Relations of Genetic Variants in Superoxide Dismutase 2 and Dystrobrevin-binding Protein 1 to Methamphetamine Psychosis among Methamphetamine Dependents in Taiwan

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Abstract

Objectives: Both *superoxide dismutase 2* gene (*SOD2*), encoding a free radical scavenger, and *dystrobrevin-binding protein 1* gene (*DTNBP1*), a candidate gene for schizophrenia, have been implicated in methamphetamine (METH) psychosis. In this study, we intended to compare the distribution of those two genes between METH users with and without psychosis and to evaluate whether the length of time of METH exposure influenced such relationship. **Methods:** The study sample consisted of 84 cases of patients with METH-induced psychosis and 187 controls (METH users without psychosis). Five single-nucleotide polymorphisms (SNPs) in *SOD2* and three SNPs in *DTNBP1* were genotyped. We did both single-locus and haplotype analyses, and adjusted for multiple comparisons with an effective number of markers, and multivariable logistic regression analyses for adjusting for age, sex, and duration of METH use. **Results:** None of the individual SNPs were associated with METH-induced psychosis after adjustment for multiple comparisons. In the subgroup analysis, both rs4880 and rs2855116 in *SOD2* were significantly associated with prolonged METH-induced psychosis ($p < 0.01$ and $p < 0.01$, respectively). Under the assumption of a codominant model, the CC genotype of rs4880 was significantly associated with METH-induced psychosis after adjustments for age, sex, and the duration of METH use ($p < 0.01$). This association was not supported by the haplotype analyses or gene–gene interactions between SOD2 and *DTNBP1*. **Conclusion:** Functional C-allele of rs4880 in *SOD2* was associated with the prolonged subtype of METH-induced psychosis in Taiwanese population. Oxidative stress mechanisms show a rôle in the development of METH-induced psychosis.

Key words: oxidative stress, polymorphism, reactive oxygen species, scavenger
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Introduction

Methamphetamine (METH) is a central nervous system stimulant that acts on several neurotransmitter systems and exerts neurotoxic effects on dopaminergic and other neurons [1, 2]. METH is highly addictive, and its use continues to be one of the most worrying threats of drug use in East and South-East Asia [3]. With the capture-recapture method on information collected from both judicial and medical systems, the prevalence of male METH use in the northern Taiwan from 1999 to 2002 has been estimated to be ranged from 1.24% to 2.47% [4]. According to the 2014 national survey in Taiwan, the lifetime use prevalence of METH is 0.6%, remaining the most frequently used illicit drug since the previous two waves of national surveys in 2005 and 2009 [5].

One severe consequence of METH consumption is METH-induced psychosis [6]. More than half of the METH users

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experience psychotic symptoms [7]. About 13% of METH users have been found to meet the diagnostic criteria for METH-induced psychosis [8, 9], which increases health service use, poor outcomes, the incidence of psychiatric symptoms across many domains over time, and premature death [10]. Genetic factors have accounted for 43% of the variance in METH use in a large twin study [11], but little is known about the contribution of genes to METH-induced psychosis. Nevertheless, circumstantial evidence suggests that a genetic component may be involved in the susceptibility to METH-induced psychosis. Patients with METH-induced psychosis and their relatives have more schizoid/schizotypal personality traits, which are indicators of genetic vulnerability to schizophrenia, than their counterpart METH users without psychosis [12]. In addition, METH-induced psychosis tends to occur following only a short period of METH consumption [12].

Several studies examined genetic factors and were found that they are associated with susceptibility to METH-induced psychosis [13]. Superoxide dismutase (SOD), as a free radical scavenger and may lessen the damage from METH-induced neurotoxicity, has been examined for its association with METH-induced psychosis [14]. A common *SOD2* missense variant (rs4880: C to T, Ala^{16}Val) decreases SOD2 enzyme activity [15]. The frequency of allele Ala is high in patients with METH-induced psychosis than healthy controls in Japanese and Taiwanese samples [16]. A second gene investigated for this association was *dystrobrevin-binding protein 1* gene (*DTNBP1*) [17], which has been reported to be associated with schizophrenia and bipolar psychosis [18]. Because of the close resemblance between METH-induced psychosis and these endogenous psychoses, *DTNBP1* was chosen in a Japanese study, and the frequencies of two single-nucleotide polymorphisms (SNPs), allele G in rs3213207 and allele T in rs2619538, in *DTNBP1* have been found to be elevated in patients with METH-induced psychosis compared with healthy controls [18].

The association of genetic variants in *SOD2* or *DTNBP1* with the increased risk for METH use *per se* or with METH-induced psychosis remains uncertain. A more appropriate comparison group should consist of individuals who have used METH but do not have psychosis. A related issue is whether the relations of genetic variants are limited to certain forms of METH-induced psychosis, such as prolonged or transient psychosis. Furthermore, the influence of some known correlates of METH-induced psychosis, such as the initiation age of METH use or the total duration of consumption [12], on these associations has not been explored in previous studies.

We did a case–control study that contrasted patients with METH-induced psychosis with METH users without psychosis in Taiwan and found that METH is one of the most frequently consumed illegal drugs [4]. In this study, we intended to compare the distribution of genetic variants in *SOD2* and *DTNBP1* between METH users with and without psychosis after adjusting for other known correlates of METH-induced psychosis and to evaluate whether the relation of these genetic variants to METH-induced psychosis is influenced by the length of psychosis.

Methods

Study participants

We recruited both cases and controls from the Taipei City Psychiatric Center and Taipei Detention Center from October 2005 to April 2010. The inclusion criteria for cases were those with (a) fulfilled criteria of METH dependence and METH-induced psychosis according to *the Diagnostic and Statistical Manual, Fourth Edition* (*DSM-IV*), (b) being Han Chinese in ethnicity, and (c) age being older than 17 years. Individuals were excluded from this study if they had experienced perceptual changes only briefly following METH intoxication. The following inclusion criteria were used for the controls: (a) fulfillment of the *DSM-IV* criteria of METH dependence but without experiencing psychotic symptoms, (b) Han Chinese in ethnicity, and (c) age being older than 17 years. Individuals were excluded from being controls in this study it they were (a) METH users with comorbid schizophrenia and (b) opportunistic users who failed to meet the criteria for METH dependence.

Written informed consents were obtained from all the study participants after a complete description of the study. Because using METH in Taiwan is illegal, all participants were informed that their legal status would not be influenced by their participation or nonparticipation in the study. This study protocol was approved by the institutional review board of Taipei City Hospital, with the requirement of obtaining informed consents from the study participants.

During the study period, we identified 593 METH users and excluded 322 (70 individuals with METH-intoxication and perceptual change, 7 patients with schizophrenia, 3 individuals with an uncertain diagnosis, and 242 opportunistic users). The final sample had 84 cases of patients with METH-induced psychosis (22 from Taipei City Psychiatric Center and 62 from Taipei Detention Center) and 187 controls of METH users without psychosis (4 from Taipei City Psychiatric Center and 183 from Taipei Detention Center).

Clinical assessments

One member of the assessment team (consisting of 2 experienced psychologists and 3 psychiatrists) administered the specific METH session of the Chinese version of Diagnostic Interview for Genetic Studies (DIGS-C) [19]. A *DSM-IV* diagnosis of METH-induced psychosis was made based on the information obtained from the DIGS-C interview. Notably, we expanded the one month psychosis duration criterion in the *DSM-IV* to six months. Patients whose psychotic symptoms were longer than 1 month but < 6 months were categorized as having prolonged psychosis, whereas patients with psychotic symptoms being < one month were categorized as having transient psychosis. Out of the 84 cases, 21 had prolonged psychosis and 63 had transient psychosis. Those patients who suffered from psychosis for > six months after discontinuing METH use were diagnosed as having schizophrenia and therefore excluded from this study.

We collected several variables relating to METH use, in addition to diagnostic information, including the initiation

age and the total and actual durations of METH use. The total duration of METH use was defined as the period from the first day the respondent used METH to the last date of such use, and the actual duration of METH use was derived by subtracting the period of abstinence from the total duration of METH use. The information on the actual duration of METH use was available for 192 (70.8%) of 271 respondents.

Single-nucleotide polymorphism genotyping

We chose five SNPs (rs 2758357, rs4880, rs2855116, rs2758330, and rs2842980) in *SOD2* [16] and three SNPs (rs26119539, rs3213207, and rs2619538) in *DTNBP1* [18, 20, 21] in this study. The genotyping was conducted using *Taq*Man probe assays (*Taq*Man™ SNP Genotyping Assays; Applied Biosystems, Foster City, California, USA) on a 7900 Fast Real-Time PCR System (Applied Biosystems). The *Taq*Man primer/probe set designed for each SNP allele was purchased from Applied Biosystems. Allelic discrimination was done automatically using SDS 2.2.2 software (Applied Biosystems).

Statistical analysis

Deviation from Hardy–Weinberg equilibrium was examined using the Chi-square test as implemented in the Procedure ALLELE in Statistical Analytic System/GENETICS, version 9.1 (SAS Institute, Cary, North Carolina, USA) among the pooled sample of cases and controls. We examined marker-trait association analyses for alleles and genotypes separately for individual SNPs and used multivariable logistic regression analyses to adjust for covariates, including age, sex, and duration of METH use.

The coefficient D′ of linkage disequilibrium (LD) among the SNPs and haplotype block structure were constructed using the software Haploview version 4.1 [22], in which blocks were merged if they had a multiallelic D' > 0.8 and the cumulative frequency of common haplotypes in the merged block was > 80% [23, 24]. The frequency of individual haplotypes and their association with METH-induced psychosis were estimated using the software THESIAS [25].

To correct for multiple tests, we used the method of Nyholt [26], in which the effective number (M_{eff}) of independent comparisons was derived from the variance of the eigenvalues of the observed marker correlation matrix. The more highly correlated the markers, the higher the variance of the eigenvalues and the lower the effect number (M_{eff}) identified. Using this method, the M_{eff} of independent SNPs was determined to be three for both *SOD2* and *DTNBP1*, and therefore, the adjusted threshold of the *p* value for a significant association was 0.0167 (i.e., 0.05/3).

Potential interactions among the eight SNPs were examined using the software generalized multifactor dimensionality reduction (GMDR) beta version 0.7 [27]. The GMDR is a nonparametric, generalized linear method that collapses

Table 1. Demographic and clinical characteristics of the study participants in a case-control study of methamphetamine-induced psychosis in Taiwan

Characteristics	Mean ± SD	
	METH users with psychosis (n = 84)	METH users without psychosis (n = 187)
Male, *n* (%)	65 (77.9)	138 (73.8)
Other substance use†, *n* (%)	5 (6.85)	4 (2.21)
Age, years	31.0 ± 6.9	31.7 ± 7.9
Onset age of METH use, years	20.7 ± 6.9	24.5 ± 7.4**
Total METH use duration, years	9.8 ± 7.4	6.8 ± 6.8***
Actual METH use duration§, years	5.4 ± 5.7	3.1 ± 4.7**

*p < 0.05; **p < 0.01; ***p < 0.001 using *t*-test or Chi-square test when appropriate (N = 271); †Including marijuana, opioids, ecstasy, and ketamine; §Information on the actual duration of METH use was available for 192 participants, including 63 cases and 129 controls. SD, standard deviation; METH, methamphetamine

Table 2. Information of the eight single-nucleotide polymorphisms genotyped in a case-control study of methamphetamine-induced psychosis in Taiwan (84 cases of methamphetamine-induced psychosis and 187 controls of methamphetamine users without psychosis)

Gene	Marker name	MAF§ in NCBI† (%)	MAF in this study (%)	Minor/major alleles	*p*-value of test for Hardy-Weinberg equilibrium
SOD2	rs2758357	25	19	T/C	0.74
	rs4880	12	15	C/T	0.98
	rs2855116	12	14	C/A	0.82
	rs2758330	42	48	T/G	0.83
	rs2842980	44	48	T/A	0.78
DTNBP1	rs2619539	40	40	G/C	0.57
	rs3213207	1	1	G/A	0.88
	rs2619538	2	2	T/A	0.81

Nonsignigicant differences were found in all items tested. §Minor allele frequency; †Those of Asian populations reported in the National Center for Biotechnology Informatics. SNPs, single-nucleotide polymorphisms

Table 3. Frequencies of the alleles and genotypes of individual single-nucleotide polymorphisms in superoxide dismutase 2 gene in a case-control study of methamphetamine-induced psychosis in Taiwan (84 cases of methamphetamine-induced psychosis and 187 controls of methamphetamine users without psychosis)

Marker	SNP	Sample	Genotype frequency, *n* (%)			*p*	Allele frequency, *n* (%)		*p*
			T/T	T/C	C/C		T	C	
SNP 1	rs2758357	Control	5 (2.67)	56 (29.95)	126 (67.38)		66 (17.65)	308 (82.35)	
		Case	6 (7.14)	27 (32.14)	51 (60.71)	0.1885	39 (23.21)	129 (76.79)	0.1293
		Brief	4 (6.35)	21 (33.33)	38 (60.320)	0.3163	29 (23.02)	97 (76.98)	0.1840
		Prolonged	2 (9.52)	6 (28.57)	13 (61.90)	0.2555	10 (23.81)	32 (76.19)	0.3271
Marker	SNP	Sample	C/C	C/T	T/T	*p*	C	T	*p*
SNP 2	rs4880	Control	1 (0.53)	48 (25.67)	138 (73.80)		50 (13.37)	324 (86.63)	
		Case	5 (5.95)	21 (25.00)	58 (69.05)	0.0195*	31 (18.45)	137 (81.55)	0.1247
		Brief	3 (4.76)	18 (28.57)	42 (66.67)	0.0565	24 (19.05)	102 (80.95)	0.1205
		Prolonged	2 (9.52)	3 (14.29)	16 (76.19)	0.0030**	7 (16.67)	35 (83.33)	0.5557
Marker	SNP	Sample	C/C	C/A	A/A	*p*	C	A	*p*
SNP 3	rs2855116	Control	1 (0.53)	47 (25.13)	139 (74.33)		49 (13.10)	325 (86.90)	
		Case	4 (4.76)	20 (23.81)	60 (71.43)	0.0572	28 (16.67)	140 (83.33)	0.2716
		Brief	2 (3.17)	17 (26.98)	44 (69.84)	0.2311	21 (16.67)	105 (83.33)	0.3186
		Prolonged	2 (9.52)	3 (14.29)	16 (76.19)	0.0032**	7 (16.67)	35 (83.33)	0.5210
Marker	SNP	Sample	G/G	G/T	T/T	*p*	G	T	*p*
SNP 4	rs2758330	Control	52 (27.96)	89 (47.85)	45 (24.19)		193 (51.88)	179 (48.12)	
		Case	22 (26.19)	44 (52.38)	18 (21.43)	0.7801	88 (52.38)	80 (47.62)	0.9144
		Brief	17 (26.98)	33 (52.38)	13 (20.63)	0.7909	67 (53.17)	59 (46.83)	0.8017
		Prolonged	5 (23.81)	11 (52.38)	5 (23.81)	0.9052	21 (50.00)	21 (50.0)	0.8171
Marker	SNP	Sample	T/T	T/A	A/A	*p*	T	A	*p*
SNP 5	rs2842980	Control	46 (24.60)	89 (47.59)	52 (27.81)		181 (48.40)	193 (51.60)	
		Case	18 (21.43)	44 (52.38)	22 (26.19)	0.7509	80 (47.62)	88 (52.38)	0.8671
		Brief	13 (20.63)	33 (52.38)	17 (26.98)	0.7608	59 (46.83)	67 (53.17)	0.7603
		Prolonged	5 (23.81)	11 (52.38)	5 (23.81)	0.9026	21 (50.00)	21 (50.0)	0.8436

*$p < 0.05$; **$p < 0.01$, not adjusting for multiple comparisons. The adjusted threshold of the *p* value for a significant association was 0.0167, which was derived using the M_{eff}[26] of independent SNPs three for *SOD2* (i.e., 0.05/3). SNPs, single-nucleotide polymorphisms; *SOD2*, superoxide dismutase 2 gene; Brief, transient psychosis (< one month; Prolonged, prolonged psychosis (2-6 months)

Table 4. Frequencies of alleles and genotypes of individual single-nucleotide polymorphisms in dystrobrevin-binding protein 1 gene in a case-control study of methamphetamine-induced psychosis in Taiwan (84 cases of methamphetamine-induced psychosis and 187 controls of methamphetamine users without psychosis)

Marker	SNP	Sample	Genotype frequency, *n* (%)			*p*	Allele frequency, *n* (%)		*p*
			GG	GC	CC		G	C	
SNP7	rs2619539	Control	32 (17.11)	94 (50.27)	61 (32.62)		158 (42.25)	216 (57.75)	
		Case	10 (11.90)	41 (48.81)	33 (39.29)	0.4104	61 (36.31)	107 (63.69)	0.1927
		Brief	8 (12.70)	33 (52.38)	22 (34.92)	0.7081	49 (38.89)	77 (61.11)	0.5082
		Prolonged	2 (9.52)	8 (38.10)	11 (52.38)	0.1859	12 (28.57)	30 (71.43)	0.0874
Marker	SNP	Sample	AA	AG	GG	*p*	A	G	*p*
SNP8	rs3213207	Control	184 (98.40)	3 (1.60)	0		371 (99.20)	3 (0.80)	
		Case	82 (97.62)	2 (2.38)	0	0.6604	166 (98.81)	2 (1.19)	0.6619
		Brief	61 (96.83)	2 (3.17)	0	0.4413	124 (98.41)	2 (1.59)	0.4436
		Prolonged	21 (100.00)	0	0	0.5588	42 (100.00)	0	0.5602
Marker	SNP	Sample	TT	TA	AA	*p*	A	T	*p*
SNP9	rs2619538	Control	0	4 (2.14)	183 (97.86)		370 (98.93)	4 (1.07)	
		Case	0	4 (4.76)	80 (95.24)	0.2381	164 (97.62)	4 (2.38)	0.2416
		Brief	0	3 (4.76)	60 (95.24)	0.2751	123 (97.62)	3 (2.38)	0.2785
		Prolonged	0	1 (4.76)	20 (95.24)	0.4568	41 (97.62)	1 (2.38)	0.4596

Nonsignificant different in all between-group comparisons. SNPs, single-nucleotide polymorphisms; *DTNBP1*, dystrobrevin-binding protein 1 gene; METH, methamphetamine; Brief, transient psychosis (< one month); Prolonged, prolonged psychosis (2-6 months)

high-dimensional genetic data into a single dimension, which permits interactions to be detected in relatively small sample sizes. As an extension of the traditional multifactor dimensionality reduction method, this method allows for unbalanced case–control sample and adjustment for covariates. We used cross-validation/permutations to minimize false-positive results. An empirical *p*-value for the prediction accuracy was calculated from 1,000 permutations. The interactive model with the highest cross-validation consistency, followed by better predictive accuracy, and the parsimony of parameter number was chosen as the "best model."

Because a previous study on the relations of *SOD2* genetic variants to METH-induced psychosis had a group of Han-Chinese nonusers as controls [16], we compared them with this study's controls of METH users without psychosis to evaluate whether the *SOD2* genetic variants were associated with METH use *per se*.

Table 5. Effects of the genotypes of individual single-nucleotide polymorphisms in superoxide dismutase 2 gene and dystrobrevin-binding protein 1 gene under a codominant model in a case-control study of methamphetamine-induced psychosis in Taiwan (84 cases of methamphetamine-induced psychosis and 187 controls of methamphetamine users without psychosis)

Polymorphism	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
SOD2		
SNP 1		
C/T versus C/C	1.19 (0.68 - 2.09)	1.36 (0.74 - 2.51)
T/T versus C/C	2.97 (0.87 - 10.15)	3.85 (0.97 - 15.28)
SNP 2		
C/T versus T/T	1.04 (0.57 - 1.89)	1.31 (0.69 - 2.52)
C/C versus T/T	11.89 (1.36 - 103.99)*	21.31 (2.15 - 211.77)**
SNP 3		
C/A versus A/A	0.99 (0.54 - 1.80)	1.38 (0.72 - 2.65)
C/C versus A/A	9.27 (1.01 - 84.65)	18.45 (1.71 - 198.74)
SNP 4		
G/T versus T/T	1.24 (0.64 - 2.38)	1.05 (0.52 - 2.11)
G/G versus T/T	1.06 (0.51 - 2.22)	0.89 (0.41 - 1.98)
SNP 5		
T/A versus A/A	1.17 (0.63 - 2.16)	1.16 (0.59 - 2.29)
T/T versus A/A	0.93 (0.44 - 1.94)	1.07 (0.49 - 2.36)
DTNBP1		
SNP 7		
G/C versus C/C	0.81 (0.46 - 1.41)	0.73 (0.39 - 1.34)
G/G versus C/C	0.58 (0.25 - 1.32)	0.62 (0.25 - 1.51)
SNP 8		
A/A versus A/G	0.67 (0.11 - 4.08)	1.17 (0.12 - 11.59)
SNP 9		
T/A versus A/A	2.29 (0.59 - 9.37)	3.45 (0.62 - 19.27)

*$p < 0.05$; **$p < 0.01$.
SNPs, single-nucleotide polymorphisms; *SOD2*, superoxide dismutase 2 gene; *DTNBP1*, dystrobrevin-binding protein 1 gene; OR, odds ratio; CI, confidence interval

Results

Table 1 shows that the distributions in age, gender, and other substance dependence were similar between the cases and controls, whereas the initiation age of METH use was younger and the duration of METH use, regardless of total or actual, was longer for cases than controls. The total and actual durations of METH use were highly correlated in respondents with available information on both, with a Pearson's correlation coefficient of 0.62.

The distributions of the genotypes of the eight SNPs were all within the values expected from Hardy–Weinberg equilibrium (Table 2). The frequencies of the alleles and genotypes for individual SNPs in the controls and cases and the two subtypes of cases are shown for *SOD2* in Table 3 and for *DTNBP1* in Table 4. For *SOD2*, none of the individual SNPs were associated with METH-induced psychosis after adjustment for multiple comparisons (Table 3). But, after stratification by the length of psychotic symptoms, both rs4880 and rs2855116 were significantly associated with prolonged METH-induced psychosis ($p < 0.01$ and $p < 0.01$, respectively). In contrast, neither allelic nor genotypic frequencies were different between the cases and controls for the three SNPs in *DTNBP1*, and the subtype analyses did not show any associations with the SNPs (Table 4).

Table 5 shows multivariable logistic regression analyses on factors of age, gender, and total duration of METH use as covariates, to adjust for the influence of potential confounders on the relationships of these SNPs to METH-induced psychosis. Under the assumption of a codominant model of SNPs that used the most common genotype as a reference, the CC genotype of rs4880 was significantly associated with METH-induced psychosis (adjusted odds ratio = 21.31, 95% confidence interval = 2.15–211.77, $p < 0.01$).

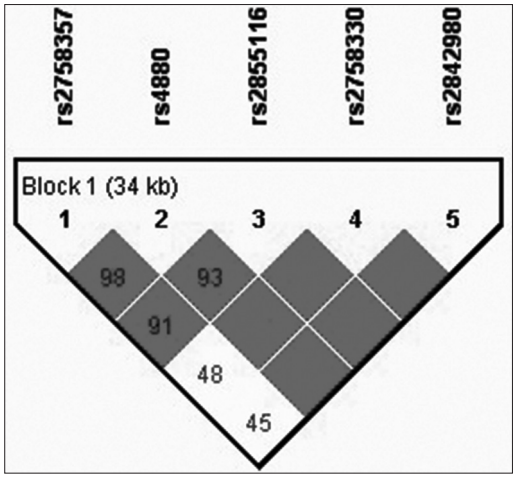

Figure 1. The linkage disequilibrium plot of five SNPs on *SOD2* in a case–control study of methamphetamine-induced psychosis in Taiwan (84 cases of methamphetamine-induced psychosis and 187 controls of methamphetamine users without psychosis). SNPs, single-nucleotide polymorphisms; *SOD2*, superoxide dismutase 2 gene.

Table 6. Distribution of haplotypes on superoxide dismutase 2 gene and dystrobrevin-binding protein 1 gene, respectively, and their association with the illness in a case-control study of methamphetamine-induced psychosis in Taiwan (84 cases of methamphetamine-induced psychosis and 187 controls of methamphetamine users without psychosis)

Gene	Haplotype	Frequencies		*p*-value for haplotype-disease association
		Cases	Controls	
SOD2	CGAGA	0.4700	0.4756	Reference
	CGATT	0.2340	0.2275	0.59
	CGATA	0.0517	0.1131	0.09
	CGCTT	0.0060	0.0081	0.97
	CACTT	0.0061	0.0001	0.99
	TGAGA	0.0538	0.0406	0.55
	TAATT	0.0238	0.0108	0.43
	TACTT	0.0991	0.1157	0.95
	TACTA	0.0555	0.0086	0.19
DTNBP1	CAA	0.6250	0.5695	Reference
	GAA	0.3512	0.4171	0.20
	GAT	0.0119	0.0053	0.48
	CGA	0.0000	0.0027	NA
	CGT	0.0119	0.0053	0.54

None of the haplotypes were significantly associated with METH-induced psychosis. The haplotypes of *SOD2* consisted of rs2758357, rs4880, rs2855116, rs2758330, and rs2842980, whereas those of *DTNBP1* consisted of rs2619539, rs3213207, and rs2619538. *SOD2*, superoxide dismutase 2 gene; *DTNBP1*, dystrobrevin-binding protein 1 gene; METH, methamphetamine

Based on the LD matrix, one haplotype block was identified for *SOD2* (Figure 1) that consisted of all five genotyped SNPs, and a three-marker haplotype was identified for *DTNBP1* (Figure 2). None of the haplotypes were significantly associated with METH-induced psychosis (Table 6).

For a variety of interactive models examined for gene–gene interactions using the GMDR method, ranging from one way to four way, the accuracy that was adjusted for study design was low, and the cross-validation consistencies were equal to 5/10 or less. None of the models reached a significant effect for the prediction accuracy (Table 7).

We compared the distributions of five *SOD2* SNPs in this study's control group of METH users without psychosis versus the nonusers of the Han-Chinese controls in a previous study to evaluate the association of METH use *per se* with the SNPs examined in this study [16]. No differences in the distributions of the markers between the two groups were observed (Table 8). Therefore, *SOD2* was most likely not associated with the initiation of METH use.

Discussion

This study compared METH users with psychosis with METH users without psychosis and clarified the association of certain genetic variants in *SOD2* or *DTNBP1* with METH-induced psychosis rather than METH use. The results (Table 2)

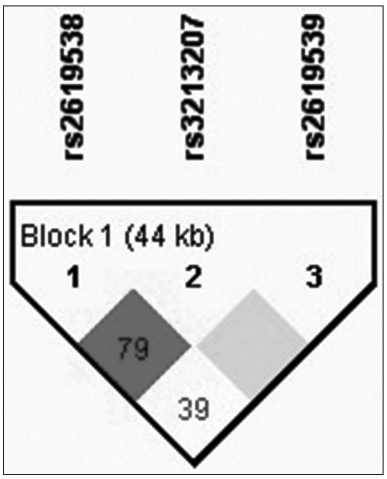

Figure 2. The linkage disequilibrium plot of three SNPs on *DTNBP1* in a case–control study of methamphetamine-induced psychosis in Taiwan (84 cases of methamphetamine-induced psychosis and 187 controls of methamphetamine users without psychosis). SNPs, single-nucleotide polymorphisms; *DTNBP1, dystrobrevin-binding protein 1* gene.

showed that only one SNP in *SOD2* was significantly associated with the prolonged subtype of METH-induced psychosis after correction for multiple comparison and adjustment for covariates ($p < 0.01$). But, no such association was found for *DTNBP1* (Table 3). We suggest that the specific association with *SOD2* rather than *DTNBP1* implies that the etiology of METH-induced psychosis is more likely related to oxidative stress following METH consumption.

Among the five SNPs in *SOD2* examined in this study (Table 2), the frequency of the CC genotype of rs4880 was significantly increased from 0.53% in the METH users without psychosis to 5.95% in the patients with METH-induced psychosis and to 9.52% in the patients with prolonged psychosis ($p < 0.05$). As also shown in Table 2, the association with the prolonged subtype of METH-induced psychosis was significant after correction for multiple comparisons ($p < 0.01$). The production of neurotoxicity or oxidative stress after METH use is dose dependent [28], and a dose–response relationship for the development of METH-induced psychosis has been observed [12, 29]. Further adjusting for this potential confounder using the duration of METH use as a proxy, we found that the association of the CC genotype of rs4880 with the prolonged subtype of METH-induced psychosis remained after adjustment for METH use duration.

We observed that other SNPs on *SOD2* did not increase the strength of the association in their haplotypes with rs4880 (Figure 1). This observation further indicates that the functional polymorphism of rs4880– from the C allele to the Ala variant– allows for a more efficient uptake of the SOD2 enzyme into the mitochondrial matrix [30], and that a more active enzyme [15] is involved in METH-induced psychosis. This hypothesis is further supported by the finding that the genetic variants in *SOD2* were not related to METH use

because their genotype distributions in METH users without psychosis in this study have been similar to the Han-Chinese normal controls of nonusers in a previous study [16].

The involvement of *SOD2* with METH-induced psychosis is supported by cell line and animal studies. First, oxidative stress plays a potent rôle in METH toxicity. The ingestion of METH increases the dopamine levels within nerve terminals, which is associated with producing harmful superoxide radicals and hydrogen peroxide during quinone redox cycling [31]. METH can disturb the balance between the capacity of antioxidant enzyme systems to scavenge reactive oxygen species (ROS) and the production of RO [32]. A thiol antioxidant, *N*-acetylcysteine, protects against METH-induced cell death [33], and giving ascorbic acid or Vitamin E also protects against METH-induced oxidative stress in animal studies [34]. Second, METH toxicity is attenuated when *SOD2* is overexpressed. For example, transgenic mice that overexpressed human *SOD2*, which results in higher SOD2 enzyme activity, protects against METH toxicity [35].

The frequency of the Ala variant-related C-allele of rs4880 in *SOD2* was higher, rather than lower, in patients with METH-induced psychosis in this study (Tables 2 and 5). The Ala variant allows for more efficient uptake of the SOD2 enzyme into the mitochondrial matrix to protect against METH-induced oxidative stress, which is contrary to expectations. But, this result agrees with that from previous studies on METH-induced psychosis [16] and other diseases (such as Alzheimer's disease, breast cancer, gastric cancer, and Parkinson's disease) [36-38]. Some explanations for this paradox may be offered. First, the SOD2 enzyme may play a dual rôle in ROS exposure. SOD2 is clearly an important scavenger of ROS, but potential harmful effects may result from its production of hydrogen peroxide (H_2O_2) under certain circumstances, causing secondary oxidative damage, especially in individuals with low catalase

Table 7. Comparison of various best models for individual single-nucleotide polymorphisms, two-way, three-way, and four-way interactions among eight single-nucleotide polymorphisms from superoxide dismutase 2 gene and dystrobrevin-binding protein 1 gene after adjustment for age, gender, and methamphetamine use duration in a case-control study of methamphetamine-induced psychosis in Taiwan (84 cases of methamphetamine-induced psychosis and 187 controls of methamphetamine users without psychosis)

Best model	Test-balanced accuracy	Cross-validation consistencies	*p*
SOD2 SNP1 (rs2758357)	0.4910	4/10	0.15
SOD2 SNP3 (rs2855116), *DTNBP1 SNP7* (rs2619539)	0.4386	5/10	0.23
SOD2 SNP3 (rs2855116), *SOD2* SNP4 (rs2758330), *DTNBP1 SNP7* (rs2619539)	0.4520	5/10	0.39
SOD2 SNP1 (rs2758357), *SOD2* SNP3 (rs2855116), *SOD2* SNP4 (rs2758330), *DTNBP1 SNP7* (rs2619539)	0.4359	5/10	0.66

p-value was derived from 1,000 permutations over the disease status.
SNPs, single-nucleotide polymorphisms; SOD2, superoxide dismutase 2 gene; *DTNBP1*, dystrobrevin-binding protein 1 gene

Table 8. Comparing the distributions of five superoxide dismutase 2 gene single-nucleotide polymorphisms in this study's control group of methamphetamine users without psychosis ($n = 187$) versus the counterparts of the Han-Chinese normal controls in Nakamura et al. (2006) ($n = 200$)

Marker	SNP	Sample	Genotype, *n* (%)			*p*	Allele, n (%)		*p*
			T/T	T/C	C/C		T	C	
SNP1	rs2758357	Normal CN	4 (2.00)	70 (35.00)	126 (63.00)		78 (19.50)	322 (80.50)	
		METH user	5 (2.67)	56 (29.95)	126 (67.38)	0.5530	66 (17.65)	308 (82.35)	0.5080
Marker	SNP	Sample	C/C	C/T	T/T	*p*	C	T	*p*
SNP2	rs4880	Normal CN	5 (2.47)	47 (23.27)	150 (74.26)		57 (14.11)	347 (85.59)	
		METH user	1 (0.53)	48 (25.67)	138 (73.80)	0.3264	50 (13.37)	324 (86.63)	0.7646
Marker	SNP	Sample	C/C	C/A	A/A	*p*	C	A	*p*
SNP3	rs2855116	Normal CN	4 (1.97)	48 (23.56)	151 (74.38)		56 (13.79)	350 (86.21)	
		METH user	1 (0.53)	47 (25.13)	139 (74.33)	0.5052	49 (13.10)	325 (86.90)	0.7774
Marker	SNP	Sample	G/G	G/T	T/T	*p*	G	T	*p*
SNP4	rs2758330	Normal CN	41 (20.50)	105 (52.50)	54 (27.00)		187 (46.75)	213 (53.25)	
		METH user	52 (27.96)	89 (47.85)	45 (24.19)	0.2305	193 (51.88)	179 (48.12)	0.1541
Marker	SNP	Sample	T/T	T/A	A/A	*p*	T	A	*p*
SNP5	rs2842980	Normal CN	55 (27.50)	104 (52.00)	41 (20.50)		214 (53.50)	186 (46.50)	
		METH user	46 (24.60)	89 (47.59)	52 (27.81)	0.2423	181 (48.40)	193 (51.60)	0.1557

Nonsignificant differences in all comparisons for genotype and allele of five *SOD2* SNPs.
Normal CN, the Han-Chinese normal controls in the study of Nakamura *et al*. (2006); METH user, this study's control group of METH users. *SOD2*, superoxide dismutase 2 gene; SNPs, single-nucleotide polymorphisms, METH, methamphetamine

or glutathione peroxidase, which induces detoxification by converting H_2O_2 into water. Second, superoxide may act as a lipid peroxidation terminator. The overscavenging of free radicals increases lipid peroxidation and causes cell injury [39].

In contrast to *SOD2*, the genetic variants of *DTNBP1* did not show any association with METH-induced psychosis in the single locus or haplotype analyses (Table 6). In contrast, a previous study using healthy nonusers as controls reported an association between the *DTNBP1* gene and METH-induced psychosis [18]. In addition to the differences in the phenotype of the controls, another possibility for this discrepancy is an ethnic difference in allele frequencies; the genotype frequency of A/G in patients with METH-induced psychosis was 2.38% in this study and 11.2% in a Japanese counterpart [18]. This hypothesis is supported by a similar lack of association of *DTNBP1* with schizophrenia in the Taiwanese population [40]. The low frequency of *DTNBP1* SNPs genotyped in this study might also account for the lack of its interactive effect with SOD2. Future studies should evaluate genetic variants with a higher frequency using deep sequencing to clarify the role of *DTNBP1* in METH-induced psychosis in the Han-Chinese population.

Study limitations

The readers are warned against overinterpreting our study results because this study has the following three limitations:

- Our study participants were recruited primarily from a detention center, and the generalizability of the results to community samples may be limited
- Some unknown population stratification may have existed despite the use of cases of Han-Chinese ancestry
- The sample size of our study did not have sufficient power to detect a genetic effect of moderate or modest magnitude, such as an odds ratio of < 1.5, or alleles of low frequency, such as < 15%.

Summary

This study is the first investigation using METH users without psychosis as controls in the assessment of the relationship between genetic variants of *SOD2* and METH-induced psychosis. Our results suggest that only the Ala variant-related C-allele of rs4880 in *SOD2* was associated with the prolonged subtype of METH-induced psychosis. This finding supports further investigation of the rôle of oxidative stress mechanisms in the development of METH-induced psychosis.

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Conflicts of Interest

There are no conflicts of interest.

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