/Del		Ins	Del	
Control	74.4%	24.7%	0.9%		86.7%	13.3%	
Patient	70.8%	25.1%	4.1%	0.11	83.3%	16.7%	0.20
DRD2 Ser311Cys	Ser/Ser	Ser/Cys	Cys/Cys		Ser	CYs	
Control	95.2%	4.8%	0.0%		97.6%	2.4%	
Patient	91.7%	8.3%	0.0%	0.17	95.9%	4.1%	0.55
DRD3 Ser9Gly	Ser/Ser	Ser/Gly	Gly/Gly		Ser	Gly	
Control	51.9%	39.0%	9.0%		71.4%	28.6%	
Patient	49.7%	43.1%	7.8%	0.79	71.2%	28.8%	0.93
DRD4 −521C>T	CC	CT	TT		C	T	
Control	17.6%	55.3%	27.0%		45.3%	54.7%	
Patient	17.6%	52.3%	30.1%	0.82	43.8%	56.2%	0.70

Table 2
VNTR of *DRD4* and methamphetamine dependence.

Group	Repeat numbers						p (by CLUMP)
	2	3	4	5	6	7	
Control	8.8%	0.7%	85.0%	3.8%	0.7%	0.7%	-
Patient	12.7%	0.3%	81.3%	3.0%	1.3%	0.3%	0.35

methamphetamine dependence was found (Tables 1 and 2). Then, the patient group was divided into three clinical phenotypes of methamphetamine psychosis: latency to onset of methamphetamine psychosis after initial methamphetamine consumption, duration of the psychotic state after therapy, and complication of spontaneous psychosis. The genotypic but not allelic distribution of the *TaqI*A polymorphism was different between the subgroups of duration of psychosis after therapy ($p=0.09$) and complication of spontaneous relapse ($p=0.025$, Table 3). The group of patients with the prolonged type of methamphetamine psychosis that continued more than 1 month even after discontinuance of methamphetamine abuse and beginning of neuroleptic therapy showed fewer A1/A1 homozygotes of *TaqI*A polymorphism of *DRD2* than the transient type with better prognosis whose psychosis improved within 1 month after therapy ($p=0.04$, A1/A1 vs. A1/A2 + A2/A2). The odds ratio of the A1/A1 homozygote of *DRD2* for poorer prognosis of methamphetamine psychosis was 0.42 (95% CI: 0.27–0.65). The group of patients complicated by spontaneous relapse of psychotic state after complete remission of methamphetamine psychosis showed fewer A1/A1 homozygotes of *DRD2* than those without the complication of spontaneous relapse ($p=0.014$). The odds ratio of the A1/A1 homozygote for risk of spontaneous relapse was 0.34 (95% CI: 0.22–0.54). The A1/A1 homozygote of *DRD2* was not associated with latency to onset of psychosis after the initial abuse of methamphetamine. Another polymorphism of *DRD2*, −141C Ins/Del polymorphism showed a significant association with the clinical phenotype of rapid onset of methamphetamine psychosis, but not poorer prognosis or complication of spontaneous relapse. Patients who developed methamphetamine psychosis within 3 years after the initial abuse of methamphetamine showed the −141C Del allele more frequently than those who developed psychosis after 3 years or more after initiation of abuse ($p=0.011$). A Del positive genotype, composed of Del/Del and Ins/Del, was a significant risk for rapid onset of methamphetamine psychosis when compared the Ins/Ins genotype ($p=0.00037$), and the odds ratio was 3.62 (95% CI: 2.48–5.28). The minor allele frequency of Ser311Cys of *DRD2* was relatively low at about 3%, and this polymorphism was not associated with clinical phenotypes of methamphetamine psychosis. Ser9Gly of *DRD3* and the two polymorphisms of *DRD4*, −521 C>T, and VNTR in exon 3 were not associated with the clinical phenotypes of methamphetamine psychosis either (Ser9Gly of *DRD3*; latency to onset of psychosis: allele distribution $p=0.23$, duration of psychosis: $p=0.66$, spontaneous relapse: $p=0.20$, −521C>T of *DRD4*; latency to onset of psychosis: allele distribution $p=0.35$, duration of psychosis: $p=0.28$, spontaneous relapse: $p=0.96$, VNTR in exon 3 of *DRD4*; latency to onset of psychosis: allele distribution $p=0.86$ by CLUMP, duration of psychosis: $p=0.87$, spontaneous relapse: $p=0.96$).

4. Discussion

We analyzed 6 polymorphisms of three D2-like dopamine receptor genes, *DRD2*, *DRD3*, and *DRD4*, and found that these loci were not associated with methamphetamine-seeking behaviors, but that two polymorphisms of *DRD2*, *TaqI*A and −141C Ins/Del polymorphisms, affect several clinical phenotypes of methamphetamine psychosis. The A1/A1 homozygote of the *TaqI*A had a significant negative risk for prolonged psychosis after therapy and complication of spontaneous relapse after remission, and reduced these risks to less than half and 1/3, respectively. On the contrary, having the −141C Del positive genotypes, Del/Del and Del/Ins, was a quite potent risk for rapid onset of methamphetamine psychosis within 3 years after beginning of abuse, and raised the risk to more than 3-fold. These two *DRD2* polymorphisms may be physiologically functional. Postmortem studies revealed that individuals with the A1 allele of *TaqI*A polymorphism of *DRD2* showed reductions of D2 dopamine receptor binding in the striatum by 30–40% (Noble et al., 1991; Ritchie and Noble, 2003; Thompson et al., 1997). *In vivo* PET studies with [^{11}C]raclopride consistently showed a statistically significant reduction in D2 receptor availability, reflecting a decrease in receptor density in the A1/A2 genotype group compared to the A2/A2 group (Jonsson et al., 1999; Pohjalainen et al., 1998). Therefore, the presence of the A1 allele of *TaqI*A polymorphism reduces D2 receptor density in the brain. *In vitro* luciferase assays indicated that the −141C Del/Ins polymorphism, which is located in the promoter region of *DRD2*, affects transcription of the gene. Thus, reporter constructs containing the −141C Del allele cloned into a luciferase reporter plasmid drove 21% (Y-79 cells) and 43% (293 cells) expression compared with the −141C Ins allele (Arinami et al., 1997). However, *in vivo* human studies indicated the opposite effects of the −141C Ins/Del polymorphism on the D2 dopamine receptor expression. A PET study showed that the presence of the −141C Del allele was associated with a higher striatal D2 dopamine receptor density in healthy human subjects (Jonsson et al., 1999). Another clinical study examined serum prolactin response to treatment with perphenazine, a D2 receptor antagonist, and evaluated the occupancy of D2 receptors by the neuroleptic in the brain (Aklillu et al., 2007). Prolactin response was higher in subjects with the −141C Ins/Ins genotype than the −141C Ins/Del genotype, which implies that the same dose of perphenazine occupied the D2 receptors of subjects

Table 3
Clinical phenotypes of methamphetamine psychosis and *DRD2* polymorphisms.

Locus	Genotype			p	Allele		p
DRD2 Taq IA	A1/A1	A1/A2	A2/A2		A1	A2	
Latency to onset of psychosis, <3 years	14.7%	47.4%	37.9%	0.41	38.4%	61.6%	1.0
Latency to onset of psychosis, ≥3 years	19.5%	37.7%	42.9%		38.3%	61.7%	
Duration of psychosis, ≤1 month	21.7%	37.7%	40.6%	0.09	40.6%	59.4%	0.33
Duration of psychosis, >1 month	10.4%	49.4%	40.3%	(0.04*)	35.1%	64.9%	
Spontaneous relapse: no	22.7%	35.5%	41.8%	0.025	40.4%	59.6%	0.19
Spontaneous relapse: yes	8.9%	49.4%	41.8%	(0.014#)	33.5%	66.5%	
DRD2 −141 Ins/Del	Ins/Ins	Ins/Del	Del/Del		Ins	Del	
Latency to onset of psychosis, <3 years	59.6%	36.2%	4.3%	0.0011	77.7%	22.3%	0.0035
Latency to onset of psychosis, ≥3 years	84.2%	11.9%	3.9%	(0.00037$)	90.1%	9.9%	
Duration of psychosis, ≤1 month	68.0%	28.1%	3.9%	0.69	82.0%	18.0%	0.57
Duration of psychosis, >1 month	73.4%	22.4%	4.0%		84.9%	15.1%	
Spontaneous relapse: no	70.0%	24.5%	5.5%	0.30	82.3%	17.7%	0.48
Spontaneous relapse: yes	71.4%	27.3%	1.3%		85.1%	14.9%	

*A1/A1 vs. A1/A2 + A2/A2.
$Ins/Ins + Ins/Del vs. Del/Del.

with the −141C Ins/Ins genotype more efficiently than those with the −141C Ins/Del genotype. Therefore, it is likely that the density of D2 dopamine receptors in the brain was higher in the −141C Ins/Ins genotype than the −141C Ins/Del genotype. This deduction was supported by the same study, which showed that prolactin response to perphenazine was higher in the A1/A1 of *TaqIA* genotype than the A1 negative genotype. Accordingly, the A1 allele of *TaqIA* and the −141C Ins allele decreased the D2 receptor density, and the A2 allele of *TaqIA* and the −141C Del allele increased D2 receptor density, at least in the human brain. Therefore, individuals with a genetically lower density of D2 dopamine receptors due to possession of the *TaqIA* A1/A1 homozygote may have a lower risk for the prolonged type of methamphetamine psychosis and spontaneous relapse, and those with higher density of D2 dopamine receptors due to possession of the −141C Del positive genotype may have a risk for rapid onset of psychosis after methamphetamine abuse. The present results are consistent with the classical D2 receptor hypothesis for mechanisms of psychosis. We previously reported that *TaqIA* polymorphism of *DRD2* showed a tendency to associate with temporal lobe size of methamphetamine abusers (Harano et al., 2004). Recently, Han et al. (2008) reported that A1 allele of *TaqIA* showed a greater score of novelty-seeking character and lower frontal executive function measured by Wisconsin Card Sorting Test in patients with methamphetamine dependence. These findings may indicate that *DRD2* polymorphisms affect not only D2 receptor density but also temporal lobe development, character and higher brain functions, those may result in susceptibility to methamphetamine taking behaviors and prognosis of methamphetamine psychosis.

An allele of 311Cys in *DRD2* was found to be a risk factor for schizophrenia for the first time in 1994 by Arinami et al. (Arinami et al., 1994), and this was confirmed by a meta-analysis in huge samples more than 9000 subjects (Glatt and Jonsson, 2006). Methamphetamine psychosis has been considered as a pharmacological model of endogenous psychosis of schizophrenia because the clinical symptoms and courses are quite similar (Ujike, 2002). It is possible that the disorders may share genetic risk factors. However, the present study showed that a Ser311Cys polymorphism was not associated with any clinical phenotype of methamphetamine psychosis. This may be the result of the lower power of the present study because the allele frequency of 311Cys was as low as about 4%, indicating a necessity of a larger sample to detect the genetic effects on methamphetamine psychosis, if they are relatively small. The *DRD3* gene was also found to confer susceptibility to schizophrenic psychosis by several meta-analyses, and the homozygosity of Ser9Gly polymorphism was determined to be a risk for schizophrenia (Jonsson et al., 2003; Spurlock et al., 1998). However, our data did not provide further evidence for the roles of *DRD3* in the emergence of psychosis because Ser9Gly was not associated with methamphetamine psychosis either. This may have resulted from population differences because meta-analysis of Ser9Gly in a Japanese population failed to show a significant association with schizophrenia (Utsunomiya et al., 2008). Several studies suggested that *DRD4* may be related to human behavior phenotypes (Benjamin et al., 1996; Ebstein et al., 1996), and the VNTR in exon 3 and −521C>T polymorphism may be associated with novelty seeking (Munafo et al., 2008), although this issue is still inconclusive (Ebstein, 2006; Kluger et al., 2002). A novelty-seeking trait should facilitate drug-seeking and drug-taking behaviors. Some preliminary studies reported a significant association of *DRD4* with heroin dependence and heavy drinking (Kotler et al., 1997; Laucht et al., 2007; Li et al., 1997), but they were followed by inconsistent results (Franke et al., 2000; Li et al., 2000). Mice lacking the *DRD4* gene showed supersensitivity to ethanol, cocaine, and methamphetamine (Rubinstein et al., 1997). However, our data did not find any effect of the polymorphisms on methamphetamine dependence or psychosis. A seven-repeat allele of the VNTR is a possible risk for novelty-seeking behaviors in Caucasians, but the seven-repeat allele is found in less than 1% of Asian populations. A Korean study reported that subjects with a two-repeat allele of the VNTR scored higher on a novelty-seeking scale (Reist et al., 2007). This may imply that the two-repeat allele plays a role in novelty-seeking behaviors in Asian populations like the seven-repeat allele in Caucasians. However, our data did not show an excess of two-repeat alleles of *DRD4* in methamphetamine dependence ($X^2 = 1.43$, df = 1, $p = 0.23$). Our negative results of *DRD3* and *DRD4* in susceptibility to methamphetamine dependence and/or methamphetamine psychosis need to be examined in larger samples and for other types of drug addiction.

Some points should be concerned in the present study. We found several significant association of *DRD2* with clinical phenotypes of methamphetamine psychosis, but and we did not apply multiple test correction. Possibility of the type 1 error should be considered. When recruiting controls and patients, we did not apply structured interviews, which may not exclude completely diverse psychiatric disorders and influence the results of association analyses.

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G72 gene is associated with susceptibility to methamphetamine psychosis

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ABSTRACT

Methamphetamine psychosis is considered as one of the pharmacological models of schizophrenia, and a hyperdopaminergic one. However, many lines of experimental evidence indicate that glutamatergic signaling is also involved in development of methamphetamine psychosis. Several genes related to glutamate function, e.g. the *DTNBP1*, *G72*, and *GRM3* genes, were shown to be associated with schizophrenia susceptibility. Recently, we found significant association of the *DTNBP1* gene with methamphetamine psychosis. This finding prompted us to examine the *G72* gene encoding the d-amino acid oxidase activator (DAOA), which metabolizes d-serine, an NMDA co-agonist, in methamphetamine psychosis. Six SNPs of the *G72* gene, which previously showed significant association with schizophrenia, were analyzed in 209 patients with methamphetamine psychosis and 291 age- and sex-matched normal controls. One SNP of M22 (rs778293) showed a significant association with methamphetamine psychosis (genotype: $p=0.00016$, allele: $p=0.0015$). Two haplotypes G-A of M12 (rs3916965)-M15 (rs2391191) ($p=0.00024$) and T-T of M23 (rs947267)-M24 (rs1421292) ($p=0.00085$) also showed associations with methamphetamine psychosis. The present findings suggest that the *G72* gene may contribute to a predisposition to not only schizophrenia but also to methamphetamine psychosis.

1. Introduction

Methamphetamine, amphetamine, and cocaine are illicit psychostimulants, and their abuse and dependence frequently cause serious social and health problems around the world. In Japan, methamphetamine is the most popular illicit drug (Ujike and Sato, 2004). Long-term consumption of large quantities of methamphetamine often causes psychotic symptoms such as delusions of persecution, reference, and poisoning, and auditory hallucination that are quite similar to those observed in paranoid-type schizophrenia (Ujike and Sato, 2004). Therefore, methamphetamine psychosis is considered a pharmacological model of schizophrenia, a hyperdopaminergic model because methamphetamine is an indirect dopamine agonist. However, many lines of evidence indicated that glutamatergic signaling also plays a crucial role in development of methamphetamine psychosis (Ujike, 2002). For example, amphetamine administration enhanced glutamate release in the accumbens (Reid et al., 1997); MK-801, an N-methyl-d-aspartate (NMDA) receptor antagonist, blocked sensitization to methamphetamine in an animal model of methamphetamine psychosis (Ohmori et al., 1994), and mice deficient in the NR1 subunit of NMDA receptors showed enhanced sensitivity to amphetamine-induced disruption of prepulse inhibition (Moy et al., 2006).

Genetic association studies have identified many schizophrenia susceptibility genes, some of which are implicated in the function of

Abbreviations: DTNBP1, dystrobrevin-binding protein 1; GRM3, metabotropic glutamate receptor 3; DAOA, d-amino acid oxidase activator; NMDA, N-methyl-D-aspartic acid; SNP, single nucleotide polymorphism; DAAO, D-amino acid oxidase; JGIDA, the Japanese Genetics Initiative for Drug Abuse; ICD-10, International Classification of Diseases-10; PCR, polymerase chain reaction; LD, linkage disequilibrium.

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glutamate and NMDA receptors and consequently support a glutamate dysfunction hypothesis of schizophrenia (Coyle, 2004). Among them, the *DTNBP1* gene encoding dysbindin, which is involved in the glutamate release mechanism (Numakawa et al., 2004), is considered a very reliable genetic risk for schizophrenia because the significant association of *DTNBP1* with schizophrenia was repeatedly replicated in diverse populations (Williams et al., 2005). Raybould et al. (2005) revealed that the *DTNBP1* gene was not associated with bipolar disorder when all cases were analyzed, but it was associated with a subpopulation of patients with bipolar disorder when classified by the presence of mood-incongruent psychotic features. These reports may indicate that the *DTNBP1* gene is likely to be implicated in susceptibility to complications of psychotic symptoms such as hallucination and delusion across psychiatric disorders rather than a specific predisposition to schizophrenia. To test this possibility, we recently examined the *DTNBP1* gene in another psychotic condition, methamphetamine psychosis, and found a strong association with methamphetamine psychosis (Kishimoto et al., 2007). Strikingly, three different type of psychotic disorders, schizophrenia, psychotic bipolar disorder, and methamphetamine psychosis shared a common protective haplotype (Kishimoto et al., 2007; Raybould et al., 2005; Williams et al., 2004). Therefore, it is possible that genes related to glutamatergic neurotransmission play a role not only in schizophrenia but also in other types of psychosis including methamphetamine psychosis.

The *G72* gene, which locates on chromosome 13q, is known as a susceptibility locus for schizophrenia (Badner and Gershon, 2002), and many studies have demonstrated significant associations with schizophrenia (Chumakov et al., 2002; Addington et al., 2004; Korostishevsky et al., 2004; Schumacher et al., 2004; Wang et al., 2004; Zou et al., 2005; Fallin et al., 2005; Ide et al., 2006; Yue et al., 2007). A yeast two-hybrid method detected G72 protein binding to D-amino acid oxidase (DAAO) (Chumakov et al., 2002), and G72 protein may act as a DAAO activator. Therefore, genetic variants of the *G72* gene may affect DAAO activity and metabolism of D-serine, one of the D-amino acids and a co-agonist of NMDA receptors, resulting in aberrant concentrations of D-serine, NMDA dysfunction, and susceptibility to schizophrenia. This hypothesis was supported by recent studies demonstrating that D-amino acid oxidase activity was increased in the cerebral cortex of schizophrenic patients (Madeira et al., 2008). Recently, Kvajo et al. (2008) failed to demonstrate functional interaction between G72 and DAAO, but they found implication of G72 in the regulation of mitochondrial function and dendritic arborization, those aberrance might be involved in pathogenesis of schizophrenia. Finally, genetic variations in the *G72* gene was associated with impairments in cognitive function such as attention and memory, and affect hippocampal and prefrontal cortex activation during a cognitive task in schizophrenic patients and high risk subjects for schizophrenia (Golberg et al., 2006; Hall et al., 2008). Based on these rationales, we examined a possible association between the *G72* gene and methamphetamine psychosis in a case-control genetic association study to test the hypothesis that glutamate-related genes may be involved in methamphetamine psychosis as well as schizophrenia.

2. Materials and methods

2.1. Subjects

The subjects comprised 209 patients with methamphetamine psychosis (171 male and 38 female; mean age ± SD, 37.4 ± 12.0) and 291 age-, sex-, and geographical origin-matched healthy controls (227 male and 64 female; mean age ± SD, 37.2 ± 13.1). All the subjects were unrelated Japanese, born and living in relatively restricted areas of Japan. All patients were out- or inpatients in psychiatric hospitals of the Japanese Genetics Initiative for Drug Abuse (JGIDA). Consensus diagnoses of the patients were made by two trained psychiatrists according to ICD-10 criteria (F15.5 or F15.7) on the basis of unstructured interviews and medical records. More detailed clinical characterizations of these patients were published elsewhere (Ujike et al., 2009). The controls had no individual or family history of drug dependence or major psychotic disorders such as schizophrenia or bipolar disorder. IQ or social class of the subjects was not examined. This study was approved by the ethics committee of each JGIDA institution. After a complete description of the study to the subjects, written informed consent was obtained.

2.2. Genotyping

Peripheral blood was obtained from the subjects, and genomic DNA was extracted from peripheral leukocytes by a standard method. Six single nucleotide polymorphisms (SNPs), (M12 (rs3916965), M15 (rs2391191), M18 (rs947267), M22 (rs778293), M23 (rs3918342), and M24 (rs1421292) that are located on the *G72* gene and flanking regions were genotyped using TaqMan®-based techniques (Applied Biosystems 7300/7500 Real Time PCR System). These SNPs were selected because they were shown to be significantly associated with schizophrenia by previous studies (Korostishevsky et al., 2004; Yue et al., 2007; Chumakov et al., 2002; Wang et al., 2004; Ma et al., 2006). The polymerase chain reaction was carried out in a total volume of 7 μl containing 3.2 μl TaqMan® 2× Universal PCR Master Mix, 0.32 μl

Table 1
Genotype and allele frequencies of six single nucleotide polymorphisms of *G72* gene in patients with methamphetamine psychosis and controls.

	N	Genotype (%)			*p*	Allele (%)		*p*
M12 (rs3916965)		A/A	A/G	G/G		A	G	
Controls	278	144(51.8)	115(41.4)	19(6.8)		403(72.5)	153(27.5)	
Patients	209	117(56.0)	75(35.9)	17(8.1)	0.45	309(73.9)	109(26.1)	0.62
M15 (rs2391191)		A/A	A/G	G/G		A	G	
Controls	279	143(51.3)	115(41.2)	21(7.5)		401(71.9)	157(28.1)	
Patients	205	111(54.1)	74(36.1)	20(9.8)	0.43	296(72.2)	114(27.8)	0.91
M18 (rs947267)		A/A	A/C	C/C		A	C	
Controls	288	122(42.4)	133(46.2)	33(11.4)		377(65.5)	199(34.5)	
Patients	206	99(48.1)	85(41.2)	22(10.7)	0.45	283(68.7)	129(31.3)	0.29
M22 (rs778293)		A/A	A/G	G/G		A	G	
Controls	287	179(62.4)	102(35.5)	6(2.1)		460(80.1)	114(19.9)	
Patients	203	109(53.7)	72(35.5)	22(10.8)	0.00016	290(71.4)	116(28.6)	0.0015
M23 (rs3918342)		T/T	T/C	C/C		T	C	
Controls	291	72(24.7)	165(56.7)	54(18.6)		309(53.1)	273(46.9)	
Patients	208	62(29.8)	98(47.1)	48(23.1)	0.11	222(53.4)	194(46.6)	0.93
M24 (rs1421292)		A/A	A/T	T/T		A	T	
Controls	282	76(27.0)	145(51.4)	61(21.6)		297(52.7)	267(47.3)	
Patients	205	51(24.9)	90(43.9)	64(31.2)	0.054	192(46.8)	218(53.2)	0.072

TaqMan® Genotyping Assays, and 20 ng of DNA. The amplification protocol was denaturation at 95 °C for 10 min, followed by 40 cycles of denaturation at 92 °C for 15 s and annealing and extension at 60 °C for 1 min.

2.3. Statistical analysis

Deviation from Hardy–Weinberg equilibrium and case-control association were tested by χ^2 test using SNPAlyze software (Dynacom Co., Japan). Linkage disequilibrium (LD) was tested using the χ^2 test, and D' and r^2 values were made the index in the authorization of LD. Case-control haplotype analysis was performed by the permutation method, and permutation p values were calculated based on 100,000 replications. The differences between groups were evaluated by Fisher's exact test. Differences were considered significant at $p<0.05$.

3. Results

Table 1 shows the genotype distributions and allele frequencies of the six SNPs of the patients with methamphetamine psychosis and control subjects. The genotype distributions of the patients and control subjects did not deviate from Hardy–Weinberg equilibrium at any of the six SNPs (M12, $\chi^2=0.01$, $p=0.92$; M15, $\chi^2=0.068$, $p=0.79$; M18, $\chi^2=0.054$, $p=0.82$; M22, $\chi^2=0.006$, $p=0.94$; M23, $\chi^2=3.64$, $p=0.056$; M24, $\chi^2=0.64$, $p=0.42$). In the single marker analysis, M22 (rs778293) of the *G72* gene showed significant differences between patients and control subjects in the frequency distribution of the genotype ($\chi^2=17.4$, $df=2$, $p=0.00016$) and allele ($\chi^2=10.0$, $df=1$, $p=0.0015$). The patient group showed significantly higher frequency of the G allele of M22, a minor one, than the control group. After application of Bonferroni correction to exclude type I errors, the differences in the frequency of genotype and allele of M22 remained significant. None of the other SNPs, M12 (rs3916965), M15 (rs2391191), M18 (rs947267), M23 (rs3918342), or M24 (rs1421292), showed significant differences in the frequency of genotype or allele between the patient and control groups.

Table 2 shows the results of the pairwise linkage disequilibrium (LD) among the six SNPs of the *G72* gene using the D' and r^2 values as an index. LD analyses indicated two LD blocks, M12-M15 and M23-M24 ($D'>0.7$, $r^2>0.3$). Our LD structure result for the *G72* gene was consistent with that of the European population reported by Detera-Wadleigh et al. (2006), who showed that M12, M13, M14, and M15 locate on one LD block and M22, M23, and M24 locate on another LD block. Then, we analyzed two marker haplotypes in the two LD blocks (Table 3). The haplotypes of M12-M15 and M23-M24 had significant global permutation p values ($p=0.0003$, and 0.010, respectively). The frequency of the G-A haplotype of M12-M15 was significantly lower in patients than in control subjects ($p=0.00024$). On the other hand, the haplotype frequency of T-T of M23-M24 was significantly higher in patients than in control subjects ($p=0.00085$). The A-G haplotype of M12-M15 showed a marginally significant difference between the patients and control, but it was not significant after Bonferroni correction.

Table 2
Pairwise linkage disequilibrium between single nucleotide polymorphisms of *G72* gene.

	M12	M15	M18	M22	M23	M24
M12		**0.712**	0.260	0.0632	0.0589	0.0718
M15	**0.864**		0.282	0.0727	0.0391	0.0588
M18	0.595	0.599		0.0409	0.000102	0.00226
M22	0.275	0.305	0.257		0.0866	0.268
M23	0.374	0.296	0.0134	0.503		**0.561**
M24	0.444	0.387	0.0677	**0.940**	**0.795**	

Upper right and lower left diagonals show r^2 and D' values, respectively. $r^2>0.3$ and $D'>0.7$ were shown in bold.

Table 3
Haplotype frequencies of *G72* gene of control subjects and methamphetamine psychosis.

	Controls (%)	Patients (%)	Permutation p
M12-M15			
A-A	0.674	0.720	0.137
G-G	0.233	0.256	0.408
A-G	0.0475	0.0220	0.0449
G-A	0.0456	0.00249	0.000240
M23-M24			
T-A	0.476	0.422	0.0936
C-T	0.421	0.422	0.978
T-T	0.0526	0.113	0.000850
C-A	0.0508	0.0439	0.659

4. Discussion

We found a significant association between the *G72* gene and methamphetamine psychosis by a single marker and haplotype-based case-control analyses. The G allele of M22, which is located in the 3' flanking region of the *G72* gene, was a risk factor for methamphetamine psychosis ($p=0.0015$). The odds ratio was 1.61 (95% CI: 1.39–1.88). In the previous schizophrenic studies, Ma et al. (2006) reported that the G allele of M22 was a risk factor for schizophrenia in Chinese ($p=0.0013$) and Scottish $p=0.022$) populations, and in the subsequent meta-analysis also showed that having the G allele of M22 was a risk for schizophrenia in an Asian population (Shi et al., 2008). However, Chumakov et al. (2002) and Korostishevsky et al. (2004) reported that the A allele of M22 was a risk factor in Canadian and Ashkenazi schizophrenics, respectively. In the haplotype analyses, the G-A haplotype of M12-M15 was significantly more frequent in control subjects than patients with methamphetamine psychosis, indicating a possible protective haplotype. In contrast, the T-T haplotype of M23-M24 was significantly more frequent in patients than controls, indicating a risk for development of methamphetamine psychosis. In previous schizophrenic studies (Chumakov et al., 2002), many significant two-, three-, or four-marker haplotypes of the *G72* gene were identified as follows: C-A-T haplotype of M21-M22-M23 in Ashkenazi schizophrenics (Korostishevsky et al., 2004), A-T haplotype of M22-M23 and G-C haplotype of M19-M22 in Anhui and Scottish schizophrenics (Ma et al., 2006), A-G-A-A and A-G-A-C of M12-M14-M15-rs1935062 in Han Chinese schizophrenics (Wang et al., 2004), A-G-A of M12-M14-M15 in Han Chinese schizophrenics (Wang et al., 2004), and A-A of M15-M18, A-G of M18-M19, and A-A-G of M15-M18-M19 in Han Chinese schizophrenics (Yue et al., 2007). However, there was no identical risk or protective haplotype of schizophrenia in our findings for methamphetamine psychosis. This may result from, at least in part, a difference between examined SNPs of the *G72* gene in the studies, but these findings may indicate that the *G72* gene is involved in susceptibility to two different types of psychosis, endogenous psychosis and substance-induced psychosis, but that significantly associated alleles or haplotypes were different between the disorders or among populations except for the G allele of M22, which was a risk factor for schizophrenia and methamphetamine psychosis in Asian populations. The hypothesis that the *G72* gene may be involved in liability to complicate psychotic symptoms may be supported by the findings of Schulze et al. (2005), who examined the *G30/G72* locus in Polish patients with bipolar disorders and found that M23 was associated with a subgroup of bipolar disorders with persecutory delusion but not with a subgroup without delusion. Unfortunately, it is unknown whether M22 is associated with delusional bipolar disorders because they did not examine it, and a subsequent study failed to replicate the Schulze study (Williams et al., 2006). However, all findings of the present and previous studies indicate that the *G72* gene

may be involved in liability to complications of delusional or psychotic symptoms in several types of psychotic disorders: psychotic bipolar disorders, methamphetamine psychosis, and schizophrenia. To confirm the possibility of involvement of the *G72* gene in liability to psychotic symptoms across psychiatric disorders, it may be useful to investigate the *G72* gene in other types of psychosis such as delusional depression or cocaine paranoia.

Although our sample size, 209 patients and 291 controls, was relatively large and our results remained significant after Bonferroni correction, we should consider the possibility of a chance finding resulting from reduced power due to small sample size. Statistical power analysis showed that our sample size had power of 0.999 to detect an effect size ($w = 0.218$) for an SNP (M22), with a significance level of 0.05 to detect significant associations in allelic analysis between control subjects and patients with methamphetamine psychosis (Faul et al., 2007). Therefore, our sample size was statistically large enough, although haplotype analyses power may be reduced because of an increase in the degree of freedom.

5. Conclusion

The present findings suggest that the *G72* gene may contribute to a predisposition to not only schizophrenia but also to methamphetamine psychosis.

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