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S100B gene polymorphisms predict prefrontal spatial function in both schizophrenia patients and healthy individuals

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ABSTRACT

Animal studies have strongly implicated a role of S100B in spatial ability and our recent study of humans found that S100B gene polymorphisms (rs9722, rs1051169, and rs2839357) were associated with schizophrenia patients' spatial ability (as assessed by a block design task and a mental rotation task). In this study, we explored the associations between these and three additional SNPs in S100B and prefrontal functions (working memory and executive control) among 434 schizophrenia patients and 412 healthy controls. Results showed that, for both schizophrenia patients and healthy controls, two SNPs were significantly associated with prefrontal functions in the spatial domain (P value threshold was set at 0.014 after correcting for multiple comparisons), with the AA genotype of rs9722 and the GG genotype of rs2839357 linked to poorer performance. No SNP was associated with prefrontal functions in the verbal domain (all P s > 0.05). These results extend our previous study and further confirm the important roles of the S100B gene in spatial abilities.

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1. Introduction

S100B is a member of the S100 calcium-binding protein superfamily. Produced primarily by astrocytes, S100B exerts autocrine and paracrine effects on neurons and glia, and thus contributes to signal transduction, cell proliferation and differentiation (Rothermundt et al., 2004). Within the brain, S100B is expressed most abundantly in the dorsolateral prefrontal cortex (DLPFC) (Steiner et al., 2008). There is evidence that elevated S100B levels may impair cognitive functions that are most closely linked to the DLPFC (e.g., working memory and executive functioning) (Ehrenreich et al., 2007; Yadavalli et al., 2008).

Elevated levels of S100B have also been suggested as a possible biomarker of schizophrenia by several studies (Lara et al., 2001; Rothermundt et al., 2001; Steiner et al., 2008; Schroeter et al., 2009; Zhang et al., 2010; see Schroeter et al., 2009 for a recent meta-analysis). For example, Steiner et al. (2008) compared schizophrenia patients and healthy controls in the expression of S100B in several brain regions and found that patients' S100B levels were significantly elevated only in the DLPFC, which indicated that the altered S100B function in this region may be crucial for the etiology of schizophrenia. Due partly to the above

evidence, the gene that encodes S100B has also been suggested as a candidate gene for schizophrenia. The S100B gene is located at chromosome 21 and has 3 exons. A previous study of Han Chinese subjects (Liu et al., 2005) found a schizophrenia risk haplotype composed of two S100B SNPs, viz. rs9722 (at 3'-UTR) and rs1051169 (a synonymous SNP at exon 2). Our study (Zhai et al., 2011) further found a correlation between these two SNPs and schizophrenia patients' visual spatial disability. It should be noted that both of these SNPs appear to be functional. Hohoff et al. (2010) genotyped 9 SNPs in the S100B gene and found that the AA genotype of rs9722 was significantly and the GG genotype of rs1051169 was marginally associated with higher S100B levels in the prefrontal cortex or peripheral blood.

Based on these studies, we hypothesized that S100B gene polymorphisms (specifically rs9722 and rs1051169), which are associated with schizophrenia and its endophenotypes such as spatial abilities, would be associated with prefrontal cognitive functions, including working memory and executive function, especially when they involve spatial stimuli. To test our hypothesis, we used four widely used cognitive tasks assessing prefrontal cognitive functions, all of which were well documented endophenotypes for schizophrenia (Ehlis et al., 2007; Liu et al., 2008; Opgen-Rhein et al., 2008; Osinsky et al., 2009; Bertolino et al., 2010). They are the backward digit span task derived from Wechsler Adult Intelligence Scale (WAIS) (verbal

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working memory), an N-back task similar to that developed by Callicott et al. (1998) (spatial working memory), a Stroop task (verbal conflict effect), and an attention network test (Fan et al., 2002) (ANT, spatial conflict effect). For S100B gene polymorphisms, we included six SNPs. In addition to rs9722 and rs1051169 mentioned above, we also included rs2839357 (at intron 1). The GG genotype of this SNP showed a marginally significant association with schizophrenia patients' poorer performance on a mental rotation task (a visual spatial cognitive task that has a component of working memory) in our previous study (Zhai et al., 2011). To cover the full length of the S100B gene, three additional SNPs, viz. rs3788266 (at 5'-UTR), rs881827 (at intron 2) and rs2839349 (at 3'-UTR), were also genotyped.

2. Materials and methods

2.1. Subjects

The sample consisted of 434 schizophrenia patients and 412 healthy volunteers. Among them, 304 patients and 196 healthy controls were included in our previous study (Zhai et al., 2011). The remaining subjects were newly added. The patients were recruited from the Ankang Hospital, a division of the Jining Medical College in Shangdong Province. All patients had been hospitalized for less than 1 month and fulfilled the ICD-10 criteria for schizophrenia based on the diagnostic consensus of two experienced psychiatrists. The positive and negative syndrome scale (PANSS) was used to assess each patient's positive (SAPS) and negative (SANS) symptoms at the time of the administration of the cognitive tests. The mean score of the patients' SAPS was 19.23 ± 6.49 and the mean SANS score was 17.29 ± 7.25 . The mean duration of illness was 5.15 ± 8.54 years, and the mean number of previous hospitalizations was 1.41 ± 2.10 . All patients were undergoing monotherapy with atypical antipsychotics and had been treated for more than 2 weeks. Exclusion criteria for the patients included a history of other psychiatric disorders, a history of severe head injury, currently having acute psychotic episodes, current substance abuse, and failure to cooperate during the cognitive tests. The healthy controls were from the same geographical region as the patients and were interviewed by experienced psychiatrists to exclude any with a history or a family history of psychiatric disorders. Additional demographic information for both the schizophrenia patients and the healthy controls is shown in Table 1. This study was approved by the Institutional Review Board of the Institute of Cognitive Neuroscience and Learning at Beijing Normal University, and all subjects gave written informed consent for this study.

2.2. Cognitive tasks

IQ was indexed using the full scale of the Wechsler Adult Intelligence Scale-Revised (WAIS-RC), which was administered by experienced examiners. Backward digit span task is a subset of WAIS-RC. The raw score of this subset task was used as a measurement of the performance on verbal working memory.

Table 1
Differences in demographic characteristics and performance on cognitive tasks between schizophrenia patients and healthy controls.

	Schizophrenia patients	Healthy controls	F(or χ^2)	P value
Age (years)	36.00 ± 9.90	24.80 ± 8.00	110.73	<0.001*
Gender (female/male)	146/288	170/242	4.382	0.036*
Education (years)	9.98 ± 2.93	10.60 ± 2.78	11.92	0.001*
IQ ^a	97.13 ± 14.87	108.52 ± 13.89	135.46	<0.001*

^a Kruskal Wallis χ^2 test.

* P value <0.05.

The N-back task assessing the spatial working memory was similar to the version introduced by Callicott et al. (1998). In this task, a white circle was presented randomly at one of the four corners of a gray diamond-shaped square in a white background on an IBM 14-inch screen notebook. The four response buttons were arranged also in a diamond-shape similar to the configuration of the white circles presented on the screen. Subjects used their right index or middle finger to press one of the four buttons to match the target stimulus. There were three task conditions: 0-, 1- and 2-back. In the 0-back task, the subjects were instructed to press the button whose position was the same as the white circle on the screen at the current trial. In the 1-back task, the subjects pressed the button corresponding to the position of the white circle presented 1 trial before the current one. In the 2-back task, the subjects pressed the button corresponding to the position of the white circle presented 2 trials before the current one. Each condition (performed in one block) included 48 trials. All subjects followed the order of 0-, 1-, and 2-back conditions. The stimulus presentation time was 200 ms and the inter-stimulus interval was 800 ms. Following Blokland et al.'s (2008) twin study, the current study used error rate (percentage of wrong responses) to index performance.

The attention network test (ANT) uses arrows pointing to the left or the right as stimuli, so it is believed to reflect the spatial conflict effect (Fan et al., 2003). More detailed descriptions of the design of this task can be found elsewhere (Fan et al., 2002). The current study used the short version of this test, which contained 144 trials and can be freely downloaded from Fan's webpage (<http://www.sacklerinstitute.org/users/jin.fan/>). This version omitted the double cue conditions and the neutral target conditions, which were irrelevant to the calculation of the conflict effect. The conflict effect was calculated by subtracting the mean reaction time (RT) of all correct trials for the congruent target condition from the mean RT of correct trials for the incongruent target condition.

We also used the classic Stroop task to assess the verbal conflict effect. Color words were presented in three different ink colors: red, green and blue. The subjects were asked to press one of the three keys to indicate the color of ink while ignoring the meaning of the color word. The ink color and the word's meaning may be congruent or incongruent. The conflict effect was measured, similar to the ANT, by subtracting the mean RT of all correct trials for the congruent target condition from the mean RT of correct trials for the incongruent target condition.

2.3. SNPs genotyping

Details of the genotyping for all SNPs (rs2839357, rs1051169, rs9722, rs2839349, rs3788266 and rs881827) can be found in our previous study (Zhai et al., 2011). Briefly, all the SNPs were genotyped using Taqman allele-specific assays on the 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, U.S.A.). The sample success rates for all SNPs were above 95% and the reproducibility of the genotyping was 100% according to a duplicate analysis of at least 2% of the genotypes.

2.4. Statistical analysis

The Hardy-Weinberg test of each SNP was done by using the PLINK program (Purcell et al., 2007). The haplotypes for each subject were estimated by phase 2.1 (Stephens and Donnelly, 2003). All other analyses were done by using SPSS version 13.0. Two-way ANOVA was used to test the overall effect of genotype on cognitive functions, followed by one-way ANOVA or Kruskal Wallis Test in patients and controls separately. In the two-way ANOVA analysis, genotype and diagnosis (patients vs. controls) were entered as fixed factors, scores of all cognitive tasks were entered as dependent variables and demographic factors including age, gender, education years and

IQ were entered as covariates. One-Sample Kolmogorov–Smirnov Test was performed to examine whether the distributions of cognitive task scores in either patients or controls were normal. For cognitive scores that showed normal distribution, the associations between cognitive function and SNPs or haplotypes were tested by a parametric test (one-way ANOVA), followed by a post-hoc analysis. For cognitive scores that did not show normal distribution, we first performed a non-parametric test (Kruskal Wallis Test), and then transformed the data to rank orders to do post-hoc analysis with ANOVA. Considering the strong linkage disequilibrium among SNPs, we used the method suggested by Nyholt (2004) to correct for multiple testing. Effective number of independent marker loci (Meff) was 3.57 and the significance threshold required to keep Type I error rate at 5% was 0.014.

3. Results

Consistent with our previous study, no deviation from Hardy–Weinberg equilibrium (all P values >0.05) was found for any SNP in healthy controls. Moreover, this study found no significant differences in genotype or allele frequencies of all SNPs between schizophrenia patients and controls (all P values >0.05).

Significant differences were found between patients and controls for all demographic factors, including age, gender, years of education and IQ (all P values <0.05 , see Table 1). Controlling for all these demographic factors, patients performed more poorly on all cognitive tasks (all P values <0.01) than did controls. For the 2-back task, three SNPs showed significant associations (for rs9722, $F = 4.599$, $P = 0.010$; for rs1051169, $F = 4.618$, $P = 0.010$; for rs2839357, $F = 3.981$, $P = 0.019$, not significant after correction). For the 1-back task, significant associations were found for two SNPs, namely rs9722 ($F = 5.310$, $P = 0.005$) and rs2839357 ($F = 5.477$, $P = 0.004$). Moreover, we also found significant associations between ANT conflict effect and both rs9722 ($F = 6.524$, $P = 0.002$) and rs2839357 ($F = 4.410$, $P = 0.013$). Significant interactions were also found for the 1-back task between diagnosis and two SNPs (rs9722, $F = 4.497$, $P = 0.011$; and rs2839357, $F = 3.992$, $P = 0.019$, not significant after correction). However, no significant association was found for the two verbal tasks (all P values >0.05) (see Table 2 and Supplementary Table S4). Consistent with our previous study, the other three SNPs, viz. rs2839349, rs3788266 and rs881827, showed no significant associations (see Table 2 and Supplementary Table S4).

To explore the nature of the genotype's main effects and interaction effects with diagnosis, we further tested the simple effects of genotype separately for patients and controls. Because no significant differences were found in all demographic factors or clinical characteristics across genotypic groups in either patients or healthy controls (all P values >0.05 , see Supplementary Table S1), the following analysis did not control for these factors. For patients, the error rates for the 1-back task and the Stroop task showed normal distribution, whereas for controls, the error rates for the 2-back task and the ANT task showed normal or near-normal distribution (all Kolmogorov–Smirnov P values >0.01). Other measures showed skewed distribution (P values <0.01 , see Supplementary Table S2).

For the 2-back task, significant associations were found at rs1051169 in patients (Kruskal Wallis $\chi^2 = 7.890$, $P = 0.019$) with G allele homozygotes showing significantly higher error rates than did C allele carriers. In controls, significant associations were found at both rs9722 ($F = 3.512$, $P = 0.031$) and rs2839357 ($F = 3.546$, $P = 0.030$) with the AA genotype of rs9722 and the GG genotype of rs2839357 showing higher error rates than did the other genotypes. For the 1-back task, significant associations were found at both rs9722 and rs2839357 in patients (for rs9722, $F = 5.490$, $P = 0.005$; for rs2839357, $F = 5.214$, $P = 0.006$), but not in controls. The direction of the association was the same as in the 2-back task. Finally, for the ANT task, rs9722 showed significant associations in both patients (Kruskal Wallis

$\chi^2 = 7.912$, $P = 0.019$) and controls ($F = 3.452$, $P = 0.033$) with the AA genotype showing a stronger conflict effect than both AG and GG genotypes. However, rs2839357 showed a significant association only in controls ($F = 3.783$, $P = 0.024$) (see Table 2) with the GG genotype showing a stronger conflict effect than the GG and GA genotypes. Only the associations between the 1-back task and both rs9722 and rs2839357 withstood the correction for multiple comparisons, suggesting that the statistical power was insufficient to analyze the patients and controls separately (also see Supplementary Table S3).

We further constructed haplotypes using rs9722 and rs2839357 (SNPs that showed significant association to both spatial tasks), and found associations at almost all the cognitive measurements in both patients and controls. Two common haplotypes (GA and AG) were observed. In healthy controls, compared to AG haplotype carriers, GA haplotype carriers showed a lower error rate for the 2-back task ($F = 6.840$, $P = 0.009$) and a weaker ANT conflict effect ($F = 6.751$, $P = 0.010$). In patients, this haplotype was significantly associated with the error rates for both 1-back ($F = 9.056$, $P = 0.003$) and 2-back tasks ($\chi^2 = 3.840$, $P = 0.050$, not significant after correction) as well as the ANT conflict effect ($\chi^2 = 6.119$, $P = 0.013$). The direction of these significant differences was the same as in controls (see Table 3).

4. Discussion

Consistent with our hypothesis, the current study found significant associations between S100B gene polymorphisms (rs9722, rs1051169 and rs2839357) and performance on prefrontal function tasks that involved spatial stimuli (i.e., the spatial N-back task and the ANT spatial conflict effect). The results were consistent across schizophrenia patients and healthy controls. In contrast, S100B gene polymorphisms did not show any significant associations with the tasks that involved verbal stimuli (the Stroop task and the backward digit span task). These results indicated that the S100B gene may play a crucial role in prefrontal cognitive function in the spatial domain.

Both spatial working memory and verbal working memory impairments are well documented in schizophrenia patients (Glahn et al., 2005). In their study of twins with probands of schizophrenia, Cannon et al. (2000) evaluated the heritability of various cognitive abilities including spatial working memory and verbal working memory. They found that spatial working memory, but not verbal working memory, was more highly correlated within MZ pairs than within DZ pairs. Another twins study (Pirkola et al., 2005) found that schizophrenia patients and their unaffected co-twins performed significantly worse than control subjects on a spatial working memory task, whereas only the schizophrenia patients performed significantly worse than the control subjects on a verbal working memory task. Kravariti et al. (2006) further suggested that an intellectual asymmetry with superior verbal skills but inferior spatial skills is a putative endophenotype for schizophrenia. These studies indicated that spatial working memory and spatial abilities in general may be a better endophenotype of schizophrenia than verbal working memory or other verbal abilities, a proposition consistent with our finding that the schizophrenia-related S100B gene was associated with spatial but not verbal working memory. It is worth adding that the distinction between verbal and spatial working memory has been demonstrated among non-clinical samples. For example, behavioral genetics studies found that 7%–30% of the genetic variance in working memory was due to modality-specific factors (i.e., spatial vs. verbal) (Ando et al., 2001). Similarly, molecular genetics studies have found that spatial working memory and verbal working memory have different molecular genetic bases (Jansen et al., 2009). Along the same line, although the ANT and the Stroop task both assess the executive control of attention, they have different neural bases (Fan et al., 2003), attention processing (Chajut et al., 2009), and heritability (Breton et al., 2011). These may be reasons for the different association results found in this study. In the following paragraphs, we

Table 2
Results of the association analysis between S100B SNPs and cognitive functions.

	Sample/ number	Mean ± SD			Case vs controls	Genotype effect	Interaction	Simple effect
		AA	AG	GG	F(P value)	F(P value)	F(P value)	F or χ^2 (P value)
<i>Rs9722</i>								
Error rate at 1-back	Case/317	0.50 ± 0.23	0.35 ± 0.22	0.40 ± 0.22	97.189 (<0.001)**	5.310 (0.005)**	4.497 (0.011)**	5.490 (0.005)**
	Control/396	0.23 ± 0.21	0.20 ± 0.16	0.19 ± 0.15				
Error rate at 2-back	Case/312	0.76 ± 0.14	0.67 ± 0.20	0.68 ± 0.19	73.796 (<0.001)**	4.599 (0.010)**	0.147 (0.863)	4.970 (0.083) ^a
	Control/396	0.56 ± 0.20	0.45 ± 0.24	0.45 ± 0.23				
Backward digit span	Case/434	4.38 ± 1.14	4.35 ± 1.25	4.50 ± 1.28	30.881 (<0.001)**	1.828 (0.161)	1.598 (0.203)	3.512 (0.031)*
	Control/412	4.97 ± 1.52	5.45 ± 1.46	5.56 ± 1.66				
ANT conflict effect	Case/283	130.09 ± 81.98	91.96 ± 52.32	110.56 ± 76.92	8.998 (0.003)**	6.524 (0.002)**	3.461 (0.032)*	7.913 (0.019)**
	Control/397	110.64 ± 54.29	85.74 ± 33.20	85.09 ± 35.97				
Stroop conflict effect	Case/119	136.88 ± 85.08	146.27 ± 148.80	159.84 ± 114.43	11.273 (0.001)**	0.237 (0.789)	0.935 (0.393)	3.452 (0.033)
	Control/399	118.91 ± 89.51	102.14 ± 70.19	100.04 ± 75.04				
<i>Rs1051169</i>								
Error rate at 1-back	Case/316	0.43 ± 0.22	0.39 ± 0.23	0.35 ± 0.21	108.440 (<0.001)**	2.054 (0.129)	1.538 (0.216)	7.890 (0.019) ^a
	Control/396	0.20 ± 0.15	0.20 ± 0.16	0.18 ± 0.16				
Error rate at 2-back	Case/311	0.72 ± 0.19	0.68 ± 0.18	0.65 ± 0.19	116.368 (<0.001)**	4.618 (0.010)**	0.414 (0.661)	2.749 (0.065)
	Control/396	0.48 ± 0.22	0.46 ± 0.24	0.40 ± 0.25				
Backward digit span	Case/432	4.48 ± 1.22	4.35 ± 1.26	4.53 ± 1.26	62.680 (<0.001)**	0.996 (0.370)	1.176 (0.309)	
	Control/412	5.39 ± 1.47	5.40 ± 1.51	5.75 ± 1.82				
ANT conflict effect	Case/282	100.95 ± 71.87	103.02 ± 59.61	116.89 ± 84.43	11.642 (0.001)**	1.189 (0.305)	1.508 (0.222)	
	Control/397	87.10 ± 43.41	87.03 ± 32.35	85.38 ± 37.39				
Stroop conflict effect	Case/120	152.10 ± 125.96	152.35 ± 138.75	151.82 ± 111.34	22.523 (<0.001)**	0.087 (0.916)	0.001 (0.972)	
	Control/399	103.31 ± 76.72	100.57 ± 70.53	107.60 ± 81.27				
<i>Rs2839357</i>								
Error rate at 1-back	Case/314	0.49 ± 0.23	0.35 ± 0.23	0.40 ± 0.22	95.777 (<0.001)**	5.477 (0.004)**	3.992 (0.019)*	5.214 (0.006)**
	Control/395	0.23 ± 0.21	0.20 ± 0.16	0.19 ± 0.15				
Error rate at 2-back	Case/309	0.67 ± 0.19	0.68 ± 0.19	0.74 ± 0.16	69.282 (<0.001)**	3.981 (0.019)*	0.369 (0.692)	0.785 (0.675) ^a
	Control/395	0.56 ± 0.20	0.45 ± 0.24	0.45 ± 0.23				
Backward digit span	Case/431	4.40 ± 1.20	4.37 ± 1.23	4.48 ± 1.29	30.032 (<0.001)**	1.770 (0.171)	1.702 (0.183)	3.546(0.030)*
	Control/411	4.97 ± 1.55	5.41 ± 1.46	5.60 ± 1.65				
ANT conflict effect	Case/280	124.34 ± 77.51	96.32 ± 60.06	109.13 ± 75.34	8.278 (0.004)**	4.410 (0.013)**	1.305 (0.272)	5.373 (0.068) ^a
	Control/396	101.26 ± 54.97	85.24 ± 32.30	85.00 ± 36.02				
Stroop conflict effect	Case/119	136.37 ± 85.26	153.36 ± 149.17	154.43 ± 115.91	10.872 (0.001)**	0.033 (0.968)	0.775 (0.461)	3.783 (0.024)*
	Control/397	121.56 ± 89.46	100.47 ± 70.81	101.27 ± 74.69				
<i>Rs3788266</i>								
Error rate at 1-back	Case/317	0.40 ± 0.24	0.41 ± 0.23	0.37 ± 0.22	55.378 (<0.001)**	0.294 (0.745)	0.153 (0.858)	
	Control/399	0.18 ± 0.14	0.20 ± 0.16	0.20 ± 0.17				
Error rate at 2-back	Case/312	0.66 ± 0.19	0.71 ± 0.17	0.67 ± 0.20	45.480 (<0.001)**	0.227 (0.797)	0.034 (0.967)	
	Control/399	0.44 ± 0.22	0.46 ± 0.25	0.46 ± 0.22				
Backward digit span	Case/434	4.61 ± 1.20	4.38 ± 1.22	4.44 ± 1.29	15.720 (<0.001)**	1.973 (0.140)	1.159 (0.314)	
	Control/408	5.58 ± 1.41	5.54 ± 1.57	5.25 ± 1.48				
ANT conflict effect	Case/280	98.79 ± 77.35	93.63 ± 79.39	100.57 ± 71.55	2.009 (0.157)	1.308 (0.271)	1.348 (0.260)	
	Control/397	67.56 ± 31.85	87.40 ± 36.04	89.03 ± 38.67				
Stroop conflict effect	Case/122	137.13 ± 96.06	164.57 ± 120.01	142.10 ± 136.53	11.468 (0.001)**	0.414 (0.661)	1.364 (0.257)	
	Control/402	98.21 ± 54.78	98.22 ± 73.84	107.12 ± 76.92				
<i>Rs881827</i>								
Error rate at 1-back	Case/316	0.40 ± 0.22	0.36 ± 0.21	0.42 ± 0.24	55.335 (<0.001)**	2.053 (0.129)	2.873 (0.057)	
	Control/396	0.19 ± 0.16	0.20 ± 0.16	0.20 ± 0.16				
Error rate at 2-back	Case/311	0.68 ± 0.18	0.66 ± 0.20	0.70 ± 0.17	52.087 (<0.001)**	1.937 (0.145)	0.291 (0.748)	
	Control/396	0.44 ± 0.19	0.44 ± 0.24	0.47 ± 0.24				
Backward digit span	Case/432	4.67 ± 1.30	4.37 ± 1.26	4.44 ± 1.24	9.254 (0.002)**	1.097 (0.334)	1.466 (0.231)	
	Control/412	50.9 ± 1.61	5.49 ± 1.52	5.51 ± 1.59				
ANT conflict effect	Case/283	104.82 ± 77.98	95.74 ± 75.23	98.10 ± 74.68	0.797 (0.372)	0.469 (0.626)	0.089 (0.915)	
	Control/397	87.82 ± 35.50	84.44 ± 34.56	87.02 ± 38.97				
Stroop conflict effect	Case/120	137.39 ± 93.72	172.17 ± 128.43	135.21 ± 124.11	4.155 (0.042)*	0.608 (0.545)	2.828 (0.060)	
	Control/389	127.27 ± 71.08	98.37 ± 69.17	108.66 ± 75.92				
<i>Rs2839349</i>								
Error rate at 1-back	Case/317	0.42 ± 0.22	0.39 ± 0.23	0.38 ± 0.22	78.51 (<0.001)**	0.016 (0.985)	0.696 (0.499)	
	Control/396	0.18 ± 0.14	0.19 ± 0.15	0.21 ± 0.18				
Error rate at 2-back	Case/312	0.71 ± 0.16	0.67 ± 0.21	0.69 ± 0.18	66.755 (<0.001)**	1.285 (0.277)	0.076 (0.926)	
	Control/396	0.47 ± 0.24	0.44 ± 0.24	0.48 ± 0.22				
Backward digit span	Case/432	4.67 ± 1.28	4.31 ± 1.24	4.46 ± 1.25	21.004 (<0.001)**	2.782 (0.062)	0.301 (0.740)	
	Control/407	5.67 ± 1.64	5.43 ± 1.53	5.39 ± 1.57				
ANT conflict effect	Case/281	97.71 ± 70.10	97.35 ± 80.53	97.92 ± 70.05	0.962 (0.327)	0.485 (0.616)	0.433 (0.648)	
	Control/396	77.27 ± 32.90	87.82 ± 35.63	89.32 ± 41.13				
Stroop conflict effect	Case/120	129.05 ± 97.22	170.65 ± 146.83	143.53 ± 109.98	13.987 (<0.001)**	1.190 (0.305)	2.110 (0.122)	
	Control/399	99.41 ± 74.48	100.37 ± 70.86	108.37 ± 78.89				

^a Kruskal Wallis χ^2 test.

* P value ≤ 0.05.

** P value ≤ 0.014.

integrate our results with those of previous human and animal studies to discuss the role of S100B in prefrontal function in spatial and verbal domains.

Using various models such as S100B knockout mice and S100B over-expression mice or infusing S100B or S100B antiserum to mice, a number of animal studies have repeatedly found a significant role

Table 3
Results of the haplotype analysis.

	rs9722–rs2839357			
	AG		GA	
	F/ χ^2	P	F/ χ^2	P
Patients:				
Error rate at 1-back	0.242	0.623	9.056	0.003**
Error rate at 2-back ^a	0.447	0.504	3.840	0.050*
ANT conflict effect ^a	0.702	0.402	6.119	0.013**
Controls:				
Error rate at 1-back ^a	0.110	0.740	0.758	0.384
Error rate at 2-back	0.764	0.383	6.840	0.009**
ANT conflict effect	0.614	0.434	6.751	0.010**

^a Kruskal Wallis χ^2 test.

* P value ≤ 0.05 .

** P value ≤ 0.014 .

of S100B in the etiology of spatial ability. For example, mutant mice with null expression of S100B exhibited enhanced hippocampus long term potentiation (LTP) and spatial ability in the Morris water maze test (Nishiyama et al., 2002), while transgenic mice with overexpression of human S100B exhibited impaired hippocampus LTP and spatial ability in the Morris water maze test (Gerlai et al., 1995). Moreover, S100B overexpression mice (Roder et al., 1996) also showed impaired spatial exploratory pattern in T-maze, reflecting impaired working memory. In our previous study (Zhai et al., 2011), we extended the link between the S100B gene and spatial abilities to humans. We found that three SNPs in the S100B gene (the same ones included in the present study) were associated with performance on spatial ability tasks (mental rotation and block design). Because of the importance of working memory in tasks such as mental rotation (Hyun and Luck, 2007), we conducted the current study to investigate whether the contribution of S100B gene polymorphisms to mental rotation may partly be due to working memory involved in mental rotation. As we anticipated, this study found the most significant associations at rs9722 with the AA genotype showing relatively poor spatial function (higher error rates in the N-back tasks and a stronger ANT conflict effect) than the GG and AG genotypes. Taken together the animal studies and our previous and current studies, it seems reasonable to postulate that S100B gene polymorphisms may play an important role in the prefrontal functions in the spatial domain or even spatial abilities in general.

As for the prefrontal cognitive functions in the verbal domain, our study did not find an association with the S100B gene. There are only two related studies to our knowledge, both of which tested peripheral S100B levels (Schmitt et al., 2005; Yadavalli et al., 2008). Consistent with our results, Schmitt et al. (2005) found no significant associations between schizophrenia patients' performance on a Stroop task and their serum S100B levels. In their study of healthy older adults, however, Yadavalli et al. (2008) found a significant correlation between S100B levels and several prefrontal cognitive functions which included the letter number sequencing task. This task is similar to the digit span task used in the current study; both of them have been used to assess verbal working memory. Many factors could have contributed to the discrepancy between our results and those of Yadavalli et al. (2008). For example, we used different samples (healthy older American adults in their study vs. schizophrenia patients and healthy young adults in China in our study), different measures of biomarkers (serum S100B levels in their study and genetic variations in our study), and different measures of prefrontal functions in the verbal domain (a letter number sequencing test in their study vs. a backward digit span test in our study). More research is needed to reconcile the different findings from Yadavalli et al. (2008) as compared to ours and those of Schmitt et al. (2005).

Finally, although we found significant associations between S100B gene polymorphisms and endophenotypes of schizophrenia (i.e.,

prefrontal functions in the spatial domain), we found no significant association between S100B gene polymorphisms and schizophrenia diagnosis. We interpret this result as indicating a superiority of the endophenotype strategy in genetic studies. It is likely that it takes a moderate sample size like ours to detect genetic factors in the endophenotypes, but it takes a much larger sample like that of Liu et al. (2005) to find the more "distant" associations between S100B gene polymorphisms and schizophrenia (~750 patients and almost the same number of controls in their study).

In conclusion, this study found significant associations between S100B gene polymorphisms and prefrontal function involving spatial stimuli in both schizophrenia patients and healthy controls. These results extend our previous study and further suggest a critical role of the S100B gene in spatial ability.

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Contributors

JL, QD and CC designed the study and wrote the protocol. JZ, LC, DJ, QZ and QS managed the literature searches and analyses. JZ, LC, QZ and QS selected the sample and evaluated patients. FJ and CL evaluated the healthy controls. MC, LG, XC, KW, XD and ZX undertook the statistical analysis. JZ, JL and CC wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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