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Association of the manganese superoxide dismutase gene Ala–9Val polymorphism with clinical phenotypes and tardive dyskinesia in schizophrenic patients

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ABSTRACT

Objective: Several recent studies that have investigated the genetic association between the manganese superoxide dismutase (*MnSOD*) gene Ala–9Val single-nucleotide polymorphism (SNP) and tardive dyskinesia (TD) have produced conflicting results. This study was to investigate whether this SNP was associated with clinical phenotypes and antipsychotic-induced tardive dyskinesia (TD) in schizophrenia in a genetically homogeneous Han Chinese inpatient population.
Methods: Genotyping was performed for the *MnSOD* gene Ala–9Val SNP in Chinese schizophrenia patients with ($n=176$) and without TD ($n=346$). The severity of TD was assessed using the abnormal involuntary movement scale (AIMS), and psychopathology using the Positive and Negative Syndrome Scale (PANSS).
Results: The frequencies of genotypes and alleles did not differ significantly between schizophrenic patients with and without TD (both $p>0.05$). Also, there was no significant difference in the AIMS total score between the Val/Val and Ala allele carrier groups ($p>0.05$). However, the PANSS negative symptom subscore was significantly higher in patients with Val/Val genotype (21.8 ± 7.3) than those with Ala alleles (20.1 ± 7.7) ($t=2.32$, $p=0.03$).
Conclusion: While the *MnSOD* gene Ala–9Val polymorphism did not play a major role in the susceptibility to TD in schizophrenic patients, it might be associated with negative symptoms of schizophrenia.

1. Introduction

Several lines of recent studies have indicated that free radicals or oxidative stress may be associated with the development of tardive dyskinesia (TD) (Lohr et al, 2003). Superoxide dismutase (SOD), the first line antioxidant defense enzyme plays a critical role in protecting cells against damage derived from free radicals. SOD has been found to be altered in TD patients (Lohr, 1991; Lohr and Browning, 1995; Lohr et al., 2003; Reddy and Yao, 1996; Yao et al., 2001). For example, SOD has been found to be lower in erythrocytes, plasma and CSF in patients with TD (Lohr et al., 1990; Yamada et al., 1997; Tsai et al., 1998; Zhang et al., 2003, 2007), and decreased SOD levels are significantly correlated with dyskinetic movements in TD patients (Zhang et al., 2003).

There are three isoforms of SOD which contain different prosthetic groups: the manganese (Mn) isoform (SOD2) is found in the mitochondria, and the copper and zinc (CuZn) isoforms are present in the cytoplasm (SOD1) as well as in the extracellular space (SOD3). Especially, the manganese SOD (MnSOD) is an intramitochondrial enzyme that scavenges the greatest number of superoxide anions produced from the electron transport systems in mitochondria, which account for more than 95% of all oxygen consumption in aerobic cells (Robinson, 1998). Also, MnSOD plays a role in neurodevelopment, specifically growth termination and differentiation initiation (Mahadik and Mukherjee, 1996). The human *MnSOD* gene is located in the long arm (6q25) of chromosome 6, previously known as a candidate region for linkage with schizophrenia (Lindholm et al., 2001). Among known

Abbreviations: AIMS, Abnormal Involuntary Movement Scale; ANOVA, one-way analysis of variance; MnSOD, manganese superoxide dismutase; PANSS, Positive and Negative Syndrome Scale; SNP, single-nucleotide polymorphism; TD, tardive dyskinesia.

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Table 1
Demographics of studies shown in the literature and present study.

Reference	Country/origin	Subject	*N*	MAF (Ala)	Ala/Ala	Ala/Val	Val/Val	*p*
Hori et al. (2000)	Japanese	TD	39	0.04	0 (0.00)	3 (0.08)	36 (0.92)	0.04
		Non-TD	153	0.14	3 (0.02)	36 (0.24)	114 (0.75)	
Zhang et al. (2002)	Chinese	TD	42	0.14	0 (0.00)	12 (0.29)	30 (0.71)	n.s
		Non-TD	59	0.18	0 (0.00)	21 (0.36)	38 (0.64)	
Akyol et al. (2005)	Tukisch	TD	23	0.35	2 (0.09)	12 (0.52)	9 (0.39)	n.s
		Non-TD	130	0.45	12 (0.09)	94 (0.72)	24 (0.19)	
Pae et al (2007)	Korean	TD	44	0.14	0 (0.00)	12 (0.27)	32 (0.73)	n.s
		Non-TD	218	0.09	0 (0.00)	37 (0.18)	181 (0.82)	
Hitzeroth et al. (2007)	Xhosa	TD	30	0.50	9 (0.30)	12 (0.40)	9 (0.30)	0.011
		Non-TD	204	0.40	22 (0.11)	121 (0.59)	61 (0.30)	
Kang et al. (2008)	Korean	TD	83	0.12	3 (0.04)	14 (0.17)	66 (0.80)	n.s
		Non-TD	126	0.08	1 (0.01)	19 (0.15)	106 (0.84)	
Pae (2008)	Korean	TD	44	0.14	0 (0.00)	12 (0.27)	32 (0.73)	n.s
		Non-TD	63	0.18	0 (0.00)	22 (0.35)	41 (0.65)	
Zai et al. (2010)	Caucason	TD	76	0.48	21 (0.28)	31 (0.41)	24 (0.32)	n.s.
		Non-TD	114	0.53	30 (0.26)	60 (0.53)	24 (0.21)	
Present study	Chinese	TD	176	0.13	2 (1.1)	43 (24.4)	131 (74.4)	n.s.
		Non-TD	346	0.15	0 (0.0)	103 (29.8)	243 (70.2)	

Abbreviations: TD = tardive dyskinesia; MAF, minor allele frequency. n.s. = non significance.

functional polymorphisms of *MnSOD*, the Ala–9Val polymorphism (rs4880) in exon 2 of the *MnSOD* gene is the most extensively studied SNP in schizophrenia, with the Ala-to-Val substitution possibly resulting in the alteration of MnSOD activity in human mitochondria (Shimoda-Matsubayashi et al., 1997). Since MnSOD plays a vital role in the antioxidant defense system as well as neurodevelopment, a change in enzyme concentration through the action of Ala–9Val, may result in free radical accumulation and hence cell injury.

The development of TD in a subset of patients (20–40%), familial nature, and concordance amongst twins indicate a possible genetic basis (Basile et al., 2002; Müller et al., 2001, 2004). Recently, several studies that have investigated the genetic association between the *MnSOD* gene Ala–9Val SNP and TD have produced conflicting results (see Table 1). For example, Hori et al. (2000) and Hitzeroth et al. (2007) confirmed an association in a Japanese and Xhosa population, while Zhang et al. (2002), Akyol et al. (2005), Pae et al. (2007), Kang et al. (2008) and Zai et al. (2010) did not find such an association in the Chinese, Turkish, Korean and Caucasian population, respectively. A recent meta-analysis by Bakker et al. (2008) found a protective effect against TD for the Val carriers using Ala/Ala homozygotes as a reference category. However, the most recent association study and meta-analysis did not reveal a significant association of the alleles or genotypes with TD occurrence (Zai et al., 2010). Thus, the picture emerging is that the association between *MnSOD* gene Ala–9Val SNP and TD deserves further examination in cases of schizophrenia.

These earlier studies have had comparatively small sample sizes, which often leads to false positive results. The number of TD patients ($n = 134$) in the meta-analysis was also not large (Bakker et al., 2008). Furthermore, the relative allelic frequencies of this polymorphism vary considerably between different ethnic groups (Zai et al., 2010), which may be attributed to the difference in genetic vulnerability to the development of TD in different ethnic populations (Kang et al., 2008). We therefore undertook the present study with a comparatively larger sample to examine these associations between TD and the *MnSOD* Ala–9Val gene polymorphism in ethnically homogeneous samples of Chinese Han patients with schizophrenia. We assessed TD using both continuous [Abnormal Involuntary Movement Scale (AIMS)] and dichotomous (TD occurrence) measures.

2. Methods and materials

2.1. Subjects

Five hundred and twenty-two schizophrenic patients (male/female = 352/170) were recruited from Beijing Hui Long-Guan hospital, a Beijing-city-owned psychiatric hospital. Diagnoses were made for each patient by two independent experienced psychiatrists on the basis of the Structured Clinical Interview for DSM-IV (SCID). All schizophrenic patients had been receiving stable doses of oral antipsychotic drugs for at least 12 months before entry into the study. All patients were chronic, with at least 5 years of illness, were Han Chinese, and were between 25 and 75 years old. The mean duration of hospitalization was 10.0 ± 9.5 years.

A complete medical history, physical examination and laboratory tests were obtained from patients. All patients were in good physical health, and any subjects with major medical illnesses or drug and alcohol abuse/dependence were excluded. All patients gave written informed consent, which was approved by the Institutional Review Board of Beijing Hui Long Guan hospital.

2.2. Clinical measures

A detailed questionnaire including general information, socio-demographic characteristics, smoking behavior, medical and psychological conditions was administered to each subject by a member of the research staff. Additional information was collected from available medical records and collateral data. Additional visits were requested for subjects with missing or ambiguous data.

Four experienced psychiatrists who were blinded to the clinical status of the patients assessed the severity of tardive dyskinesia using the AIMS. TD was diagnosed using the criteria of Schooler and Kane (1982). The first seven items determine severity of abnormal movements for each of seven respective regions of the body. When a patient had one AIMS score of at least 3 (moderate degree) in any body part or with at least 2 (mild degree) in two or more body parts in seven AIMS items mentioned above, he/she was considered to have TD, and the others were assumed not to have TD. At the same time, the same four psychiatrists assessed the patients' psychopathology using the Positive and Negative Syndrome Scale (PANSS).

All these raters for the AIMS and the PANSS rating scales had simultaneously attended a training session in the use of the AIMS and the PANSS before this study start. After training, repeated assessments documented that a correlation coefficient greater than 0.8 was maintained for the PANSS or AIMS total score. All evaluations were carried out prior to the laboratory procedures.

2.3. Genetic analysis

DNA was extracted using standard protocols. *MnSOD* genotyping was performed by a polymerase chain reaction (PCR) under modified

Table 2
Demographics of the patients with schizophrenia ($n=522$) according to MnSOD polymorphism.

	Genotypes	
	Ala/Ala + Ala/Val	Val/Val
Sex (male/female)	102/46	250/124
Age (years)	49.0 ± 10.1	50.8 ± 9.9
Age at onset (yeaars)	24.4 ± 6.7	24.7 ± 7.0
Number of hospitalization	3.4 ± 2.2	3.8 ± 3.1
Duration of current antipsychotics (years)	10.0 ± 9.8	9.4 ± 8.5
Current antipsychotic dose (chlorpromazine equivalent)	513 ± 551	483 ± 508
PANSS total score	59.8 ± 13.6	62.0 ± 14.5
P subscore	13.1 ± 5.2	13.1 ± 5.6
N subscore	20.1 ± 7.7[a]	21.8 ± 7.3
G subscore	26.6 ± 5.2	27.1 ± 6.1
AIMS score	3.3 ± 3.7	3.6 ± 4.3

[a] Indicates the comparison between Val/Val and Ala carrier groups; $p<0.05$.

conditions, according to a previously reported method (Zhang et al., 2002). The PCR product (246 bp) was digested with 5 units of BsaW, and then underwent electrophoresis in a 3.5% agarose gel, with ethidium bromide, giving fragments of 246 bp for the allele (Ala-9) or 164 bp and 82 bp for the allele (Val-9). Genotyping was duplicated and carried out blind to the clinical status.

2.4. Statistical analysis

Deviations from Hardy–Weinberg equilibrium (HWE) were assessed using the HWSIM program (Cubells et al., 1997). Differences of the *MnSOD* Ala–9Val allele and genotype frequencies between patients with TD and without TD were evaluated using χ^2 tests. Group differences were compared using Student's two-sample *t*-test or one-way analysis of variance (ANOVA) for continuous variables and chi-squared for categorical variables. A logistic regression analysis was also performed to adjust potential confounding factors for TD using TD as a dependent variable and genotype, sex, age, duration of illness, age of onset, type (atypical *vs* typical antipsychotics drug) and dose of drugs (equivalent to chlorpromazine) and duration of antipsychotic treatments as independent variables. All statistical analyses were two-tailed, and the level of statistical significance was set at $p<0.05$.

3. Results

The Val/Val, Val/Ala, and Ala/Ala genotypes of the *MnSOD* gene Ala–9Val SNP were found in 374 (71.6%), 146 (28.0%) and 2 (0.4%) of the 522 patients, respectively. The overall genotype distribution of *MnSOD* gene Ala–9Val SNP conformed with the Hardy–Weinberg equilibrium ($p>0.05$).

Demographic variables, clinical variables, and AIMS scores in the *MnSOD* Ala–9Val genotype groups are shown in Table 2. The PANSS total score and its subscale scores were normally distributed across the patients and among the genotype categories (all $p>0.05$). They were treated as a continuous variable for the *t*-test. There was a significant difference in the negative symptom subscore of the PANSS between patients with Val/Val genotype and Ala allele (Val/Ala + Ala/Ala) ($t=2.32$, $p=0.03$; effect size: Cohen's $d=0.23$). However, there was no significant difference between these two groups in terms of age, sex, duration of illness, schizophrenia subtype, current neuroleptic doses, duration of antipsychotic treatment, number of hospitalization, smoking status or the PANSS total and the other subscore scores (all $p>0.05$).

The frequencies of genotypes ($\chi2=5.41$, df = 2, $p=0.067$) and alleles ($\chi2=0.45$, df = 1, $p=0.51$) did not differ significantly between schizophrenic patients with and without TD (Table 3). Total AIMS scores in patients with the two groups were 3.6 ± 4.3 for Val/Val, and 3.3 ± 3.7 for Val/Ala + Ala/Ala ($t=0.81$, $p=0.42$; effect size: Cohen's $d=0.075$).

As presented in Table 4, there was a significant difference in age ($t=7.36$, $p<0.0001$), duration of illness ($t=6.29$, $p<0.0001$), and numbers of hospitalization ($t=3.58$, $p<0.0001$) between those with and without TD. One hundred and seventeen patients (22.4%) received typical antipsychotic drugs and 405 patients (77.6%) received atypical antipsychotic drugs. More patients who were receiving typical antipsychotic drugs developed TD than patients who were received atypical antipsychotic drugs (43.6% *vs.* 30.9%; $\chi2=6.58$, df = 1, $p<0.05$). Moreover, TD patients had been taking antipsychotic drugs longer than those without TD ($t=5.12$, $p<0.0001$). However, there was no a significant difference in doses of antipsychotics between patients with and without TD. In addition, patients with residual type schizophrenia had a higher rate of TD than all the other types ($\chi2=15.4$, $p=0.004$). Smoking was more common in patients without TD (48.0%) than with TD (36.4%; $\chi2=5.97$, $p=0.015$). Fewer women (22.4%, 38 of 170) than men (39.2%, 138 of 352) suffered from TD ($\chi2=14.6$, $p<0.0001$). However, no significant differences in current occupation, marital status, and education level were found between those with and without TD.

The logistic regression analysis was used to examine whether or not any of demographic variables, clinical variables and *MnSOD* genotype was a risk factor for TD. We found that greater negative symptom ($p=0.003$, OR = 1.07, CI = 1.02–1.12), male [$p=0.006$, odds ratio (OR) = 2.38, 95% confidence interval [CI] = 1.33–4.35), and the old age ($p=0.012$, OR = 1.06, CI = 1.01–1.10) were the risk factors for TD. This logistic regression model, however, showed no association between *MnSOD* gene Ala–9Val SNP and TD ($p=0.69$). As we used Val/Val genotype as a reference in logistic regression, we found that the odds ratio for developing TD was 0.92 ($p=0.73$, 95% CI = 0.57–1.47) in subjects with Val/Ala and Ala/Ala genotypes.

In addition, we tested for differences in AIMS scores between *MnSOD* genotypes in the TD group. Independent *t* tests revealed no differences in the AIMS total ($t=0.75$, $p=0.46$), orofacial ($t=0.79$, $p=0.42$), and limb–trunk ($t=0.37$, $p=0.72$) scores among the two genotype groups.

4. Discussion

This study had three major findings. (1) There was no significant association between TD and the *MnSOD* Ala–9Val gene polymorphism. (2) A significantly higher negative symptom score was observed in patients with Val/Val genotype than those with Ala alleles. (3) Negative symptoms, male and the old age were major risk factors for TD.

Table 3
Comparison of the MnSOD Ala–9Val genotype and allele frequencies between schizophrenia with ($n=176$) and without TD ($n=346$).

	Genotypes			χ^2	*p*	Allele frequencies		χ^2	*p*
	Ala/Ala	Val/Ala	Vla/Vla			Val	Ala		
TD	2 (1.1%)	43(24.4%)	131(74.4%)	5.41	0.067	305(86.6%)	47(13.4%)	0.45	0.51
Non-TD	0 (0.0%)	103(29.8%)	243(70.2%)			589(85.1%)	103(14.9%)		

MnSOD: manganese superoxide dismutase; TD: tardive dyskinesia.

Table 4
Characteristics of patients with schizophrenia with or without TD.

Characteristic	Patients with TD ($n=176$)	Patients without TD ($n=346$)	t or $\chi 2$	p value
Gender			14.6	0.000
Female	38 (21.6%)	132 (38.2%)		
Male	138 (78.4%)	214 (61.8%)		
Age (years)	54.4 ± 8.6	47.0 ± 11.4	7.36	0.000
Duration of illness (years)	29.5 ± 9.8	22.9 ± 11.5	6.29	0.000
Age of onset	25.2 ± 6.9	24.2 ± 7.2	1.53	n.s.
Antipsychotics			6.58	0.01
Atypicals	125 (71.0%)	280 (80.9%)		
Typicals	51(29.0%)	66 (19.1%)		
Daily AP dose (mg)				
(CPZ equivalent)	479 ± 468	505 ± 577	0.49	n.s.
Duration of treatment	155 ± 113	101 ± 107	5.12	0.000
Hospitalization numbers	4.1 ± 2.6	3.2 ± 2.8	3.58	0.000
Subtypes of schizophrenia			15.4	0.004
Disorganized type	8 (4.5%)	21 (6.1%)		
Paranoid type	56 (31.2%)	143 (41.3%)		
Undifferentiated type	12 (6.8%)	18 (5.2%)		
Residual type	94 (53.4%)	154 (44.5%)		
Others	6 (3.4%)	10 (2.9%)		
Smokers/nonsmokers	59/103 (36.4%)	160/173 (48.0%)	5.97	0.015

Several previous studies investigated the association of the *MnSOD* polymorphism with schizophrenia or TD, with inconsistent results (Hori et al., 2000; Zhang et al., 2002; Akyol et al., 2005; Hitzeroth et al., 2007; Pae et al., 2007; Kang et al., 2008). For example, Hori et al. (2000) found a significant association between the *MnSOD* gene Ala–9Val SNP and TD, and suggested that the –9Ala allele played a role in protecting against susceptibility to TD in Japanese schizophrenic patients. While Hitzeroth et al., (2007) reported that the *MnSOD* Ala–9Val polymorphism was involved in the development and severity of TD, the –9Val allele could protect against the development of TD in Xhosa schizophrenic patients. However, Zhang et al. (2002), Pae et al. (2007) and Zai et al. (2010) did not find any significant differences in Ala–9Val SNP genotypes or alleles between schizophrenic patients with and without TD among the Chinese, Korean and Caucasian population. Several factors, such as different ethnicity, sample size, differences in definitions of TD (the Glazer–Morgenstern criteria *vs* the Schooler–Kane criteria), treatment history, gender of subjects, environmental factors (such as diet) and other factors that are associated with varying degrees with the expression of TD or the biological heterogeneity of TD may be responsible for the discrepancy.

The previous studies mostly involved small samples of TD patients ($n=30$–83) for genetic association studies (Hitzeroth et al., 2007; Hori et al., 2000; Kang et al., 2008; Pae et al., 2007; Zhang et al., 2002;). The number of TD patients ($n=134$) in the meta-analysis was also not large (Bakker et al., 2008), and the sample comprised different ethnic groups, with subjects from Turkey, Xhosa, Japan, China, and North America (Akyol et al., 2005; Hitzeroth et al., 2007; Hori et al., 2000; Kang et al., 2008; Zhang et al., 2002; Zai et al., 2010). While the increased power of such meta-analysis can provide a more precise estimate, the inclusion of multiple ethnicities is likely to cause population stratification bias (Kang et al., 2008).

It is important to note that the genotype frequency of the *MnSOD* gene Ala–9Val SNP differs considerably with ethnicity. For example, there were no or only a very small number of Ala/Ala genotypes in the Asian samples, while patients from Turkey, Xhosa and Caucasian showed higher frequencies of the –9Ala allele and Ala/Ala genotype frequencies (Akyol et al., 2005; Hitzeroth et al., 2007; Kang et al., 2008; Zai et al., 2010). Thus, interethnic differences in the genotype frequencies of the *MnSOD* gene Al–9Val polymorphism may play an important role that may account for the inconsistent results seen in different samples from the different populations. In our present study, we recruited a comparatively large sample size in a genetically more homogeneous Han Chinese inpatient population to avoid such bias. We found no significant association between TD and the *MnSOD* gene Ala–9Val SNP in Chinese schizophrenic patients, suggesting that it is unlikely that this polymorphism might play a major role in the pathogenesis of TD, at least in Chinese population.

Interestingly, our results suggest that the presence of the –9Ala allele was associated with fewer negative symptoms in schizophrenia. To our knowledge, this is the first report showing that *SOD* genotype might be associated with the clinical symptoms of schizophrenia. However, the –9Val allele is thought to be a wild type allele, while the –9Ala allele is thought to be a mutant allele. In fact, the –9Val allele was much more common, with an allele frequency of 0.86, than the –9Ala allele, with a frequency of 0.14, in patients in our present study. Consequently, it may be difficult to interpret these findings. In a previous study, however it was shown that the –9Ala *MnSOD* allele played a role in protecting against susceptibility to TD in schizophrenia (Hori et al., 2000). Our present study and some other studies have shown that negative symptoms are the important risk factors for the development of TD (Caligiuri et al., 1997; van Os et al., 2000). Furthermore, the association between negative symptoms and TD may be an intrinsic part of the psychotic disease process (McCreadie et al., 1996; van Os et al., 1997). Therefore, our finding of the association between the presence of the –9Ala allele and fewer negative symptoms might have a similar underlying mechanism as Horri et al.'s finding that the –9Ala *MnSOD* allele may play a role in protecting against susceptibility to TD in schizophrenia. In addition, previous studies have suggested that the Ala allele is associated with higher activity, which results in more efficient transport of MnSOD into the mitochondria (Hiroi et al., 1999; Shimoda-Matsubayashi et al., 1996), thereby leading to an increased capacity for defense against superoxide radicals and decreased oxidative damage. Some studies have shown that oxidative stress may play an important role in the pathological mechanisms of negative symptoms of schizophrenia (Buckman et al., 1990). Additionally, SOD has been found to protect against neuronal degeneration induced by the glutamatergic mechanism (Tsai et al., 1998), which is thought to be relevant to the pathophysiology of schizophrenia. Based on these results, we speculate that the reason that the –9Ala allele was more associated with a low negative symptom than the –9Val allele, was that the former may induce higher MnSOD activity, thereby leading to an increased capacity for defense against oxidative damage, which in turn reduce the negative symptoms. However, previous studies have shown that SOD was lower in erythrocytes, plasma and CSF in patients with TD (Lohr et al., 1990; Yamada et al., 1997; Tsai et al., 1998; Zhang et al., 2003, 2007), and decreased SOD levels are significantly correlated with dyskinetic movements in TD patients (Zhang et al., 2003), as shown in the Introduction section. Hence, regarding protective function of MnSOD–9Ala alleles against free radicals and lower levels of SOD in patients with TD reported in previous studies seems to be contrasting. We could not offer a reasonable explanation regarding this association of the *MnSOD* genotype and negative symptoms in schizophrenia, which seems to be conflicting with lower SOD reported previously. Perhaps some other associated factors may be involved in the relationships between *MnSOD* genotype, MnSOD activity and negative symptoms, which deserve further investigation using the longitudinal study. In addition, it is also noteworthy that the genetic contribution of the Ala9Val polymorphism in the *MnSOD* gene to negative symptoms was relatively weak in the present study. The frequency of the –9Ala allele among subjects with schizophrenia was only 0.14, and thus the protective effect of this allele against TD must be small. Furthermore, the significance is modest, with a p value of 0.03. Attributing the risk to just one locus, with the nominal odds as in this study, may be inappropriate. Therefore, the association between the *MnSOD* Ala–9Val gene polymorphism and negative symptoms need to be replicated in a larger sample size from other population before the firm conclusion could be made.

The study has several limitations. First, the relatively small sample size limits the generalizability of our findings. A replication study for our association between negative symptoms of schizophrenia and the genotype for high MnSOD activity should be needed. Second, hidden population stratification of our samples could be confounders, although the Chinese in Beijing are ethnically relatively homogenous. A replication study with genomic controls or a family-based population study would help address this limitation. Third, only one polymorphism was genotyped in the study, which does not fully capture all the genetic variants in *MnSOD* gene. More SNPs of the *MnSOD* gene, preferably tags for the Chinese population, should be genotyped to include more information (Pae et al., 2007; Zai et al., 2010).

In summary, the *MnSOD* Ala–9Val polymorphism does not appear to be a risk factor for the development and expression of TD, but may play a role in the negative symptom profile of schizophrenia. Further adequately powered studies involving candidate genes associated with oxidative system, gene–gene interactions and other polymorphisms of *MnSOD* gene in a larger sample will be needed.

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