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Brief report

Manganese superoxide dismutase (MnSOD: Ala–9Val) gene polymorphism may not be associated with schizophrenia and tardive dyskinesia

Chi-Un Pae [a,b,*], Tae-Suk Kim [c], Ashwin A. Patkar [b], Jung-Jin Kim [a], Chang-Uk Lee [a], Soo-Jung Lee [a], Tae-Youn Jun [c], Chul Lee [a], In-Ho Paik [a]

[a] *Department of Psychiatry, Kangnam St. Mary' Hospital, The Catholic University of Korea College of Medicine, Seoul, South Korea*
[b] *Department of Psychiatry and Behavioral Sciences, Duke University, Durham, NC, USA*
[c] *Department of Psychiatry, St. Mary' Hospital, The Catholic University of Korea College of Medicine, Seoul, South Korea*

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Abstract

There has been increasing evidence that the alteration of antioxidant enzymes such as manganese superoxide dismutase (MnSOD) might be implicated in the development of schizophrenia and/or tardive dyskinesia (TD). This study investigated the association of a MnSOD gene (*MnSOD*) polymorphism (Ala–9Val) with schizophrenia as well as its involvement in TD. Patients with schizophrenia ($n=262$) and healthy controls ($n=263$) were enrolled in this study and genotyped by a polymerase chain reaction-based method. The distribution of the *MnSOD* genotypes and alleles was not significantly different between patients and controls. Logistic regression analysis also failed to reveal any association between *MnSOD* genotypes and TD. Taken together, these results suggest that the *MnSOD* polymorphism does not contribute to the development of schizophrenia and/or TD, at least in the Korean population.

Keywords: Schizophrenia; Tardive dyskinesia; Manganese superoxide dismutase; Polymorphism; Korean

1. Introduction

Alteration of the antioxidant system has been suggested to be associated with the pathogenesis of schizophrenia and also the development of tardive dyskinesia (TD) during antipsychotic treatment. Several oxidative stress parameters such as albumin, bilirubin and uric acid and total antioxidant plasma levels were reported to be associated with schizophrenia (Yao et al., 2001; Pae et al., 2004), and suggested to be involved in the intermediate or final common pathogenic processes of its development (Michel et al., 2004). Furthermore, antioxidant enzymes are involved in the generation of superoxide radicals, which are believed to exert a critical role in neurotoxicity (Hori et al., 2000), though not unequivocally (Mahadik and Mukherjee, 1996).

* Corresponding author. Department of Psychiatry, Kangnam St. Mary' Hospital, The Catholic University of Korea College of Medicine, 505 Banpo-Dong, Seocho-Gu, Seoul 137-701, South Korea and Department of Psychiatry and Behavioral Sciences, Duke University, Ben Franklin Blvd., Suite 700, Durham, NC 27704, USA. Tel.: +82 2 590 2780; fax: +82 2 536 8744.
E-mail addresses: pae@catholic.ac.kr, chiun.pae@duke.edu (C.-U. Pae).

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Superoxide radicals could also be associated with the pathophysiology of TD, through modifications of dopamine turnover, excitotoxic damage and loss of GABAergic neurons (Lohr et al., 2003). Recent controlled trials supported the hypothesis of a direct involvement of the failure of the antioxidant system in TD (Lohr and Caligiuri, 1996; Zhang et al., 2004). Another study demonstrated that free radical metabolism and the severity of TD were positively correlated (Zhang et al., 2003a).

Manganese superoxide dismutase (MnSOD) is an interesting intramitochondrial antioxidant enzyme that has a crucial role in the detoxification of superoxide radicals, linked to the prevention of the formation of toxic free radicals in the brain. It has been shown to be involved in the turnover of superoxide radicals (Yao et al., 1998; Zhang et al., 2003a,b; Dakhale et al., 2004; Michel et al., 2004), although some disagreement exists (Parikh et al., 2003; Ranjekar et al., 2003).

The MnSOD gene (*MnSOD*) mapped on chromosome 6q25, previously known as a candidate region for linkage with schizophrenia (Lindholm et al., 2001). Among known functional polymorphisms of *MnSOD*, the Ala–9Val polymorphism was extensively investigated for association with schizophrenia, producing conflicting results in different ethnic groups (Hori et al., 2000; Zhang et al., 2002; Akyol et al., 2005). Therefore, we carried out a case–control association study between the *MnSOD* polymorphism and schizophrenia/TD in the Korean population.

2. Methods

2.1. Subjects

Subjects comprised 262 inpatients with schizophrenia, and 263 voluntary controls. The diagnosis was based on the consensus between two board-certified psychiatrists (C.U.P.; C.U.L.), according to the DSM-IV criteria (American Psychiatric Association, 1994) along with the structured Clinical Interview, DSM-IV Axis I Disorders-Clinician Version (SCID-I-CV, First et al., 1997). The Positive and Negative Syndrome Scale (PANSS) was obtained at the time of hospitalization (Kay et al., 1988). Family history, age of onset and other clinical variables were collected when possible. The evaluation of TD was made by using the TD criteria of Schooler–Kane (Schooler and Kane, 1982) and the Abnormal Involuntary Movement Scale (AIMS) (Guy, 1976).

The voluntary controls were recruited from the personnel of The Catholic University of Korea College of Medicine and Kangnam St. Mary's Hospital. They were administered a direct semi-structured interview (by C.U.P.; C.U.L.) to exclude individuals with a current or past history or familial history of psychiatric illnesses.

All subjects were biologically unrelated native Koreans residing in Korea. They all were given information about the study, and written informed consent was obtained. The institutional review board of Kangnam St. Mary's Hospital approved this study.

2.2. Genotyping

The DNA was extracted from whole blood, using the standard method, and *MnSOD* genotyping was performed by a polymerase chain reaction (PCR), under modified conditions, according to a previously reported method (Zhang et al., 2002). Briefly, the genotyping was carried out in a Perkin Elmer 9600 thermocycler (Foster City, CA), using the sense and antisense *MnSOD* primers: 5′-AGC CCA GCC TGC GTA GAC-3′ and 5′-TAC TTC TCC TCG GTG ACG-3′, respectively. After an initial denaturation step at 94 °C for 6 min, the samples were processed through 35 cycles at 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 45 s. A final extension at 72 °C for 10 min was then performed. The PCR product (246 bp) was digested with 5 units of *Bsa*W, 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).

2.3. Statistics

Comparisons of the *MnSOD* genotype and allele distribution between patients and controls were performed by Fisher's exact test. Continuous variables were analyzed by the Mann–Whitney test, as the Kolmogorov–Smirnov test showed a skew of the data from normality. Comparison of the *MnSOD* genotype between patients according to presence or absence of TD was performed by Fisher's exact test. A logistic regression analysis was also performed to adjust potential confounding factors for TD using TD as a dependent variable and genotype, duration of antipsychotic treatment, current dose of antipsychotic (in chlorpromazine equivalents), age and sex as independent variables. *P* values less than 0.05 were considered significant. The 95% confidence interval (CI) was provided where appropriate. All statistical tests were performed using SPSS *v*10.0 software (SPSS Inc., Chicago, IL).

The power of our sample to detect differences between variants was calculated, using a two-tailed alpha value of

0.05. With these parameters, considering the allele frequencies in our sample, power analysis showed that our sample size had a power (0.80) to detect an effect size (w=0.13), which corresponded to a difference of 13% between the two alleles (Odds Ratio [OR]=1.702).

3. Results

Males accounted for 178 (67.9%) of the patients with schizophrenia (n=262) and for 163 (62.0%) of the controls (n=263), with mean ages of 44.7 (S.D., 9.6) and 42.9 (S.D., 13.2), respectively. The gender and age distributions in the two groups showed no significant differences (χ^2=2.049, df=1, P=0.170; Mann–Whitney U=31983.5, P=0.770, Table 1). The prescribed antipsychotics were haloperidol (n=48), chlorpromazine (n=19), sulpiride (n=17), risperidone (n=79), olanzapine (n=78), quetiapine (n=20) and clozapine (n=1). The mean daily dose of antipsychotics was 878.6±167.4 mg/day of chlorpromazine equivalent. The genotype distributions in the patients (χ^2=2.788, df=1, P=0.096) and controls (χ^2=3.442, df=1, P=0.063) were in Hardy–Weinberg equilibrium (HWE). The distributions of the *MnSOD* genotypes and alleles in the schizophrenia patients were not different from those in the controls (χ^2=2.79, df=1, P=0.573; χ^2=2.69, df=1, P=0.604, respectively, Table 1).

Table 1
Demographics of the patients with schizophrenia and the controls according to *MnSOD* polymorphism

	Genotypes	
	Ala/Val	Val/Val
Patients (n=262)		
Sex [a] (male/female)	31/18	147/66
Age [b] (years)	48.6±10.0	43.9±9.4
Age at onset [c] (years)	25.8±6.6	26.4±7.4
Number of admission [d]	5.8±3.2	5.7±3.3
Duration of antipsychotic treatment [e] (years)	19.3±8.3	17.9±8.9
Current antipsychotic dose [f]	872.3±169.5	863.3±200.5
AIMS score [g]	4.5±6.8	5.0±7.3
Controls (n=263)		
Sex	31/23	132/77
Age	41.0±11.4	43.4±13.6

Data represent mean±S.D. or number.
[a] χ^2=0.282, df=1, P=0.658.
[b] Mann–Whitney U=4206.0, P=0.193.
[c] Mann–Whitney U=4808.0, P=0.639.
[d] Mann–Whitney U=4735.5, P=0.936.
[e] Mann–Whitney U=4632.5, P=0.235.
[f] Chlorpromazine-equivalent dose (mg/day), Mann–Whitney U=4063.0, P=0.579.
[g] Mann–Whitney U=5118.5, P=0.662, AIMS, Abnormal Involuntary Movement Scale.

Table 2
Demographics and *MnSOD* polymorphism in patients with and without tardive dyskinesia (TD)

	Patients (n=262)	
	With TD (n=44)	Without TD (n=218)
Sex [a] (male/female)	26/18	152/66
Age [b] (years)	48.6±10.0	43.9±9.4
Age at onset (years) [c]	25.8±6.6	26.4±7.4
Number of admissions [d]	5.8±3.2	5.5±3.2
Duration of antipsychotic treatment (years) [e]	22.1±8.8	17.4±8.6
Current antipsychotic dose [f]	892.3±202.5	853.6±189.5
AIMS score [g]	13.0±6.6	3.2±6.2
Genotype [h]		
Ala/Val	12 (27.3)	37 (17.0)
Val/Val	32 (72.7)	181 (83.0)

Data represent mean±S.D. or number with percentage in parentheses.
[a] χ^2=1.901, df=1, P=0.215.
[b] Mann–Whitney U=3495.0, P=0.003.
[c] Mann–Whitney U=4667.0, P=0.639.
[d] Mann–Whitney U=4544.0, P=0.571.
[e] Mann–Whitney U=3317.5, P=0.002.
[f] Chlorpromazine-equivalent dose (mg/day), Mann–Whitney U=4149.0, P=0.223.
[g] Mann–Whitney U=1180.5, P<0.0001.
[h] χ^2=2.555, df=1, P=0.110.

The demographics of the patients with and without TD are shown in Table 2. As shown in Table 2, there was no significant difference of genotype distribution between the two groups. Logistic regression analysis also did not reveal any association between *MnSOD* genotypes and TD (Wald=1.123, P=0.233, 95% CI=0.700–3.315).

In the subgroup analyses, the distributions of the genotypes and alleles were not different between the positive and negative subgroups as defined by PANSS score (data available on request). Subgroup analyses according to family history did not find any significant differences (data available on request).

4. Discussion

Several previous studies (Hori et al., 2000; Zhang et al., 2002; Akyol et al., 2005) investigated the association of the *MnSOD* polymorphism with schizophrenia or TD. The Japanese and Chinese studies failed to find an association of the *MnSOD* polymorphism with schizophrenia, as well as the present study. However, Hori et al. (2000) reported that −9Ala appeared to be protective against the occurrence of TD, according to its biological

role. In fact, the –9Ala allele may be linked to higher activity of the human MnSOD, as the –9*Val* substitution results in an intracellular miscommunication and in an aberration of MnSOD activity (Shimoda-Matsubayashi et al., 1996; Hori et al., 2000). It has been shown that the helix forming potential predicting the typical amphiphilic helical structure was preserved in the –9Ala allele but not in –9*Val* allele, and this fact may reflect the functional significance of the –9Ala allele (Shimoda-Matsubayashi et al., 1996; Hiroi et al., 1999). However, the Japanese study (Hori et al., 2000) was not replicated in a Chinese sample (Zhang et al., 2002). More recently, Akyol et al. (2005) found a positive association of the *MnSOD* polymorphism with schizophrenia. This was in contrast to the previous two studies. Thus, we could not reach a definite conclusion about the role of the *MnSOD* Ala–9Val polymorphism in schizophrenia and in the development of schizophrenia and TD. Moreover, the study by Zhang et al. (2002) and the present study did not have –9Ala homozygous subjects as did Hori et al. (2000) and Akyol et al. (2005). An ethnic difference in the *MnSOD* Ala–9Val polymorphism allele frequencies has been suggested as an explanation, as well as an ethnic difference in the genetic involvement in TD (Swartz et al., 1997). In particular, Caucasian populations present higher –9Ala allele frequencies than Asian populations (Van Landeghem et al., 1999; Hori et al., 2000; Zhang et al., 2002; Akyol et al., 2005). Methodological differences in the measurement of TD and subject characteristics should also be considered, as well as the presence of unknown genes in linkage disequlibrium with the *MnSOD* Ala–9Val polymorphism. Finally, the patient sample of Akyol et al. (2005) deviated from HWE, and this could be a critical limitation for the actual significance of their results. A marginal significance on HWE deviation in the controls was also seen in the present study as in the study of Akyol et al. (2005). Population stratification is primarily caused by anthropological origins. One may assume the possibility of sample bias, but it is less likely to be relevant because our subjects were composed of samples collected from only native Koreans, who are known to be genetically homogeneous (Cavalli-Sforza, 1994). The pattern of genotype in the present study, that is, no presence of *Ala*/*Ala* in the whole sample and a slight excess of *Ala*/*Val* in the controls, should also be taken into consideration.

However, it was found that a combination of *MnSOD*–9Val and dopamine receptor D3 9ser alleles was associated with TD, suggesting that studies regarding synergistic interaction between candidate genes might be a useful approach, in which more complex mechanisms could be determined in the development of schizophrenia (Zhang et al., 2003c). Similar findings have been consistently reported in relation to gene–gene interaction, which are in the same direction with the finding that schizophrenia should be considered a complex disorder in which a number of genes are involved in combination in the etiology of schizophrenia (Freedman et al., 2001).

The major limitation of the present study is the intrinsic pitfalls of case–control association studies, such as stratification.

This study suggests that the *MnSOD* polymorphism may not be associated with the development of schizophrenia and TD, at least in the Korean population.

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