have indicated that brain CB1 receptors mediate the behavioral and neurochemical properties of cannabis, e.g., marijuana, including the rewarding effect, tolerance, and physical dependence [9,24,30]. CB1 receptors and endogenous agonists are involved not only in cannabinoid dependence but also dependence on other classes of drugs, such as alcohol, morphine, and cocaine [11,22,23,38]. Interaction between cannabinoids and amphetamines, including methamphetamine, has also been reported. Thus, the cannabinoid receptor antagonist AM251 inhibited methamphetamine self-administration by reduction of methamphetamine withdrawal [37]. Another antagonist, SR141716A, potentiated the stimulating effects of amphetamine [18]. The CB1 agonist WIN 55, 212-2 or delta 9-tetrahydrocannabinol, a main ingredient of cannabis, induced cross-sensitization to amphetamine [14,21]. These findings implicate the endocannabinoid system as one of the most important signaling pathways in drug abuse and dependence.

In the 1980s, fatty acid amide hydrolase (FAAH), the integral membrane cannabinoid enzyme, was identified [27]. FAAH is widely expressed in neuronal cells in the CNS, predominantly in the neocortex, hippocampal formation, amygdala, and cerebellum [10,31]. FAAH serves as a primary catabolic regulator of the endogenous cannabinoid ligand anandamide and related fatty acid amide-signaling molecules [15,16]. It has been reported that the metabolic activity of FAAH plays important roles in the CNS by ensuring rapid termination of specific signaling processes of the cannabinoid system [3–5]. Mice lacking FAAH (FAAH −/− mice) are severely impaired in their ability to degrade anandamide. As a consequence, the brain level of anandamide in FAAH −/− mice is increased 15-fold, and they exhibit CB1 receptor-dependent behavioral responses, including less sensitivity to several pain stimuli, hypomotility, hypothermia, analgesia, and catalepsy [2]. Mutation of the FAAH gene may induce dysregulation of the endogenous cannabinoid system, and result in alternation in brain addiction/reward pathways. Recently, Sipe et al. [28] reported that the presence of a polymorphism of the FAAH gene that converts a conserved proline residue to threonine at the 129 position (Pro129Thr) is a risk factor for problem substance abuse and dependence in a Caucasian population. We tried to determine if a genetic association of the Pro129Thr nonsynonymous polymorphism of the FAAH gene in patients with methamphetamine dependence in a Japanese population.

Furthermore, acute and chronic cannabis exposure can precipitate a psychotic state, with hallucinations and delusions resembling schizophrenia. Cannabis consumption also worsens positive symptoms of schizophrenia, and could result in a poor outcome and liability to relapse [12,13,20,29,32]. Two cohort studies showed cannabis use in adolescence increased greatly the risk of schizophrenia in adulthood [1,39]. In addition, recent studies showed an increased density of CB1 receptors in the prefrontal cortex and an increased level of anandamide in the CSF in schizophrenia [6,17,40]. These findings led to a cannabinoid hypothesis of schizophrenia [34]. Therefore, we examined a possible association between Pro129Thr polymorphism of the FAAH gene and schizophrenia.

The subjects were 153 patients with methamphetamine dependence (124 males and 29 females; mean age, 37.8 years; S.D. 12.1 years) meeting the ICD-10-DCR criteria (F15.2), who were outpatients or inpatients in psychiatric hospitals of the Japanese Genetics Initiative for Drug Abuse (JGIDA), and 200 age-, gender-, and geographical origin-matched normal controls (designated Control-1, 162 males and 38 females; mean age, 37.3 years; S.D. 12.1 years), who were mostly medical staff members without a past individual or family history of drug dependence or psychotic disorders. One hundred and forty-one of patients also suffered from methamphetamine psychosis (F15.5, 118 males and 23 females; mean age, 38.5 years; S.D. 12.1 years). The patients with methamphetamine dependence and/or psychosis were divided into several subgroups by clinical features according to age at first consumption, latency of psychosis, prognosis, and multi-substance abuse status. Seventy-six patients (49.7%) had consumed methamphetamine before the age 20 years, and 77 patients (50.3%) had first consumed methamphetamine after they were 20 years old. The latency of psychosis was less than 3 years after the first methamphetamine consumption in 60 patients (42.6%) and 3 or more years in 81 patients (57.4%). Forty-eight patients (31.4%) had abused only methamphetamine during their lifetime, and 105 patients (68.6%) had abused drugs other than methamphetamine in the past or present. Besides methamphetamine, organic solvents were the most frequently abused drugs, followed by marijuana. Cocaine and heroin were rarely abused. The prognosis of methamphetamine psychosis varied among patients, and some patients showed continuous psychotic symptoms even after methamphetamine discontinuance, as previously reported [25,26]. Therefore, patients were divided into two categories of psychosis, the transient type and the prolonged type, based on the duration of the psychotic state after methamphetamine discontinuance, as described in our previous study [36]. Patients with the transient type showed remission of psychotic symptoms within one month after the discontinuance of methamphetamine consumption and beginning of treatment with neuroleptics, and those with the prolonged type had psychosis that continued for more than one month even after this discontinuance of methamphetamine consumption and beginning of neuroleptic treatment. In this study, 85 patients (60.3%) were the transient type and 56 patients (39.7%) were the prolonged type. It has been well-documented that once methamphetamine psychosis has developed, patients in the remission state become liable to spontaneous relapse without reconsumption of methamphetamine [25,26,35]. Such enhanced liability to relapse may result from a sensitization phenomenon developed during methamphetamine abuse, and may be affected by genetic traits [33]. Therefore, the patients were divided into two groups according to the presence or absence of spontaneous relapse. The number of patients with a history of

spontaneous relapse was 65 (46.1%) and those without were 76 (53.9%).

To determine the relationship between the Pro129Th polymorphism and schizophrenia, we examined 260 patients (151 males and 109 females; mean age, 44.5 years; S.D. 13.1 years) fulfilling the ICD-10 diagnostic criteria for schizophrenia. Assessment for diagnosis and subtype of schizophrenia was performed by trained psychiatrists on the basis of all available information, including hospital notes. One hundred and twenty-seven patients (68 males and 59 females; mean age, 39.1 years; S.D. 13.1 years) were diagnosed with the paranoid type, 127 (82 males and 45 females; mean age, 39.6 years; S.D. 13.9 years) with the hebephrenic type, 2 with the catatonic type, and 4 residual type of schizophrenia. Age-, gender-, and geographical origin-matched control subjects for the schizophrenia patients were recruited (designated Control-2, 194 males and 143 females; mean age, 47.2 years; S.D. 11.8 years). Subjects with a positive personal or familial history of major psychiatric disorders were excluded from the control group. This study was performed after obtaining approval from the ethics committees of each institute of JGIDA, and all subjects provided written informed consent for the use of their DNA samples in this research.

The genomic DNA was extracted from peripheral leukocytes using the standard phenol/$CHCl_3$ method. A Pro129Thr polymorphism of the FAAH gene was amplified by polymerase chain reaction (PCR), with 3% dimethyl sulfoxide and 0.75 units of Taq DNA polymerase in a total volume of 15 μl reaction mixture using the following primer sets: 5′-ATG TTG CTG GTT ACC CCT CTC C-3′ and 5′-TCA CAG GGA CGC CAT AGA GCT G-3′. Initial denaturation was performed for 5 min at 95 °C. Then, 35 cycles were performed (30 s of denaturing at 95 °C, 30 s of annealing at the appropriate temperature, and 30 s of extension at 72 °C), followed by a final extension at 72 °C for 5 min. The PCR products were then digested with EcoO109I and analyzed on 3.0% agarose gels.

Deviation of the genotype counts from Hardy–Weinberg equilibrium was tested using a chi-square goodness-of-fit test. The statistical significance of differences in the genotype distribution and allele frequency between patients and controls was assessed by a chi-square test or Fisher's exact test at a significance level of 0.05. All genotyping was performed in a blinded fashion, with the control and case samples mixed randomly. Allele frequencies were calculated using allele-counting methods.

The genotype distribution and allele frequencies of Pro129Thr polymorphism of the FAAH gene for patients with methamphetamine dependence/psychosis or schizophrenia, Control-1, and Control-2 are shown in Tables 1 and 2. The genotype distribution of all patients and controls subjects did not deviate significantly from Hardy–Weinberg equilibrium at the polymorphic locus. No significant differences were found in the frequency of the genotype or allele of the Pro129Thr polymorphism between patients with methamphetamine dependence and Control-1 (genotype, $\chi^2 = 1.22$, d.f. = 2, $P = 0.57$; allele, $\chi^2 = 0.22$, d.f. = 1, $P = 0.68$). No significant differences were found in the frequency of the genotype or allele between subcategories of methamphetamine-dependent patients whose age at first methamphetamine consumption was less 20 years or more than 20 years (genotype, $\chi^2 = 1.80$, d.f. = 2, $P = 0.45$; allele, $\chi^2 = 0.65$, d.f = 1, $P = 0.45$). Nor was there a significant difference between patients with and without multiple substance abuse (genotype, $\chi^2 = 2.01$, d.f. = 2, $P = 0.49$; allele, $\chi^2 = 0.60$, d.f = 1, $P = 0.52$), or patients whose latency of methamphetamine-induced psychosis was less and more than 3 years (genotype, $\chi^2 = 0.66$, d.f. = 2, $P = 0.75$; allele, $\chi^2 = 0.51$, d.f = 1, $P = 0.52$), or between patients with transient and prolonged psychosis (genotype, $\chi^2 = 3.26$, d.f. = 2, $P = 0.19$; allele, $\chi^2 = 3.08$, d.f = 1, $P = 0.11$), or patients with and without spontaneous relapse of psychotic symptoms (genotype, $\chi^2 = 4.56$, d.f. = 2, $P = 0.16$; allele, $\chi^2 = 4.36$, d.f = 1, $P = 0.06$).

No significant differences were found in the frequency of the genotype or allele of the Pro129Thr polymorphism between schizophrenia patients and Control-2 (genotype, $\chi^2 = 0.18$, d.f. = 2, $P = 0.91$; allele, $\chi^2 = 0.01$, d.f. = 1, $P = 0.94$). With regard to the subcategories of schizophrenia, no significant differences were found in the frequency of the genotype or allele between patients with paranoid type schizophrenia and Control-2 (genotype, $\chi^2 = 0.67$, d.f. = 2, $P = 0.63$; allele, $\chi^2 = 0.02$, d.f = 1, $P = 0.92$), or patients with hebephrenic type schizophrenia and Control-2 (genotype, $\chi^2 = 1.15$, d.f. = 2, $P = 0.61$; allele, $\chi^2 = 0.32$, d.f. = 1, $P = 0.62$).

FAAH is a primary and rapid catabolizer of endocannabinoids, such as anandamide and 2-arachidonoyl glycerol, and FAAH knockout mice show a robust increase in brain anandamide. Therefore, homozygosity of a mutant allele of the gene may induce hyperactivity of cannabinoid signaling in the brain. The present study showed no significant association between methamphetamine dependence/psychosis and the Pro129Thr nonsynonymous polymorphism of the FAAH gene. Sipe et al. [28] reported that the Pro129Thr polymorphism is strongly associated with street-drug use and problem drug/alcohol use, especially with illegal drug use by Caucasians. They found that the odds ratios of the mutant 129Thr/Thr homozygote for risk of problem drug/alcohol use and street-drug use in individuals were 4.5 and 2.2, respectively [28]. The Pro129Thr mutation did not significantly impact the catalytic properties of FAAH, but it was found to produce a significantly greater sensitivity to proteolytic degradation, and may have direct effects on the regulation of the FAAH proteins [28]. These findings suggest that dysfunction of FAAH and enhanced endocannabinoid level due to genetic mutation may constitute a risk factor for problem drug use. However, our data indicated that dysfunction of FAAH did not affect the risk of methamphetamine dependence/psychosis in a Japanese population. We also examined a possible association between clinical features of methamphetamine dependence/psychosis, such as age at first methamphetamine

Table 1
Fatty acid amide hydrolase (FAAH) genotype distributions and allele frequency in patients with methamphetamine (METH) dependence/psychosis

Group	N	Genotype				Allele		
		Pro/Pro (%)	Pro/Thr (%)	Thr/Thr (%)	P-value	Pro (%)	Thr (%)	P-value
Control-1	200	139 (69.5)	58 (29.0)	3 (1.5)		336 (84.0)	64 (16.0)	
METH dependence/psychosis	153	105 (68.6)	43 (28.1)	5 (3.3)	0.57	253 (82.7)	53 (17.3)	0.68
Age of first use								
<20 years	76	49 (64.5)	25 (32.9)	2 (2.6)		123 (81.0)	29 (19.1)	
≥20 years	77	56 (72.7)	18 (23.4)	3 (3.9)	0.45	130 (84.4)	24 (15.6)	0.45
Multi-substance abuse								
No	48	30 (62.5)	17 (35.5)	1 (2.1)		77 (80.2)	19 (19.8)	
Yes	105	75 (71.4)	26 (24.8)	4 (3.8)	0.49	176 (83.8)	34 (16.2)	0.52
Latency of psychosis								
<3 years	60	43 (71.7)	16 (26.7)	1 (1.7)		102 (85.0)	18 (15.0)	
≥3 years	81	54 (66.7)	24 (29.6)	3 (3.7)	0.75	132 (81.5)	30 (18.5)	0.52
Prognosis of psychosis								
Transient	85	53 (62.4)	29 (34.1)	3 (3.5)		135 (79.4)	35 (20.6)	
Prolonged	56	43 (76.8)	12 (21.4)	1 (1.8)	0.19	98 (87.5)	14 (12.5)	0.11
Spontaneous relapse of pyschotic symptoms								
No	76	47 (61.8)	26 (34.2)	3 (3.9)		120 (78.9)	32 (21.1)	
Yes	65	50 (76.9)	14 (21.5)	1 (1.5)	0.16	114 (87.7)	16 (12.3)	0.06

Numbers in parentheses indicate percentages. Statistical analysis was performed by a chi-square test of Fisher's exact test.

Table 2
FAAH genotype distributions and allele frequency in schizophrenia patients

Group	N	Genotype				Allele		
		Pro/Pro (%)	Pro/Thr (%)	Thr/Thr (%)	P-value	Pro (%)	Thr (%)	P-value
Control-2	337	233 (69.1)	99 (29.4)	5 (1.5)		565 (84.0)	109 (16.0)	
Schizophrenia	260	180 (69.2)	75 (28.8)	5 (1.9)	0.91	435 (83.7)	85 (16.3)	0.94
Paranoid type	127	90 (70.9)	34 (26.8)	3 (2.4)	0.63	214 (84.3)	40 (15.7)	0.92
Hebephrenic type	127	83 (65.4)	43 (33.9)	1 (0.0)	0.61	209 (82.3)	45 (17.7)	0.62

Numbers in parentheses indicate percentages. Statistical analysis was performed by a chi-square test of Fisher's exact test.

consumption, latency to onset of psychosis, prognosis, spontaneous relapse, and multi-substance abuse status but found no significant association with any clinical feature. The discrepancy between the previous and present findings may result from differences of substance class. The present study analyzed methamphetamine abusers, however, Spine et al. analyzed "street-drug users". Although, they did not specify the kinds of drugs in their paper, marijuana use has been epidemic in the US, followed by cocaine and morphine. It is possible that the majority of patients examined in the previous study abuse marijuana and that is why the "street-drug use" of the previous study was associated with a mutant allele of the FAAH gene. This hypothesis should be addressed in future study. Alternatively, the ethnicity of the subjects must be considered. Our subjects were Japanese, and theirs were Caucasian. Japanese controls showed the 129Thr allele at 14.6–16.0%, compared to Caucasians at 28.3–30.6%. Homozygosity of the mutant allele was observed in only 1.5% of Japanese subjects. The rarity of the mutant homozygote in our Japanese population may result in a lack of genetic risk of the FAAH gene for Japanese "street-drug users".

No significant association with the Pro129Thr nonsynonymous polymorphism of the FAAH gene with schizophrenia was revealed. This is consistent with Sipe's study of a Caucasian population. Previously, we reported that a triplet repeat polymorphism of the CNR1 gene, which encodes the human CB1 cannabinoid receptor, was significantly associated with patients with schizophrenia, especially the hebephrenic subtype [36]. Several clinical studies have shown that exogenous cannabinoid ligands could precipitate schizophrenia, worsen prognosis, and induce relapse. Our present and previous genetic findings of the endocannabinoid system indicated that variants of cannabinoid receptors, rather than an altered endogenous agonist produced by the FAAH variant, may be important in the etiology of schizophrenia.

The power analysis showed that the present sample size had a power of 0.89 and 0.98 to detect a small effect size (w = 0.12) at an alpha value of 0.05 to detect significant allelic associations between Control-1 and total methamphetamine patients and between Conrol-2 and total schizophrenic patients, respectively. The present total sample size can be therefore considered to be large enough statistically. However, the

statistical power deteriorated in the analysis with regard to the subgroups of patients, and our results must be verified with a larger sample to conclude.

In conclusion, the cannabinoid pathway may be implicated in drug abuse, addiction, and also the pathophysiology of schizophrenia, but the Pro129Thr nonsynonymous polymorphism of the FAAH gene is not significantly associated with either methamphetamine dependence/psychosis or schizophrenia, at least in a Japanese population.

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Association Between the Glutathione S-Transferase M1 Gene Deletion and Female Methamphetamine Abusers

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Several lines of evidence suggest that increased generation of auto-oxidized dopamine (DA) *o*-quinone is associated with the neurotoxicity of methamphetamine (MAP) in the brain, and that, as a cellular defenses against DA-derived quinines, glutathione S-transferase (GST) detoxifies auto-oxidized DA *o*-quinone in the brain. Glutathione S-transferase M1 (GSTM1) of the mu-class of GSTs catalyzes reaction between glutathione and catecholamine *o*-quinones under physiological conditions. This study was undertaken to investigate the role of the *GSTM1* gene deletion polymorphism in the neuropathology of MAP abuse. One hundred fifty-seven MAP abusers and 200 healthy comparison subjects were tested for a genetic polymorphism of GSTM1. The difference in the frequency of deletion (D)/non-deletion (N) alleles between the female abusers and female controls was close to statistical significance ($P = 0.071$), although there was no statistical difference ($P = 0.651$) between male abusers and male controls. Furthermore, the number of female abusers with deletion alleles was significantly ($P = 0.007$, odds ratio: 2.77, 95% CI 1.30–5.89) higher than that of male abusers with deletion alleles. These findings suggest that *GSTM1* gene deletion may contribute to a vulnerability to MAP abuse in female subjects, but not in male subjects.

KEY WORDS: methamphetamine; drug abuse; glutathione S-transferase; gender difference

INTRODUCTION

Abuse of methamphetamine (MAP) is a growing problem worldwide. Some lines of evidence have suggested strong genetic contributions to drug abuse vulnerability [Uhl et al., 2002]. The application of brain imaging techniques to the study of drug abuse have demonstrated that the density of dopamine (DA) transporters is significantly reduced in the caudate/putamen of MAP abusers [Sekine et al., 2001; Volkow et al., 2001], suggesting that long-term use of MAP causes damage to dopaminergic neurons in the human brain. Furthermore, it has been shown that MAP-induced neurotoxicity in the brain has been shown to require striatum DA and to involve mechanisms associated with oxidative stress [Cadet and Brannock, 1998]. It is also known that DA is auto-oxidized and the corresponding DA *o*-quinone (aminochrome) is subse-

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quently generated; moreover, aminochrome and its subsequent product, DA *o*-semiquinone, elicit redox cycling which leads to the generation of reactive oxygen species, which in turn degenerate dopaminergic neurons [Graham et al., 1978; Smythies and Galzigna, 1998]. DA oxidation also results in the formation of DA *o*-quinone, which readily participates in nucleophilic addition reactions with sufhydryl groups on free cysteine, glutathione, or cysteine found in protein including DA transporter [Graham et al., 1978; Hastings and Zigmond, 1994; Smythies and Galzigna, 1998; Whitehead et al., 2001]. In addition, it has been reported recently that DA auto-oxidation contributes to MAP-induced neurotoxicity to DA terminals, adding support to the role of DA and oxidative stress in this model [LaVoie and Hastings, 1999]. Taken together, it is likely that increased generation of DA *o*-quinone by DA auto-oxidation is associated with the neurotoxicity of MAP in the brain.

Glutathione S-transferase M1 (GSTM1) is a subtype of GSTs that detoxify xenobiotics by conjugating glutathione. It has been reported that GSTM1 catalyzes a glutathione conjugate of catecholamine *o*-quinones such as aminochrome [Smythies and Galzigna, 1998]. GSTM1 has an entire gene deletion polymorphism and its enzymatic activity is classified into three grades, i.e., a highly active genotype (homozygous non-deletion alleles; NN), a moderately active genotype (heterozygous non-deletion alleles; DN), and a null genotype (homozygous deletion alleles; DD) [McLellan et al., 1997]. Recently, it has been reported that the frequency of D allele of *GSTM1* gene in the patients with schizophrenia was significantly ($P = 0.0075$) higher than that of normal controls, suggesting that *GSTM1* gene may be associated with an increased susceptibility to schizophrenia [Harada et al., 2001a]. Thus, it seems that differences in the GSTM1 genotype may contribute to the development of MAP abuse. In order to verify a potential role of the *GSTM1* gene in the neuropathology by MAP abuse, we analyzed a polymorphism of the *GSTM1* gene in subjects with diagnosed MAP-related disorders and in control groups.

MATERIALS AND METHODS

The research was performed after obtaining approval from the ethics committees of each institute of Japanese Genetics Initiative for Drug Abuse (JGIDA), and all subjects provided written informed consent for the use of their DNA samples for this research. The subjects were 157 patients (125 males, age: 37 ± 11 years (mean ± SD), age range: 19–69 years; and 32 females, age: 28 ± 5 years (mean ± SD), age range: 21–47 years) with MAP dependence and psychotic disorder meeting ICD-10-DCR criteria (F15.2 and F15.5) who were outpatients or inpatients of psychiatric hospitals of JGIDA, and 200 age-, gender- and geographical origin-matched normal controls (157 males, age: 37 ± 11 years (mean ± SD), age range: 19–69 years; and 43 females, age: 36 ± 10 years (mean ± SD), age range: 21–58 years) mostly consisted of medical staffs who had no past history and no family history of drug dependence or psychotic disorders. All subjects were Japanese, born and living in restricted arrears of Japan including northern Kyusyu, Setouchi, Tyukyou, Toukai, and Kantou.

The polymorphism studied in this project was the deletion of the entire *GSTM1* gene. Genotyping for this gene was performed by a combination of two types of polymerase chain reaction (PCR) amplification as reported previously [Harada et al., 2001a,b]. The first type of PCR was used for the detection of a non-deletion allele with the appropriate primers (forward: 5′-CTTCACGTGTTATGGAG GTTC-3′, reverse: 5′-GCGAGTTATTCTGTGTGTAGC-3′). The other type of PCR was used for the detection of a deletion allele with suitable primers (forward: 5′-ACAGAGGAAGGGTG-CATTTGATA-3′, reverse: 5′-GACATTCATTCCCAAA-GCGACCA-3′); both types of PCR were followed by agarose gel electrophoresis with ethidium bromide staining. Allele frequencies were calculated by gene counting and the differences between groups were evaluated by Fisher's exact test. The odds ratio (OD) and 95% confidence intervals were calculated to evaluate the effects of the different genotypes.

RESULTS

The GSTM1 genotypes and the allele frequencies in MAP abusers and controls are shown in Table I. The genotype distribution in both abusers and controls was within the Hardy–Weinberg equilibrium. We found that a difference in the frequency of deletion (D)/non-deletion (N) alleles between the female abusers and female controls was a trend toward a statistical significance ($P = 0.071$). In contrast, there was no significant difference between male abusers and male controls ($P = 0.651$). The frequency of carrying the D allele among female abusers was significantly higher than that in male abusers ($P = 0.007$, odds ratio: 2.77, 95% CI 1.30–5.89), whereas no gender difference was shown among control subjects ($P = 0.297$, odds ratio: 1.36, 95% CI 0.80–2.31). The genotype distribution difference between female abusers and female controls was significant ($P = 0.032$), whereas no significant difference between male abusers and male controls was shown ($P = 0.819$).

DISCUSSION

Our findings suggest that a deletion of the *GSTM1* gene may contribute to MAP abuse vulnerability in female, but not in male, subjects. Based on the role played by GSTM1 in the antioxidant system preventing neurotoxicity, *GSTM1* gene deletion might lead to an excess of catecholamine *o*-quinones (e.g., aminochrome) that are neurotoxic in the brain, including DA neurons. The reason underlying this gender difference is currently unclear. However, recent evidence has been suggestive of gender differences in course of drug dependence and drug abuse [Lynch et al., 2002]. It has been reported that females enter treatment programs after fewer years of amphetamine use, and that females also take less time to become addicted after initial use than do males [Westermeyer and Boedicker,

TABLE I. Allele and Genotype Frequencies of the *GSTM1* Gene Deletion Polymorphism in MAP Abusers and Controls

	Male		Female	
	Abusers (n = 125)	Controls (n = 157)	Abusers (n = 32)	Controls (n = 43)
GSTM1 allele frequency				
D	172 (68.8%)	210 (66.9%)	55 (85.9%)	63 (73.3%)
N	78 (31.2%)	104 (33.1%)	9 (14.1%)	23 (26.7%)
	$P = 0.651$		$P = 0.071$	
GSTM1 genotype frequency				
DD	58 (46.4%)	67 (42.7%)	24 (75.0%)	21 (48.8%)
DN	56 (44.8%)	76 (48.4%)	7 (21.9%)	21 (48.8%)
NN	11 (8.8%)	14 (8.9%)	1 (3.1%)	1 (2.3%)
	$P = 0.819$		$P = 0.032$*	

GSTM1, glutathione S-transferase M1; MAP, methamphetamine; D, deletion allele; N, non-deletion allele.
*$P < 0.05$.

2000]. In addition, positive subjective effects of D-amphetamine are enhanced during the follicular phase, which correlates with changes in estrogen levels [Lynch et al., 2002].

It has been suggested that gonadal hormones such as estrogen play a role in the differences between males and females regarding responses to drugs of abuse [Lynch et al., 2002]. In females, there is an accelerated transition from controlled to uncontrolled use, namely, dependence, and that gonadal hormones, particularly estrogen, may play a role in these processes [Justice and De Wit, 2000]. In studies using rats, estrogen has been revealed to enhance the behavioral and neurochemical responses to MAP by increasing stimulated DA release [Becker, 1999]. Furthermore, recent studies using brain imaging technique revealed that women have higher levels of DA transporters [Mozley et al., 2001] and lower DA D_2 receptor affinity in the striatum than men [Pohjalainen et al., 1998], suggesting a lower baseline of dopaminergic tone and elevated levels of DA released by MAP in females. Therefore, it is likely that gonadal hormones and gender differences in dopaminergic systems may be implicated in gender differences related to susceptibility to addiction to psychomotor stimulants. Thus, it appears that excess DA released by MAP might generate an excess of DA *o*-quinone, rendering it especially difficult for persons with low-activity GST to detoxify a sufficient amount of DA *o*-quinone. Furthermore, the GSTM1 deletion would influence the susceptibility of females to MAP abuse.

In conclusion, our findings suggest that *GSTM1* gene deletion may contribute to a vulnerability to MAP abuse in female subjects, but not in male subjects.

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Clinical Features of Sensitization to Methamphetamine Observed in Patients with Methamphetamine Dependence and Psychosis

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ABSTRACT: **Metamphetamine (METH) has been the most popular drug of abuse in Japan for more than 50 years, resulting in serious health and social issues. Most adult abusers in Japan consume only METH; multiple-substance abusers are rare. This unusual aspect of drug abuse makes it possible to observe clearly the sequential alteration of psychiatric symptoms induced by METH without modification by other illegal drugs. Clinical investigation reveals three core characteristics of METH abuse: (1) progressive qualitative alteration in mental symptoms from a nonpsychotic to a prepsychotic to a severely psychotic state; (2) enhanced vulnerability to relapse of psychosis; and (3) very long duration of the vulnerability to relapse. These findings indicate that the phenomenon of sensitization to METH develops during abuse and plays a key role in the susceptibility to and onset of psychosis and in the refractory process. Molecular findings using animal sensitization models may facilitate a better understanding of, and open the way for innovative therapies for, METH psychosis and also chronic schizophrenia.**

KEYWORDS: **sensitization phenomenon; drug abuse; methamphetamine psychosis; vulnerability to relapse**

INTRODUCTION

Abuse of amphetamines has been a serious health and social concern for more than 50 years in Japan. Among amphetamine derivatives, methamphetamine (METH) is the primary one consumed in Japan. Police records of the total number of people arrested each year for illegal METH consumption or possession reveal three epidemics of METH abuse (FIG. 1). The first epidemic broke out just after World War II and continued until 1957. In this epidemic, a METH product synthesized by a domestic pharmaceutical company was distributed in ampoules on the open market; it was 100% pure and injected intravenously. This epidemic reached a peak in 1954. In that year, more than 55,000 persons were arrested, and the estimated

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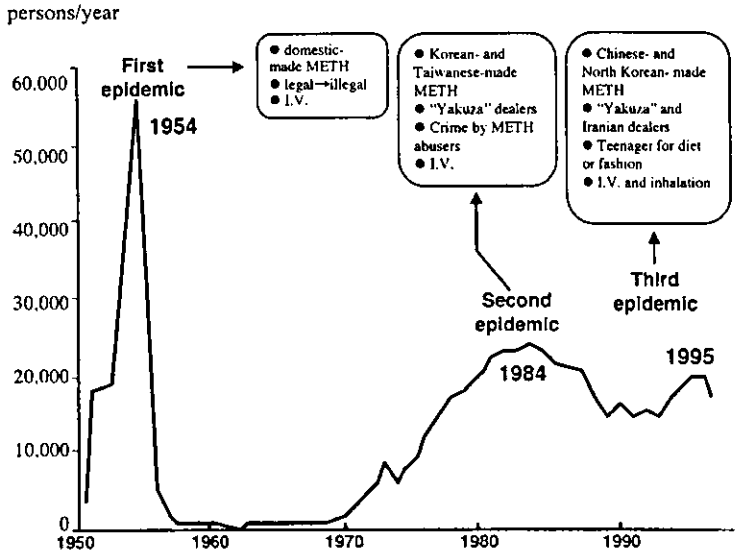

FIGURE 1. Methamphetamine (METH) abuse in Japan. Based on Japanese police records of the number of persons arrested annually for illegal drug abuse.

number of METH abusers was over half a million. The Japanese government sounded a note of warning against METH abuse with the slogan "METH brings national ruin." After 1954, the police tightened drug controls, and METH abuse dramatically subsided. However, METH abuse began to rise again from about 1970, and the second epidemic peaked in 1984. The second epidemic was characterized by promotion by Yakuza, members of the Japanese Mafia. They smuggled illegally made Korean or Taiwanese METH in crystal form into Japan and supplied it to abusers. It was dissolved in water and injected intravenously. Heinous crimes by METH abusers increased and became a serious social problem. After 1984, METH abuse appeared to decrease slowly, but it started to increase again from 1995, becoming the third epidemic, which continues up to the present. The third epidemic is characterized by the entry of Iranian drug dealers using cellular phones into the market, in addition to the Yakuza. This new distribution method made drug control very difficult and resulted in an increase in hidden abusers. A further factor was that the Iranian dealers were friendlier to teenagers than the Japanese Yakuza. Therefore, it seemed safe and easy to get drugs from them, and high school students started to use METH casually to lose weight or just for fun.

On the other hand, multiple-substance abuse has been rare in Japan. METH has been the most popular illicit drug for at least the last 10 years (FIG. 2), followed by organic solvent inhalation. Although recent reports emphasize a rapid increase of cannabinoid, morphine, and cocaine abuse in Japan, the total number arrested for abuse of these drugs is still quite small—less than 10% of that of METH abusers.

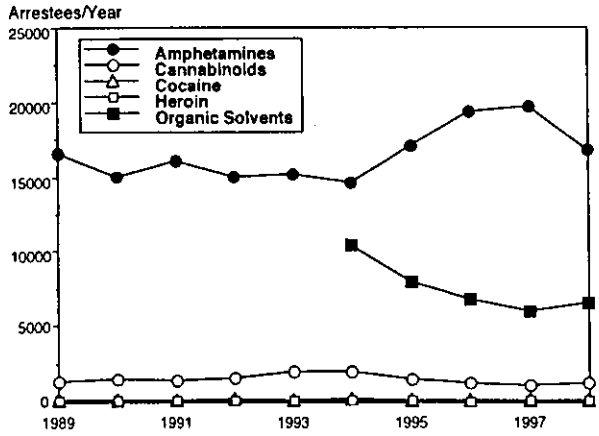

FIGURE 2. Substances abused in Japan.

Organic solvents are inhaled mostly by young and middle teenagers, rarely by adults. These facts indicate that most adult Japanese drug abusers have been consuming METH exclusively. This characteristic of Japanese drug abuse makes it possible to observe the sequential alteration of psychiatric responses to METH and the clinical course of the "pure form" of METH psychosis. Many excellent clinical studies have been published,[1–4] unfortunately mostly in Japanese; and they consistently reveal that longer and more frequent consumption of METH induces psychosis at a higher rate, more prompt relapse due to subsequent consumption, more frequent spontaneous relapse, and a worse prognosis. These clinical findings indicate development of sensitization during METH abuse and that this sensitization plays a central role in the etiology of METH psychosis. We present here three typical cases of METH psychosis and extract core clinical features of the sensitization observed in individual cases suffering from METH psychosis.

CASE REPORTS

Case 1: Male, 28 Years Old

At the age of 21, he began to inject intravenous METH 2–3 times a day (40–150 mg/injection).[5] After injecting, he felt relaxed and as if he were floating. He craved stimulation and felt generous. He drove cars at high speed for the thrill. In withdrawal, he crashed and became lethargic. Three years after beginning METH abuse (at the age of 24), he began to feel something was wrong. He felt that someone was hiding upstairs or that policemen were surrounding his house, but he found no one there. At the age of 25, he still abused METH periodically. His abnormal experiences increased at every injection. He became sure that policemen were surround-

ing his house and trying to arrest him. He became frantic and finally set a house on fire to escape.

In this case, the initial dose of METH induced stimulating effects that were enhanced by subsequent METH doses; but after three years of METH abuse, METH injections produced delusions that increased over time.

Case 2: Male, 46 Years Old

At the age of 13, he inhaled an organic solvent several times. At the age of 24, he began to inject METH intravenously.[5] After injection, he felt his hair stand on end. His libido and sexual sensations were greatly enhanced. He became garrulous and felt able to concentrate much better. During METH abuse, he participated enthusiastically in gambling and sexual intercourse. At the age 25, he was arrested several times but continued the abuse. He did not suffer from hallucinations or delusions at that time. At the age of 26, he had an auditory hallucination of a lady's voice while he was taking a shower. He heard her say, "Don't take drugs. You are worthless. Kill yourself." Because of the auditory hallucinations, he tried to stop using METH but failed. At the age of 36, he began to hallucinate after every injection, and the hallucinations lasted more than 24 hours.

One day, he encountered a space alien and found 11 holes in his own body after injection. The space alien entered his body through the holes and talked to him. He punched the alien, but he later found that his aunt was dead. He mistook his aunt for an alian under hallucination. At the age of 43, he married and stopped using METH for three years. His psychotic symptoms completely disappeared. However, at the age of 46, he consumed METH again, and his auditory and visual hallucinations recurred after only a couple of METH injections. He went on a rampage because he mistook policemen for aliens.

This case also shows that repeated METH injection produced stimulating effects at first but, later, hallucinations and delusions. The latency between the initial consumption of the drug and the onset of psychosis was two years in the first episode; but in the relapse after abstinence, only a few injections were enough to induce psychosis. Accordingly, susceptibility to relapse had developed in his brain during abuse of the drug.

Case 3: Male, 29 Years Old

At the age of 22, he began to inject METH (about 40 mg/injection) intravenously.[5] Just after injection, he felt very clear headed and repeated certain behaviors compulsively. While using METH, he was absorbed in gambling. When he consumed an overdose of METH, he felt anxious and noticed signs that people might be attacking him. At the age of 23, he injected several times a day because it highly enhanced his sexual sensation. He found that merely inhaling METH stimulated him. At the age of 24, he became aware of a voice saying, "Are you all right?" after injecting METH. At that time, he was uncertain whether the voice was illusory or real. However, at the age of 25, he began to suffer from auditory hallucinations after every METH injection. He heard a voice say, "Your wife had an affair. Kill her!"

At the age of 27, he was arrested and jailed. In prison, his hallucinations reappeared despite complete abstinence. At the age of 28, he was released from prison.

Although he has not abused METH since, his hallucinations have not disappeared. He has complained, "The TV is talking to me," "Somebody is trying to attack me," "My reflection in the window turns into the devil, and my wife sees it and runs away," and "My food has been poisoned by someone." He was arrested again, but a urine test in police custody did not detect METH.

This person suffered a relapse of psychosis while he was jailed. Japanese jails are strictly controlled, and prisoners are never able to get drugs. Therefore, he suffered from spontaneous relapse without resumption of METH use, demonstrating that longer and heavier abuse increases susceptibility to spontaneous relapse.

CORE CLINICAL FEATURES OF SENSITIZATION TO METH IN PATIENTS

Three core features of the sensitization phenomenon in METH psychosis are observed in these three cases: (1) induction of a progressive qualitative alteration in mental symptoms, (2) enhancement of vulnerability to relapse of the psychosis, and (3) very long duration of the vulnerability to relapse. These features reflect different clinical aspects of the sensitization induced by METH abuse and were observed in detail in our recent clinical investigation of patients with METH dependence and psychosis in the third epidemic.

Progressive Qualitative Alteration in Induced Mental Symptoms

The clinical course shown in these three cases is illustrated in FIGURE 3.[6] Repeated consumption of METH induces psychotomimetic effects such as feelings of stimulation and increased concentration in the abuser initially, and these effects are gradually enhanced by subsequent doses of METH. However, after a certain point, further consumption of METH no longer induces psychotomimetic effects, but psychotic symptoms. Prepsychotic abnormal experiences without certainty, such as delusional moods or ideas of reference, are seen first and then shift to a completely psychotic state consisting of potent delusions—for example, delusions of reference, persecution, and poisoning; and auditory and visual hallucinations after further use of METH. Accordingly, METH-induced psychiatric symptoms show progressive quantitative alteration from a nonpsychotic to a prepsychotic and finally to a psychotic state. The rate of psychosis due to METH is reported to be as high as 76–92%.[1–3,7,8] Unexpectedly, the rates in the first and second epidemic were almost the same despite the fact that a 100% pure pharmaceutical company product and an impure, illicitly manufactured, underground METH were consumed in the first and second epidemics, respectively. Connell[9] reported similarly high rates of psychosis due to oral amphetamine consumption. The period of latency between the initial use and the onset of psychosis varies from as little as several weeks to as much as 20 years because it is greatly influenced by the dose of METH, frequency of consumption, route of administration (intravenous injection, oral, inhalation), circumstances of abuse, and individual vulnerability to psychosis. Our present study showed that the average latency was 5.2 ± 5.9 years (N = 149) and that the risk of psychosis seemed to rise steeply when the duration of METH use exceeded 6 months (TABLE 1). Actually, case 2 developed psychosis after 2 years abuse of METH.

TABLE 1. Clinical course of methamphetamine-induced psychosis in three epidemics

	Epidemic 1	Epidemic 2	Epidemic 3
Study	Tatetsu[2]	Sato[8]	Ujike
Year	1956	1982	2003
Latency from METH abuse to initial psychotic episode			
Number of patients	$N = 100$	$N = 35$	$N = 149$
<1 week	—	0	1
1–4 weeks	6	0	7
1–3 months	19	9	7
4–6 months	23	9	4
6–12 months	27	3	17
>1 year	25	79	64
Time for recovery from psychosis after METH discontinued			
Number of patients	$N = 74$	$N = 82$	$N = 170$
0–10 days	77	64	59
11–30 days		18	
>1 month[a]	23	18	41[b]

[a]Prolonged type.
[b]11% in 1–3 months, 2% in 3–6 months, and 28% >6 months.

Enhanced Vulnerability to the Relapse of Psychosis

METH-induced psychosis usually disappears shortly after the discontinuance of METH consumption and the beginning of neuroleptic treatment. However, even after complete recovery, relapse develops very promptly after subsequent doses of METH. We found that the relapse latency from reconsumption of METH was less than one week for 60% of patients and less than one month for 80% of patients ($N = 101$), intervals that are much shorter than the latency of the initial psychosis, which was more than one year for the majority of patients (TABLE 1). As shown by case 2, the initial psychosis occurred after two years of METH use, but relapse occurred only one week after resumption of METH consumption. These data demonstrate that severe vulnerability to relapse of METH psychosis must develop during METH abuse. As shown in case 3, longer METH abuse can induce a spontaneous relapse due to an unspecific stressor, such as jail detention, without METH consumption. It is reported that spontaneous relapse may also occur after severe insomnia or heavy alcohol consumption. Our data showed that patients with spontaneous relapse usually had a history of more than two years of METH abuse. Our data also showed that the longer the duration of METH abuse, the worse the prognosis of psychosis. Users usually recover from METH psychosis within one week, or one month at the longest, after cessation of METH consumption; this is called transient-type METH psychosis. However, recovery of patients with longer METH consumption from psychosis may sometimes be delayed to over one month, or occasionally over 6 months, which are called the prolonged and persistent types, respectively. The rate of the prolonged/

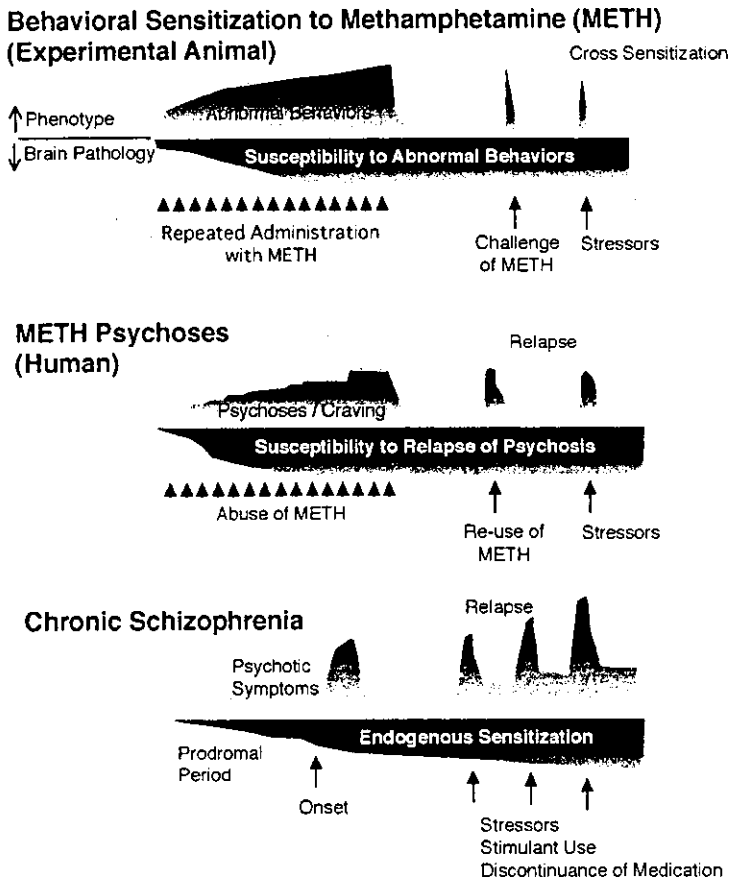

FIGURE 3. Longitudinal profiles of behavioral sensitization to methamphetamine (METH) in experimental animals, methamphetamine-induced psychosis, and patients with chronic schizophrenia.[6]

persistent type of METH psychosis was 23% in the first epidemic,[2] but it was decreased to 18% in the second epidemic[8] by the introduction of neuroleptics. However, our study of the third epidemic showed that the rate of prolonged/persistent METH psychosis rose to 41%, more than twice that of the second epidemic. We found that considerable numbers of patients in the third epidemic had consumed METH periodically since the second epidemic, which resulted in an increase in the number of patients who abuse METH for more than 10 years. This is why the percentage of patients with a worse prognosis for METH psychosis has increased in the third epidemic. Thus, longer and heavier abuse of METH delays recovery and worsens the prognosis for METH psychosis.

Very Long Duration of Vulnerability

The last characteristic of the sensitization observed in patients with METH psychosis is the very duration of vulnerability to the relapse of psychosis, which is the most serious clinical issue in the therapy for substance abuse. A patient who is in remission from METH psychosis even for several years or decades can easily relapse due to a few injections of METH. Sato *et al.*[10] reported a patient who developed a rapid relapse of psychosis after only a single METH injection after abstinence for 60 months. Therefore, the vulnerability to the relapse of psychosis developed during METH abuse does not seem to decrease over time.

CONCLUSION

As shown in FIGURE 3,[6] two other distinct conditions, chronic schizophrenia in humans and behavioral sensitization in rodents, showed temporal profiles quite similar to that of METH dependence and psychosis. This implies that these conditions share a common molecular mechanism, the sensitization phenomenon. Basic studies using animal models of behavioral sensitization have provided many divergent neurochemical and molecular findings[6,11] that probably underlie the mechanisms of sensitization. Findings derived from animal models are very useful for understanding the onset process, relapse mechanisms, and chronic and refractory courses in schizophrenia and METH dependence and psychosis, and may open the way for development of innovative and radical therapies for them.

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No association was found between a functional SNP in ZDHHC8 and schizophrenia in a Japanese case–control population

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Abstract

ZDHHC8 is a new and attractive candidate for a schizophrenia-susceptibility factor. First, several lines of linkage studies showed that 22q11, on which ZDHHC8 is located, is a "hot" region. Second, fine linkage disequilibrium mapping revealed a significant association around ZDHHC8. Moreover, a very recent study reported that one single nucleotide polymorphism (SNP: rs175174) in ZDHHC8 might affect the splicing process, the ZDHHC8 knock-out mice showed the gender-specific phenotype, and the transmission disequilibrium test (TDT) using this SNP also showed significant association with human female schizophrenia. Thus, we attempted a replication study of this SNP using relatively large Japanese case–control samples (561 schizophrenics and 529 controls). No association was found between schizophrenia and controls even after dividing samples by gender. Because our sample size provided quite high power, ZDHHC8 may not play a major role in Japanese schizophrenia. And our results did not support the gender-specific effect of this SNP.

Keywords: Chromosome 22q11; Gender difference; Candidate gene

The 22q11 region (OMIM: #600850 SCZD4) is associated with increased risk for schizophrenia [2]. Two independent meta-analyses of linkage studies showed the linkage around 22q11 [1,5], although one negative result was also reported [8]. This chromosome region contains at least three genes, COMT [12], PRODH2 and DGCR6 [7], implicated as susceptibility genes for schizophrenia.

Recently, ZDHHC8 was reported as a new and attractive candidate gene on 22q11 from the evidence of a genetic association study and animal study [6,9]. In the initial genetic association study, Liu et al. showed that three single nucleotide polymorphisms (SNPs) in ZDHHC8 were associated with schizophrenia. One of these SNPs (rs175174), which was located in intron 4 of ZDHHC8, showed the most highly significant *P* value [6]. This intronic SNP seemed to modify ZDHHC8 expression by causing imperfect splicing, intron retention and reduced enzyme activity. In addition, *Zdhhc8* knockout mice had a gender-dependent dimorphic deficit in prepulse inhibition similar to schizophrenia and reactivity to the psychomimetic *N*-methyl-D-aspartate (NMDA) receptor blocker dizocilpine. In the light of these findings, the transmission disequilibrium test (TDT) divided samples according to gender differences, revealing that human female schizophrenia was significantly associated with this SNP [9]. Thus, we here provide a replication study of rs175174 in ZDHHC8 using Japanese case–control samples.

A total of 561 patients with schizophrenia (259 female; mean age ± standard deviation (S.D.) 49.6 ± 16.4 years: 302

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male; 47.0 ± 14.9) and 529 controls (270 female; 39.7 ± 15.4 years: 259 male; 34.9 ± 12.4 years) were genotyped for association analysis of rs175174. Moreover, in additional linkage disequilibrium (LD) mapping around this SNP, 95 schizophrenic patients (50 female and 45 male) and 96 controls (44 female and 52 male), part of each sample used in association analysis, were genotyped for three SNPs. The general characterization of these subjects and a description of their psychiatric assessment according to identical criteria were published elsewhere [13]. After explaining the study to all subjects, written informed consent was obtained from each. This study was approved by the Ethics Committee of the Nagoya University Graduate School of Medicine and Fujita Health University.

Genomic DNA was extracted from peripheral blood of all subjects. For rapid genotyping of SNPs, rs175174 and additional three SNPs for LD mapping (rs175169, rs175175 and rs2292570), polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) assays were developed. The information of PCR primers is available on request. The PCR reactions of all SNPs were carried out in a 10 μl volume containing 10 ng genomic DNA, 0.4 M of each primer, 200 μM of dNTP, 1 × PCR Gold Buffer, 1.5 mM $MgCl_2$ and 0.25 U of Amplitaq Gold™ (Applied Biosystems Japan Ltd., Tokyo, Japan), using the GeneAmp™ PCR system 9700 (Applied Biosystems Japan Ltd.). PCR cycling conditions consisted of an initial denaturation step at 95 °C for 9 min, followed by 45 cycles of 95 °C for 15 s, 56 °C for 20 s, 72 °C for 30 s, and ending with a final extension step at 72 °C for 7 min. PCR product was digested using appropriate restriction enzymes according to the manufacture's recommendation (New England Biolabs, England, UK) (Table 1). DNA fragments were resolved by electrophoresis in a 6% acrylamide gel stained with ethidium bromide.

Genotype deviation from the Hardy–Weinberg equilibrium (HWE) was evaluated by χ^2 test. Marker-trait association analysis was also evaluated by χ^2 test (SPSS 10.0J, SPSS Japan Inc., Japan). To evaluate pairwise LD matrices among SNPs (by D' and r^2), we used the software HAPLOVIEW version 2.05 (developed in Mark Daly's lab., URL; http://www.broad.mit.edu/personal/jcbarret/haploview/index.php). This software also defined "LD blocks" by reasonable criteria based on 95% confidential bounds on D' values [4]. Power calculation was performed using a statistical program prepared by Ohashi et al. [10]. The significance level for all statistical tests was 0.05.

In view of the gender differences in gene effects, we included analyses of samples divided according to the gender. Both in cases and controls, genotype frequencies of total, female and male samples were not significantly different from HWE.

In association analysis, we could not find associations of rs175174 with schizophrenia in either male or female (Table 2).

Next, to test whether rs175174 is representative for ZDHHC8 or not, we performed LD mapping using three additional SNPs around ZDHHC8 (Fig. 1). LD matrices between each pair of SNPs showed strong LD both in cases and controls (Table 1). Even after dividing samples according to the gender, all LD patterns showed the same trends (data not shown). These findings may suggest that the LD pattern of ZDHHC8 is a block-like pattern and that rs175174 is the "representative SNP" of this gene.

The power based on genotype relative risk (GRR) was calculated to evaluate the non-significant results due to type II error. When we set the GRR at 1.28, 1.42 and 1.40 in all, female and male samples, respectively (multiplicative model), our sample size provided powers of more than 80%.

We could not replicate an original positive association using TDT of ZDHHC8 with schizophrenia by the present case–control association analysis among Japanese. Nor could we replicate the gender-specific effect of the risk SNP. In this association analysis, our sample sizes provide enough power to deny the hypothesis. We also performed the fine LD mapping of Japanese samples and showed that the LD pattern of ZDHHC8 was the same block-like pattern as one of the samples from the United States and South Africa. The results provide evidence that not only rs175174 but also ZDHHC8 would not be a susceptibility factor for schizophrenia in either Japanese females or males. The discrepancy between Japanese and the samples from the United States and South Africa may derive from ethnic differences.

A couple of limitation should be addressed to discuss the present results. Initially, the mean age of controls is much younger than that of patients in the present study. This means that a number of young controls, although not more than five subjects given a lifetime morbidity risk of 0.8–1.0%, may go on to develop schizophrenia. This confounding factor might weaken the power of the present study. Another limitation

Table 1
SNPs in LD mapping and pairwise LD matrices

SNP ID	D'				Restriction enzyme
	rs175169	rs175174	rs175175	rs2292570	
rs175169		0.97 (0.78)	1.0 (0.29)	1.0 (0.67)	*Bsl*I
rs175174	0.97 (0.80)		1.0 (0.36)	1.0 (0.58)	*Bse*RI
rs175175	1.0 (0.26)	1.0 (0.31)		1.0 (0.21)	*Alw*NI
rs2292570	0.93 (0.76)	0.97 (0.70)	1.0 (0.23)		*Tsp*RI

Upper diagonal figures are D' (r^2) of controls and lower diagonal figures are D' (r^2) of schizophrenia.

Table 2
Association analysis of rs175174

Samples	Number	G/G	G/A	A/A	*P* value (genotype)	MAF[a]	*P* value (allele)
Total							
SCZ	561	238	245	78		0.357	
CON	529	205	259	65	0.213	0.368	0.618
Female							
SCZ	259	114	106	39		0.355	
CON	270	112	130	28	0.133	0.344	0.714
Male							
SCZ	302	124	139	39		0.359	
CON	259	93	129	37	0.457	0.392	0.260

SCZ: schizophrenia; CON: control.
[a] Minor allele frequency.

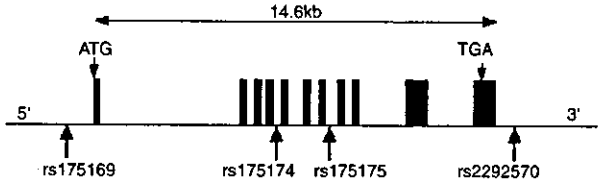

Fig. 1. Genomic structure of ZDHHC8 and SNPs used in association analysis and LD mapping. Vertical bars represent exons of ZDHHC8, and each number under arrows represents SNP ID.

which must be exercised is that the other candidates related to the neurodevelopmental and neuroprotective effect of ZDHHC8 would be in locus heterogeneity [11]. For example, ZDHHC8 encodes a putative transmembrane palmitoyltransferase modulating numerous classes of neuronal proteins including proteins important for neuronal development, neurotransmitter receptors such as NMDA [3]. Thus, the combined effect between ZDHHC8 and the other genes might be a stronger predisposing factor. Further genetic analysis including related candidate genes would definitely be required for a conclusive result.

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