Cognitive Function, Plasma MnSOD Activity, and MnSOD Ala-9Val Polymorphism in Patients With Schizophrenia and Normal Controls

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Excessive reactive oxygen species are thought to produce oxidative damage that underlies neurodegeneration and cognitive impairment in several disorders including schizophrenia. The functional Ala-9Val polymorphism of the mitochondrial enzyme manganese superoxide dismutase (MnSOD), which detoxifies superoxide radicals to hydrogen peroxide, has been associated with schizophrenia. However, no study has reported its role in cognitive deficits of schizophrenia as mediated through MnSOD activity. We recruited 923 schizophrenic inpatients and 566 healthy controls and compared them on the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS), plasma MnSOD activity, and the MnSOD Ala-9Val polymorphism. We assessed patient psychopathology using the Positive and Negative Syndrome Scale. We showed that the MnSOD Ala-9Val polymorphism may not contribute directly to the susceptibility to schizophrenia. The Ala variant was associated with worse attention performance among chronic schizophrenic patients but not among normal controls. Plasma MnSOD activity was significantly decreased in patients compared with that in normal controls. Moreover, MnSOD activity among the schizophrenic Ala allele carriers was correlated with the degree of cognitive impairments, especially attention and RBANS total score. We demonstrated an association between the MnSOD Ala-9Val variant and poor attention in schizophrenia. The association between higher MnSOD activity and cognitive impairment in schizophrenia is dependent on the MnSOD Ala-9Val polymorphism.

Key words: schizophrenia/manganese superoxide dismutase/cognition/genotype/polymorphism/association

Introduction

Schizophrenia is characterized by deficits in executive function, attention, working memory, and long-term memory.1 These disturbances in cognition appear to be core features of the illness.2-4 They can occur before the onset of the other symptoms of schizophrenia,5,6 and generally persist during the course of the schizophrenia.7,9 Moreover, functional outcome measures correlate more closely with the extent of cognitive deficits than with the severity of psychotic symptoms.10 Poor functional outcomes includes performance of basic activities of daily living, social skills acquisition, social problem solving, occupational functioning, community outcome, and quality of life.11,12 However, cognitive deficits are generally not responsive to currently available pharmacotherapies, and the pathophysiological mechanisms underlying these cognitive deficits are still unclear.4,13,14

Increasing evidence points to reactive oxygen species (ROS) as important factors in schizophrenia and suggests that the oxidative stress caused by ROS is related to disease severity.15-17 The excess ROS that are not scavenged by the antioxidant defense mechanism can interact with lipids, proteins, and nucleic acids, resulting in cellular dysfunction or cell death.20,21 Several studies show that patients with schizophrenia have altered antioxidant enzyme activities and increased levels of lipid peroxidation that will damage neurons. As an example of altered enzyme activities, superoxide dismutase (SOD) activity has been consistently high in chronic schizophrenic patients22-24 and low in neuroleptic naive, first-episode schizophrenic patients.25 SOD is the key antioxidant defense enzyme that detoxifies the superoxide radical (O2-) and generates hydrogen peroxide (H2O2), which in turn is detoxified.26 The 3 isoforms of SOD contain different
prosthetic groups: the manganese (Mn) isoform (SOD2) is found in mitochondria, and the copper and zinc (CuZn) isoforms are cytoplasmic (SOD1) and extracellular (SOD3). Mitochondrial manganese SOD (MnSOD) is the main antioxidant defense because mitochondria produce 95% of ROS and superoxide anions from oxygen consumption.\textsuperscript{33–35}

Disrupted MnSOD activity is consistently related to schizophrenia. A key finding is that MnSOD levels are significantly increased in the frontal cortex and substantia nigra of postmortem brains from patients with schizophrenia spectrum disorders.\textsuperscript{30} A genetic linkage study that independently identified the long arm (6q25) of chromosome 6 as a candidate region for linkage with schizophrenia also has suggested a genetic contribution to this MnSOD disruption because the human MnSOD gene is located in this same gene region.\textsuperscript{31} The Ala-9Val polymorphism (rs4880) in exon 2 of the \textit{MnSOD} gene is the most extensively studied single nucleotide polymorphism (SNP), with the Ala-to-Val substitution possibly resulting in a conformational change in the target sequence from beta sheet to alpha helix, which can induce a 30\%-40\% increase in mitochondrial MnSOD activity.\textsuperscript{32} Because MnSOD plays a vital role in the antioxidant defense system and neurodevelopment, a change in enzyme concentration through the action of Ala-9Val, may result in ROS accumulation and hence cell injury. This functional Ala-9Val polymorphism of MnSOD has been extensively investigated for association with schizophrenia but with conflicting results in different ethnic groups.\textsuperscript{33–35} Moreover, our meta-analysis of the MnSOD Ala-9Val polymorphism from 5 studies (schizophrenia patients = 861, controls = 813) did not find it associated with schizophrenia (fixed-effects and random-effects model: both \(P > .05\)).\textsuperscript{35–39} and a meta-analysis from 10 studies (schizophrenia with tardive dyskinesia [TD] = 614, without TD = 1478) did not find it associated with TD (fixed-effects and random-effects model: both \(P > .05\)).\textsuperscript{33–44}

Numerous studies have explored the relationship between ROS and cognitive function. Much of the work elucidating the relationship has focused on aging. Aging is accompanied by the loss of cognitive function, especially in certain types of learning and memory. Oxidative damage produced by excessive ROS is thought to underlie aging-related cognitive impairment and neurodegeneration.\textsuperscript{45,46} Superoxide has been shown to be critically involved in several pathological manifestations of aging animals. Furthermore, 1 recent work revealed that extracellular SOD overexpression improves hippocampal synaptic plasticity and memory-related behavioral performance in aged mice.\textsuperscript{37} However, studies with transgenic mice that overexpress superoxide scavengers show that certain types of memory function and underlying neuronal processes are impaired under conditions of severely reduced superoxide signaling,\textsuperscript{47} suggesting that superoxide also can act as a signaling molecule to modulate signal transduction cascades required for hippocampal synaptic plasticity. These findings provide the evidence supporting the dual role of ROS as cellular messenger molecules in normal learning and memory and their role as damaging toxic molecules in the age-related impairment of learning and memory.\textsuperscript{39}

Thus, we undertook this study to examine the relationship between the core cognitive deficits in schizophrenia and MnSOD activity because it might be altered through the Ala-9Val polymorphism. We hypothesize that the MnSOD Ala-9Val polymorphism would lead to lower MnSOD activity in neurons and thereby play a role in cognitive deficits of schizophrenia. We have 4 purposes of this study. (1) We directly measured the impact of MnSOD Ala-9Val polymorphism on plasma MnSOD activity in schizophrenic patients and normal controls. (2) We examined the relationship between plasma MnSOD activity and cognitive function in these groups. (3) We tested whether the MnSOD Ala-9Val polymorphism influences cognitive function in schizophrenic patients and normal controls. (4) We examined whether the relationship between plasma MnSOD activity and cognitive function is dependent on the MnSOD Ala-9Val polymorphism.

\textbf{Methods}

\textit{Subjects}

We recruited 923 schizophrenic inpatients from Beijing Hui-Long-Guan hospital, a Beijing-city-owned psychiatric hospital, and HeBei Province Veteran Psychiatric Hospital in BaoDing city, 50 mi from Beijing. All patients met the DSM-IV diagnosis of schizophrenia, which was confirmed by 2 psychiatrists based on the Structured Clinical Interview for DSM-IV. All schizophrenic patients were Han Chinese and between 25 and 75 years old (average: 48.1 ± 9.6 years). They were of the chronic type, with at least 5 years of illness (average: 24.6 ± 9.8 years). The mean duration of hospitalization was 9.8 ± 9.3 years. All patients had been receiving stable doses of oral antipsychotic drugs for at least 12 months before entry into the study. Antipsychotic drug treatment consisted mainly of drug monotherapy including clozapine (\(n = 416\)), chlorpromazine (\(n = 65\)), sulpiride (\(n = 48\)), perphenazine (\(n = 45\)), quetiapine (\(n = 39\)), haloperidol (\(n = 31\)), aripiprazole (\(n = 27\)), and others (\(n = 51\)). Mean antipsychotic dose (in chlorpromazine equivalents) was 446 ± 413 mg/day.

We also recruited 566 normal controls from the local community. None of them had any personal or family history nor demonstrated any clinical psychiatric disorders. All were Han Chinese from the Beijing area. All subjects were in good physical health, and any subjects with major medical illnesses or drug and alcohol abuse/dependence were excluded. All subjects gave
written informed consent, which was approved by the Institutional Review Board of Beijing Hui-Long-Guan hospital.

Clinical Measures

A detailed questionnaire including general information, sociodemographic characteristics, smoking behavior, and medical and psychological conditions was administered to each subject by a member of the research staff. Additional information was collected from available medical records.

The Repeatability Battery for the Assessment of Neuropsychological Status (RBANS, Form A) was individually administered to measure cognitive functioning. The RBANS comprised 12 subtests that are used to calculate age-adjusted index scores and a total score. Test indices are immediate memory (comprising List Learning and Story Memory tasks); visuospatial/constructional (comprising Figure Copy and Line Orientation tasks); language (comprising Picture Naming and Semantic Fluency tasks); attention (comprising Digit Span and Coding tasks); and delayed memory (comprising List Recall, Story Recall, Figure Recall, and List Recognition tasks). Our group previously translated RBANS into Chinese and the clinical validity and its test-retest reliability established among controls and schizophrenic patients. The total and 5 index scores reported in this study were standard scores.

Four psychiatrists, who had simultaneously attended a training session in the use of the Positive and Negative Syndrome Scale (PANSS) before the study began, assessed the patient’s psychopathology on the day of the blood sampling using the PANSS. After training, repeated assessment showed that these psychiatrists maintained an interobserver correlation coefficient greater than 0.8 for the PANSS total score.

Plasma MnSOD Activity Measurement

We collected venous blood from the forearm vein between 0700 and 0900 am following an overnight fast. MnSOD activity was analyzed using established procedures. Our previous report gives a full description of the assays. Briefly, activity of plasma MnSOD activity was expressed as units per milliliter plasma (U/ml). The inter- and intraassay coefficients of variation for SOD were 8% and 6%, respectively. A research assistant blind to the clinical situation assayed all samples. A code number maintained by the primary investigator indicated the identity of subjects.

Genotyping

DNA was extracted using standard protocols. The genotypes of the MnSOD Ala-9Val polymorphism were identified as reported in a previous study. Genotyping was duplicated and carried out blind to the clinical status.

Statistical Analysis

Deviations from Hardy-Weinberg equilibrium (HWE) were assessed using the HWSIM program. The MnSOD Ala-9Val allele and genotype frequencies were compared between patients with schizophrenia and healthy controls using \( \chi^2 \) tests. Group differences were compared using Student’s 2-sample \( t \)-test or one-way ANOVA for continuous variables and chi square for categorical variables. Correlation between variables was studied using Pearson product moment correlations.

We tested associations between the MnSOD Ala-9Val polymorphism and the cognitive measures or plasma SOD activity using a general factorial design in PASW statistical software version 18.0 (SPSS Inc). Because the homozygous variant Ala/Ala genotype was almost absent in all of the patients and occurred in only 0.7% of controls, the Ala/Ala genotype was combined with Val/Ala genotype in the analyses.

For the main models, the MnSOD genotype (Ala/Ala + Ala/Val vs Val/Val) and diagnosis (cases vs controls) were entered as fixed effects. Scores for each cognitive domain and the total scores of RBANS were entered as the dependent variables, with sex, age, and education included as covariates as appropriate. In each model, the main effect of diagnostic group, the main effect of genotype, and diagnostic group × genotype interaction were tested. The diagnostic group × genotype interaction term in the model detects the differential effects that alleles might have on cognitive scores between diagnostic groups. Similarly, the main effect of the MnSOD genotype on plasma MnSOD activity was also analyzed using ANCOVA. We applied Bonferroni corrections to adjust for multiple testing.

Lastly, we performed exploratory regression analyses to examine whether the relationships between MnSOD plasma activity and cognitive function were different across MnSOD genotype groups. Stepwise multiple regression analysis used RBANS total or Index scores as dependent variables, with MnSOD activity as the independent variable in each MnSOD genotype group. Covariates in these stepwise forward entry models included age, gender, education, smoking, duration of illness, age of onset, antipsychotic medication dosage, type (typical vs atypical antipsychotics), and duration.

Results

Demographic and clinical characteristics by MnSOD Ala-9Val genotype are summarized in table 1. No differences were observed in age, years of education, or sex between the genotype groups for the whole sample and when patients and controls were considered separately. We found no differences between genotypes on any of the symptom factors. We also found no differences in medication dosage between MnSOD Ala-9Val genotypes.

The MnSOD allele and genotype frequencies are summarized in table 2. The genotypic distributions of
$MnSOD$ genes in patients and normal controls were consistent with HWE (both $P > .05$). The $MnSOD$ genotype and allele distributions between the patients and normal controls did not differ. We observed no significant sex differences in $MnSOD$ genotype and allele distributions among all subjects or when the patients and normal controls were analyzed separately (all $P > .05$).

**Genotype Effects on Cognitive Functioning Between Cases and Controls**

RBANS data were available from 579 patients and 304 healthy controls. RBANS total and index scores and the effects of the $MnSOD$ Ala-9Val polymorphism on the RBANS total and index scores are summarized in table 3. As expected, patients performed significantly below controls on the total scores and all indexes (all $P < .0001$; Bonferroni corrected all $P < .001$), except for the visuospatial/constructual index ($P > .05$). These significant differences were still significant when adjusting for sex, age, education, and smoking (all $P < .001$; Bonferroni corrected all $P < .01$), except for the visuospatial/constructual index ($P > .05$).

Table 3 showed a significant genotype × diagnosis effect on attention ($F = 4.58, df = 4.876; P = .031$). However, this significance did not survive Bonferroni correction ($P > .05$). Patients who were Val homozygous had higher attention index scores than patients who carried the Ala allele ($F_{1,87} = 8.12, P = .001$; Bonferroni corrected $P = .006$). When adjusting for gender, age, education, smoking, illness course, age of onset, medication type (atypical vs typical antipsychotics), dose (chlorpromazine equivalents), and duration of treatment, this difference still remained significant ($F_{1,556} = 7.17, P = .006$; Bonferroni corrected $P < .05$). However, we found no group differences in attention among normal controls ($F_{1,299} = 0.16, P = .75$). Effect size estimates (partial eta squared) indicated that in cases, genotype explains 1.6% of attention. There were no significant genotype effects or genotype × diagnosis effects for other cognitive domains or total score of RBANS.

**Genotype Effects on Plasma MnSOD Activity Between Patients and Controls**

Plasma MnSOD activity data in addition to genotype and RBANS score were available from 313 patients and 287 healthy controls. As shown in table 3, we observed a significant main effect of diagnostic group on plasma MnSOD activity ($F = 5.48, df = 4.595$, adjusted $P = .02$). There was no main effect of genotype ($P > .05$) nor genotype × diagnosis effect ($P > .05$) on plasma MnSOD activity. We observed no significant differences in MnSOD activity between genotypic subgroups (Val/Val vs Val/Ala+Ala/Ala) in the combined groups or when the schizophrenic patients and normal controls were examined separately (both $P > .05$; table 3). These results indicated that the decreased activity of MnSOD in patients with schizophrenia was independent of the genotype.

**Relationships Between Plasma MnSOD Activity and Cognitive Functioning: Associations With MnSOD Ala-9Val**

We explored whether the cognitive function may be related to plasma MnSOD activity and whether such relationships differed between genotype groupings. In
Table 3. Cognitive Analysis and Plasma MnSOD Activity According to MnSOD Ala-9Val Genotype of Cases and Controls

<table>
<thead>
<tr>
<th>Cognitive Index</th>
<th>Schizophrenia</th>
<th>Normal Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Val/Ala+Ala/Ala</td>
<td>Val/Val</td>
</tr>
<tr>
<td>Immediate memory</td>
<td>57.2±16.3 (n=153)</td>
<td>55.6±14.3 (n=426)</td>
</tr>
<tr>
<td>Attention</td>
<td>68.6±17.6</td>
<td>69.9±17.2</td>
</tr>
<tr>
<td>Language</td>
<td>80.2±16.8</td>
<td>75.6±15.5</td>
</tr>
<tr>
<td>Visuospatial/</td>
<td>77.8±17.3</td>
<td>76.0±20.1</td>
</tr>
<tr>
<td>constructional</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delayed memory</td>
<td>65.6±20.1</td>
<td>64.5±18.7</td>
</tr>
<tr>
<td>Total</td>
<td>63.4±15.4</td>
<td>60.9±13.3</td>
</tr>
<tr>
<td>MnSOD (U/ml)</td>
<td>15.9±11.8</td>
<td>17.2±13.9</td>
</tr>
</tbody>
</table>

*There was a significant genotype × diagnosis effect on Attention (F 4,476 = 4.58, P = .031). Patients who were Val homozygous had significantly higher Attention index scores than patients who were Ala homozygous (F 1,478 = 8.12, P = .001; adjusted F 1,575 = 7.13, P = .006). There were no genotype differences in Attention among healthy controls (F = 0.16, P > .05).

Table 4. Relationships Between MnSOD Activity and Cognitive Domain Scores Across MnSOD Genotype Groupings in Schizophrenia Patients

<table>
<thead>
<tr>
<th>Cognitive Domains</th>
<th>Val Homozygotes (n = 236)</th>
<th>Ala Carriers (n = 77)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pearson</td>
<td>Pearson</td>
</tr>
<tr>
<td></td>
<td>Partial r</td>
<td>P</td>
</tr>
<tr>
<td>Immediate memory</td>
<td>−0.09</td>
<td>.18</td>
</tr>
<tr>
<td>Attention</td>
<td>−0.11</td>
<td>.08</td>
</tr>
<tr>
<td>Language</td>
<td>−0.03</td>
<td>.72</td>
</tr>
<tr>
<td>Visuospatial/</td>
<td>−0.03</td>
<td>.68</td>
</tr>
<tr>
<td>Constructional</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delayed memory</td>
<td>−0.03</td>
<td>.64</td>
</tr>
<tr>
<td>Total</td>
<td>−0.11</td>
<td>.09</td>
</tr>
</tbody>
</table>

patient group, Pearson correlations were significant between MnSOD and RBANS total score (r = −0.154, df = 313, P = .006), with the main contributions from the attention (r = −0.162, df = 313, P = .004) and immediate memory domains (r = −0.135, df = 313, P = .016). Of these 313 patients who had both RBANS and MnSOD activity measures, almost the same proportion (138/313 = 44.1%) as the total patient group (416/923 = 45.1%) was on clozapine. After controlling for gender, age, education, smoking, duration of illness, age of onset, medication type (clozapine vs nonclozapine treatment), dose (chlorpromazine equivalents), and duration of treatment, further partial correlations were significant between MnSOD and RBANS total score (r = −0.145, df = 282, P = .009) and between MnSOD and the attention (r = −0.157, df = 282, P = .007).

Further, as shown in table 4, the Ala allele carriers showed a significant correlation between MnSOD activity and RBANS total score (r = −0.30, df = 77, P = .011), with the main contributions from the immediate memory (r = −0.29, df = 77, P = .013) and attention domains (r = −0.25, df = 77, P = .036). Among Val homozygotes, there were no significant correlations between MnSOD activity and any cognitive domain scores (table 4). Furthermore, regression analyses among the Ala allele carriers found a significant negative association of MnSOD activity levels with the RBANS total score (r = −2.59, P = .016), immediate memory (r = −2.37, P = .03), and attention (r = −2.26, P = .04). Thus, the MnSOD Ala alleles mainly determined the correlations between MnSOD activity and cognition.

In control groups, no significant correlation was found between MnSOD activity and any RBANS index or total scores. Moreover, no significant correlation between MnSOD activity and any RBANS index or total scores was observed when being grouped by MnSOD genotype (all P > .05), indicating that no significant effects of MnSOD activity, MnSOD genotype, and their interactions on cognitive performance were found in the control group.

Discussion

This study had 4 major findings. (1) The MnSOD Ala-9Val polymorphism may not contribute directly to the susceptibility to schizophrenia. (2) The Ala variant was associated with worse attention performance among chronic schizophrenic patients but not among normal controls. (3) Plasma MnSOD activity was significantly decreased and correlated with the degree of cognitive impairments in schizophrenic patients. (4) This correlation of MnSOD activity with cognition was present only among schizophrenic patients with the MnSOD-9Ala polymorphism.

Several previous studies investigated the association of the MnSOD polymorphism with schizophrenia or TD, with conflicting results. For example, Akyol et al found that the MnSOD Ala-9Val polymorphism was less frequent among schizophrenia. Furthermore, Hori et al reported that -9Ala appeared to be protective against...
the occurrence of TD.\textsuperscript{33} However, like this study, several studies did not replicate these earlier associations,\textsuperscript{36-38,43} including studies in Han Chinese populations.\textsuperscript{35,44} One possible explanation for the inconsistency is a difference in ethnic background because the allele frequency distribution of Val-9Ala varied significantly between Asian and Caucasian subjects. The Ala allele frequency was around 15\% in Asian\textsuperscript{35,38,44} but around 50\% in Caucasian subjects.\textsuperscript{43} In this study, the Ala allele frequency was 11.4\% in control subjects, which is similar to other studies from Asia.\textsuperscript{35,38,44} Thus, interethnic differences in the genotype frequencies of the \textit{MnSOD} Ala-9Val polymorphism may play an important role in accounting for the inconsistent results across the different populations. Several other factors may also account for these divergent results, eg, small gene effects, heterogeneity of the schizophrenia diagnosis, and population stratification. However, it is noteworthy that although our data does not support \textit{MnSOD} Ala-9Val polymorphism as a risk variant for schizophrenia, the \textit{P}-values in the chi square tests were <.10 for both allelic and genotypic comparisons. Given the effect sizes reported for risk alleles in the schizophrenia literature, this negative finding is just as likely due to an underpowered sample.

\textbf{Effects of \textit{MnSOD} Ala-9Val Genotype on Cognitive Dysfunction in Schizophrenia}

We systematically investigated the role of \textit{MnSOD} in cognition among patients with schizophrenia using RBANS and its index that measures immediate verbal memory, visual spatial/constructional abilities, attention, language skills, and delayed verbal and visual memory. We found significant differences in cognitive scores in nearly all of the comparisons except for visuospatial/constructional index between schizophrenia and controls. These data replicate numerous studies, indicating that patients with schizophrenia perform worse than controls on a range of cognitive tasks.\textsuperscript{2,4} In fact, we found that the absolute difference between mean RBANS total scores of the 2 groups was more than 16 points (78.6 vs 61.6). These results are similar to a recent result by Dickerson et al.\textsuperscript{53}

This study indicated that the \textit{MnSOD}-9Ala variant may have a specific role in schizophrenic patients’ attentional dysfunction. Unlike controls, patients with schizophrenia who carry the Ala allele consistently performed worse than their Val-homozygous counterparts on attention performance. The -9Ala allele is thought to be a mutant allele, which causes a conformational change in the mitochondrial targeting sequence (MTS) that misdirects intracellular trafficking of the protein.\textsuperscript{43} This study suggests that the mutant -9Ala allele may produce a detrimental effect on cognitive performance, especially attention in schizophrenia. However, the mechanisms by which the \textit{MnSOD} Ala-9Val genotype affects cognitive performance, especially attention, but not other cognitive indices are unclear and need further investigation. Also, it is noteworthy that the primary significant genotype \texttimes diagnosis interaction on attention (\textit{P} = .031) did not survive correction. Moreover, effect size estimates (partial eta squared) indicated that in cases, genotype explains 1.6\% of attention. These results suggest that the \textit{MnSOD} Ala-9Val genotype may produce a small effect on attention.

On the other hand, this result suggests that the effects of a common SNP on cognitive function in patients with schizophrenia may be different from those in healthy controls. Among SNPs known to contribute to variance in human cognition, polymorphisms in the catechol-O-methyltransferase (\textit{COMT}), brain-derived neurotrophic factor (\textit{BDNF}), metabotropic glutamate receptor (\textit{GRM3}), and disrupted-in-schizophrenia (\textit{DISCI}) genes appear to have similar effects in healthy controls and in patients with schizophrenia.\textsuperscript{54,55} It is clear we need to replicate our finding that the \textit{MnSOD}-9Ala variant may confer attentional dysfunction specifically in schizophrenic patients and not in normal controls.

\textit{Decreased Plasma \textit{MnSOD} Activity and Cognitive Impairment: Relationship to \textit{MnSOD} Val-9Ala}

Higher \textit{MnSOD} activities were associated with worse cognitive function in patients with schizophrenia. The effects of \textit{MnSOD} on cognitive function may be different in schizophrenic patients than in healthy controls. Furthermore, the \textit{MnSOD} Val-9Ala polymorphism affected the correlation between \textit{MnSOD} activity and different aspects of cognitive function only in schizophrenic patients, not in controls.

Our finding that decreased plasma activities of \textit{MnSOD} in patients with a chronic form of schizophrenia is consistent with some,\textsuperscript{55,56} but not all, studies.\textsuperscript{57} Numerous factors may have contributed to these differences among studies, such as clinical status of the patients, type of antipsychotic drugs taken, dosage and length of administration, the subtypes of schizophrenic patients recruited, or biological and ethnic heterogeneity.

Interestingly, altered \textit{MnSOD} activity was a risk factor for impaired attention and immediate memory, but the association was opposite our expected direction. However, high \textit{MnSOD} activity being associated with impaired cognitive function in schizophrenia, is consistent with a previous study showing high total antioxidant status or lipoperoxides and cognitive impairment in older healthy adults.\textsuperscript{58} In order to understand this previous finding, the authors noted that an imbalance between the production and removal of free radicals can produce DNA damage during aging.\textsuperscript{59} In that study and a replication study of older age people, those with normal antioxidant levels showed a higher frequency of DNA damage, while subjects with low antioxidant levels showed little DNA damage.\textsuperscript{59,60} Thus, DNA damage leads to an increase in
antioxidant levels in order to prevent further damage and promote restoring mechanisms. Moreover, comparatively high MnSOD being associated with more cognitive impairment in schizophrenia needs to be viewed in the context of overall lower MnSOD in these patients compared with that in normal controls. On the other hand, Down syndrome (DS) or trisomy 21 is a genetic disorder that represents one of the best documented cases of a human disorder etiologically related to the redox imbalance that has long been attributed to overexpression of Cu,Zn-superoxide dismutase (SOD1), which is encoded by trisomic chromosome 21.\textsuperscript{51} A number of studies have demonstrated that, in individuals with DS, levels of SOD are elevated in a variety of cell types and organs.\textsuperscript{63} SOD overexpression in DS negatively modifies the equilibrium between SOD and glutathione peroxidase + catalase, which may ultimately lead to increased hydroxyl radical formation and an increased oxidative damage. This damage has been proposed as a pathogenic mechanism of DS neurodegeneration and of DS progression toward dementia.\textsuperscript{61} Taken together, overexpression of SOD through unknown mechanisms may also play a role in cognitive impairment in schizophrenia.

Yet, it is noteworthy that the source of circulating MnSOD remains unknown. These oxidative stress indices originate from various sources in the body and therefore whether such peripheral findings may accurately mirror the state of oxidative stress in the brain is still unknown. Although our study showed that the \textit{MnSOD}-9Ala variant may be associated with attentional dysfunction in schizophrenia, and that MnSOD activity was associated with impaired cognitive function in attention and immediate memory in schizophrenia, we found no significant difference in MnSOD activity between the 2 \textit{MnSOD} Val-9Ala genotypes. One interpretation of the results might be that another unknown polymorphism, which is in linkage disequilibrium with the \textit{Val}-9Ala polymorphism and contributes to cognitive deficits, may affect MnSOD activity. Thus, further studies will be needed to detect another functional polymorphism that is more suitable for use in association studies.

The \textit{MnSOD} Val-9Ala genotype groupings had a significant impact on the relationship between MnSOD activity and cognitive impairments in schizophrenia. The correlations between MnSOD activity and cognition, especially attention and immediate memory, were only observed for Ala allele carriers but not for the Val/Val genotype. These divergent plasma MnSOD-cognitive function relationships across \textit{MnSOD} genotypes in schizophrenia may also provide a clue about the unusual finding of greater MnSOD activity with more cognitive impairment. It has been reported that the -9Ala allele of the MTS in the \textit{MnSOD} gene has an alpha-helix structure, whereas the -9Val allele does not.\textsuperscript{29} Because an amphiphilic helix structure is an essential requirement for efficient transport into the mitochondria, the MnSOD precursor protein with the -9Ala-type signal peptide may be more easily transported into mitochondria than the precursor with the -9Val-type signal peptide. Hence, this functional polymorphism may facilitate MnSOD entry into mitochondria, where the damaging ROS are produced. Therefore, greater production of the Ala-\textit{MnSOD} will result in more entering the mitochondria, but this facilitated mitochondrial entry may not be possible with the Val/Val version of MnSOD. Thus, more MnSOD is not being made in the Val/Val patients who get neuronal damage; only the Ala-\textit{MnSOD} patients make the additional MnSOD needed to address the increasing neuronal damage as evidenced from the greater cognitive impairment. Hence, it is likely that the \textit{MnSOD}-9Ala variant may have a specific role for conferring cognitive dysfunction in schizophrenia by affecting MnSOD expression within mitochondria. Further longitudinal studies should seek to clarify this speculation and the brain correlates of peripheral MnSOD activity changes and cognitive changes and further explore the functional significance of the \textit{MnSOD} genotype for mitochondrial functioning as cognitive deficits increase over time among schizophrenia patients.

Several limitations of this study should be noted. First, we measured only MnSOD activity, which is a single antioxidant defense parameter. Effective antioxidant protection is provided by the cooperative and sequential actions of the very complex antioxidant defense system, which includes antioxidant enzymes and nonenzyme antioxidant molecules. The level of this 1 antioxidant parameter provided only partial insights into free radical-mediated central nervous system neuronal dysfunction. Moreover, an increase or decrease of SOD does not necessarily indicate oxidative stress but may be a compensatory response.\textsuperscript{63,64} Second, we measured MnSOD activity in plasma, not in cerebral spinal fluid. It is still uncertain whether peripheral MnSOD reflects similar changes in the central nervous system. Third, the ability to generalize our study is limited by our sample of chronically hospitalized patients, who had more severe psychopathology and a long duration of illness averaging 24.6 years. Moreover, heterogenous antipsychotics and doses are limitations, especially the uncommonly high proportion of the patients treated with clozapine in this study. Fourth, although the RBANS is becoming a widely used screening instrument in neuropsychological assessment, it also has some limitations. For example, because it is a brief test battery, it is unable to evaluate all of the cognitive functions that may be altered in patients, such as motor abilities or executive functioning. However, the instrument takes some of the difficulties inherent in the assessment of older patients into account.\textsuperscript{46} It has been proven that level of education would account for a statistically significant portion of the variance across performance on the RBANS and its individual indices.\textsuperscript{65-67} Moreover, the mean education level in our study was comparatively low, which may affect the actual cognitive
performance in schizophrenia patients. Additionally, the RBANS has not been used widely in China, and the applicability and the potential use of the RBANS in Chinese individuals and schizophrenic patients needs to be confirmed further. Fifth, these patients had a wide age range including older adults more than 75 years of age. The sample was in general an older one with the mean age of 48 and a SD of 10 years. The impact of aging on cognitive decline is clear. Also, these patients had a long history of treatment with antipsychotics, which may have influenced the association of MnSOD and cognitive performance to some degree. However, after statistical control for these effects of age and antipsychotic treatment, the association between MnSOD and cognition remained significant. Nevertheless, analysis in a younger and a less chronic patient population would be worthwhile.

In summary, we have provided new evidence to support the association between the MnSOD Val-9Ala variant and attentional performance in patients with schizophrenia and suggest a specific role of MnSOD-9Ala variant in some aspects of poor cognitive function among schizophrenic patients but not among normal controls without cognitive impairment such as from aging. This study showed that plasma MnSOD activity was significantly decreased and correlated with the degree of cognitive impairments in schizophrenic patients, indicating that peripheral MnSOD activity may be a biomarker of general cognitive function in schizophrenia. The association between plasma MnSOD activity and the degree of cognitive impairment in schizophrenia patients appears to be dependent on the presence of MnSOD-9Ala polymorphism. However, this study is limited by its sample of chronically hospitalized patients with heterogenous antipsychotics and doses, more severe psychopathology and longer duration of illness than typical psychotic outpatients or first-episode and drug-naive patients with schizophrenia. In addition, it is worthy of mentioning that the allele frequency differences of Val-9Ala varied significantly between Asian and Caucasian subjects, showing that the Ala allele frequency (11.4%) in this study is nearly 5 times less common compared with Caucasian individuals (50%). It is possible that our findings that the MnSOD-9Ala variant may have a specific role in cognitive dysfunction, especially attentional dysfunction, in schizophrenia may be specific to Chinese people or individuals with Asian ancestry and may not apply to western patients. Future studies are needed to examine the relationship between the MnSOD-9Ala variant and cognitive dysfunction in schizophrenia in a larger prospective sample and in different ethnic populations, such as in Caucasian population.

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References


