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Research report

Risk variants in the S100B gene, associated with elevated S100B levels, are also associated with visuospatial disability of schizophrenia

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ABSTRACT

Rs9722 and rs1051169 have been reported as affecting the levels of S100B in the serum or the brain, and haplotypes containing these two SNPs have been associated with schizophrenia. The current study investigated the role of the S100B gene in an endophenotype of schizophrenia—spatial disability. 304 schizophrenia patients and 196 healthy controls were given a block design task and a mental rotation task. Results showed that the two aforementioned SNPs and related haplotypes were associated with the spatial disability of schizophrenia patients. Specifically, risk factors for the elevated S100B levels, including the A allele of rs9722, the G allele of rs1051169, and the AG haplotype, were associated with a poorer performance on both tests of spatial ability, especially the mental rotation task. These results implicate a role for S100B gene polymorphisms in the cognitive functions of schizophrenia patients and encourage further investigation into spatial disability as an endophenotype of schizophrenia.

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1. Introduction

S100B is a calcium-binding protein involved in the regulation of energy metabolism in brain cells [34] and the proliferation and differentiation of neurons and glia [1]. Because schizophrenia patients are reported to show elevated levels of S100B in their peripheral blood [13,19,23,24,28], cerebral spinal fluid [22], and cortical brain tissue [27] (see [24] for a comprehensive meta-analysis), the S100B gene has been considered as a candidate gene in the etiology of schizophrenia. More importantly for this study, Liu and colleagues [14], using 384 schizophrenia patients and 401 controls of Han Chinese origin, found that schizophrenia was significantly associated with haplotypes composed of rs1051169 and rs9722 (two SNPs in the S100B gene) and marginally associated with rs1051169 alone. Interestingly, both of these SNPs have been found to affect S100B gene expression [12]. It is not known, however, whether these potentially functional SNPs can also affect cognitive functions in schizophrenia patients.

Some animal studies found that transgenic mice with over-expression of the human S100B gene exhibited impaired spatial ability [8], whereas mutant mice with null-expression of the S100B gene exhibited enhanced spatial ability [17]. Both of these findings strongly indicated that S100B may modulate spatial ability. Moreover, Wiltfang et al. [32] reported an association between serum S100B levels of liver cirrhosis patients and their performance on the block design test. These results suggest that spatial ability may mediate the association between S100B gene polymorphisms and schizophrenia.

In fact, there is evidence that spatial ability is a reasonable endophenotype of schizophrenia. Researchers have typically used two tasks to measure spatial ability [16]. The first is the Wechsler block design test, which primarily assesses visuospatial construction. This task requires participants to compose replicas of original patterns from several colored cubes. This test satisfies at least four of the five endophenotypic criteria proposed by Gottesman and Gould [9]. First, multiple studies indicated that schizophrenia patients were impaired in this task [31]. Second, a recent meta-analysis showed that unaffected first-degree relatives of schizophrenia patients were also impaired in this task [26]. Third, several twin studies showed that the heritability of ability measured by this test was in the range of 0.44–0.68 [21,29]. Fourth, schizophrenia patients' impairment on this test has been demon-

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Table 1
Differences in the demographic data, performance on mental rotation and block design tests between schizophrenia patients and healthy controls.

	Schizophrenia patients	Healthy controls	F(or χ^2)	P value
Age (years)	28.1 ± 8.0	24.8 ± 8.0	20.3	<0.001 ^a
Gender (female/male)	99/205	89/107	9.547	0.008 ^a
Education (years)	10.0 ± 3.0	11.6 ± 3.2	33.2	<0.001 ^a
IQ	97.4 ± 15.3	115.3 ± 10.0	211.9	<0.001 ^a
Mental rotation ^a				
ER				
0° (%)	3.9 ± 0.8	4.8 ± 0.6	0.602	0.438
60° (%)	5.5 ± 0.7	4.5 ± 0.9	0.618	0.432
120° (%)	16.2 ± 1.1	12.5 ± 1.4	3.825	0.051
180° (%)	28.6 ± 1.3	26.5 ± 1.6	0.934	0.334
240° (%)	12.0 ± 1.1	16.2 ± 1.4	1.323	0.251
300° (%)	5.3 ± 0.7	4.7 ± 0.9	0.212	0.645
RT				
0° (ms)	1622.4 ± 1115.3	965.7 ± 324.8	15.242	<0.001 ^a
60° (ms)	1862.9 ± 1135.9	1120.7 ± 521.0	20.784	<0.001 ^a
120° (ms)	2633.8 ± 1905.3	1465.4 ± 818.2	26.495	<0.001 ^a
180° (ms)	3447.6 ± 3001.4	1948.9 ± 1238.7	28.928	<0.001 ^a
240° (ms)	2702.1 ± 2216.3	1519.2 ± 896.5	23.409	<0.001 ^a
300° (ms)	2059.9 ± 1298.2	1198.4 ± 697.3	24.969	<0.001 ^a
Block design ^b	27.7 ± 8.9	36.5 ± 8.3	73.020	<0.001 ^a

^a Age, gender, education and IQ as covariates.

^b Age, gender and education as covariates.

^{*} P value <0.05.

strated as occurring from the pre-illness period to after the onset of illness [25] and has been showed to remain relatively stable during 2.5 years of follow-up [2,3]. The remaining criterion for establishing an endophenotype, namely, cosegregation of the cognitive measure and schizophrenia, has not been addressed to the best of our knowledge.

The other task that has been used to measure spatial ability is the mental rotation test, in which participants judge whether two objects are the same after one of them is appropriately rotated. This test primarily assesses visuospatial intuition. Schizophrenia patients have also been reported to be impaired in this task [6,11]. Unfortunately, few studies have discussed whether this task satisfies the other criteria for an endophenotype, although some gene polymorphisms have been reported to be associated with performance on this task [4].

Considering all the above evidences, we designed this study to test whether S100B gene polymorphisms may affect spatial ability. This study, to our knowledge, is the first to explore the association of S100B gene polymorphisms with the cognitive functions of schizophrenia.

2. Method

2.1. Subjects

The sample included 304 schizophrenia patients and 196 healthy volunteers. The patients were recruited from the Ankang Hospital in Shangdong province, a division of the Jining Medical College. 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 19.1 ± 6.5 and the mean score on the SANS was 17.2 ± 7.1. The mean duration of illness was 3.7 ± 5.3, and the mean number of previous hospitalizations was 1.7 ± 3.7. All patients were undergoing monotherapy with atypical antipsychotics and had currently been using these 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, low IQ (less than 75 on the Chinese version of the Wechsler Adult Intelligence Scale-Revised (WAIS-RC)), 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. More detailed 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, and all subjects gave written informed consent for this study.

2.2. Cognitive tasks

All the patients and healthy volunteers finished the block design task and the mental rotation task. The block design task was part of the WAIS-RC and was performed in accordance with the WAIS-RC manual. The raw score was used in this study.

A modified (easier) mental rotation task was used in this study because schizophrenia patients typically have difficulties with the classic mental rotation tests (e.g., Shepard's 3-D tasks). Based on pilot results, we chose the task that requires subjects to judge whether the digit "5" (the number "5" in Times New Roman font) is in its normal form or a mirror image after appropriate 2-D rotation. This mental rotation task was run on an IBM 14-in. screen notebook. Each trial began with a fixation point at the center of the screen lasting for 1000 ms, followed by a 100 ms blank screen and then the stimulus. Each stimulus could appear either as a mirror image or in the normal form at one of six angular orientations including 0°, 60°, 120°, 180°, 240°, and 300°. The stimulus remained on the screen until the subject responded or until 4000 ms had lapsed. In total, 48 trials were performed with an equal number of stimuli of each form and orientation. The order of presentation was pseudo-randomized to avoid consecutive trials of the same form and/or the same orientation. The subjects were instructed to determine whether the stimulus was in the mirror form or in the normal form. They were asked to press a key labeled "mirror" or "normal" on the keyboard with the right forefinger or middle finger. Both accuracy and speed were emphasized. The response times of correct responses (RT in ms) and the accuracy were recorded by the computer.

2.3. SNPs genotyping

Genomic DNA was extracted from a 200 µl venous blood sample from each subject using the QuickGene-Mini80 equipment and QuickGene DNA whole blood kit S (Fujifilm, Tokyo, Japan).

Genotypic data for the S100B gene and its 2 kb upstream and downstream regions in both Chinese and Japanese populations were downloaded from HapMap. Only SNPs with a minor allele frequency (MAF) >10% were considered. Data in HapMap showed that the SNPs in this gene were highly linked with one another. We selected tags from this subset of SNPs using the Tagger program [5] implemented in the Haploview program (version 4.2). We forced rs1051169 and rs9722 to be included in the list of tags, and then used pairwise tagging and a r^2 threshold of 0.85 to obtain another four SNPs (rs2839349, rs3788266, rs2839357, and rs881827).

All the SNPs were genotyped using Taqman allele-specific assays on the 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Genotypes were read automatically. Ambiguous genotypes were excluded if repeat assays could not be read automatically. The sample success rate was 100% and the reproducibility of the genotyping was 100% according to a duplicate analysis of 2% of the genotypes.

2.4. Analysis

The PLINK program [20] was used to perform the Hardy-Weinberg test of each SNP in the control sample, to examine the association between cognitive function and each SNP, to generate a haplotypic estimate for each patient, and to examine the association between cognitive function and each haplotype. This program uses a regression model to do both SNP-based and haplotype-based analyses. A linkage dis-

Table 2
Genotypes and allele frequencies for each SNP.

	Genotype			χ^2	P	Allele		χ^2	P
	1	2	3			Minor	Major		
rs2839349	CC	CT	TT	1.140	0.566	C	T	0.940	0.332
Case	50	140	114			240	368		
Control	35	97	64			167	225		
rs9722	AA	AG	GG	2.828	0.243	A	G	0.157	0.692
Case	34	115	155			183	425		
Control	17	89	90			123	269		
rs881827	AA	AG	GG	2.698	0.259	A	G	2.118	0.146
Case	33	119	152			185	423		
Control	13	76	107			102	290		
rs1051169	GG	GC	CC	1.155	0.561	G	C	0.640	0.424
Case	56	139	109			251	357		
Control	37	98	61			172	220		
rs2839357	GG	GA	AA	3.056	0.217	G	A	0.208	0.649
Case	34	117	153			185	423		
Control	17	91	88			125	267		
rs3788266	AA	AG	GG	1.578	0.454	A	G	0.924	0.336
Case	29	124	151			182	426		
Control	19	91	86			129	263		

equilibrium of the SNPs was performed using Haploview Version 4.2. Non-genetic factors, including age, gender, IQ and years of education among genotypic or haplotypic groups or between patients and controls were compared using a one-way ANOVA or a chi-square test in SPSS13.0. Factors that showed significant differences between patients and controls were used as covariates; however, IQ was not used as a covariate for the analyses of the block design task because the block design task is a part of the IQ test. The main effects of genotype and group (patients or controls) together with their interaction in the cognitive task performance were analyzed by a multivariate analysis of variance (MANOVA). In this analysis, demographic characteristics were used as covariates. The significance level was 0.0083 for each SNP (six SNPs) and each haplotype (six common haplotypes) after the Bonferroni correction.

3. Results

One-way ANOVA and chi-square tests revealed significant differences between the patients and controls in all demographic characteristics, including gender, age, years of education and IQ (all *P* values <0.01, see Table 1). After controlling for all these factors, the patients showed a significantly higher mean RT on the mental rotation task than did the healthy volunteers (all *P* values <0.001, see Table 1). The error rates of the mental rotation were comparable between patients and controls (all *P* values >0.05, see Table 1). The patients had a significantly lower raw score on the block design test ($F = 73.020, P < 0.001$, see Table 1). So, the subsequent genetic analyses focused on the mean RTs in the mental rotation task and the raw scores of the block design task.

The allele frequencies for each SNP in the controls did not show any deviation from Hardy-Weinberg equilibrium (all *P* values >0.05, see supplementary Table S1). *D'* values were calculated in patients

and controls separately and yielded similar results. Except for a low *D'* between rs2839349 and rs1051169 (for patients, *D'* = 0.36, *P* value <0.05; for controls, *D'* = 0.34, *P* value <0.05), a strong linkage disequilibrium among the other pairs of SNPs was observed (all *D'*s > 0.8, *P* value <0.05). No alleles or genotypes showed a significant association with schizophrenia (all *P* values >0.05, see Table 2).

In the patients, the demographic characteristics, with the single exception of gender, did not differ across the genotype groups of each SNP (all *P* value >0.05, see supplementary Table S2). Gender showed a significantly different distribution among the rs881827 genotype groups ($\chi^2 = 6.058, df = 2, P = 0.048$). In the mental rotation task, rs9722 showed a significant association with mean RTs at all degrees except 180° (all *P* value <0.05, see Table 3). Post-hoc analysis showed that the mean RTs of the AA genotype were higher than those of the GG and/or AG genotypes at all degrees except 180°. No difference was found between the mean RTs of the AG and GG genotypes. Another SNP, the rs1051169, showed a significant association with the mean RTs at 0° (*P* = 0.0008) and 60° (*P* = 0.003) with the GG genotype displaying higher mean RTs at these two degrees than GC and/or CC genotypes. Moreover, rs2839357 showed a significant association with the mean RTs at 0° (*P* = 0.009) and 180° (*P* = 0.027), and the GG genotype displayed higher mean RTs than both AA and GA genotypes. After the Bonferroni correction, only the association between rs9722 and the mean RTs at 0° and 240° and the association between rs1051169 and the mean RTs at 0° and 60° remained significant. For the block design task, rs9722 also showed a significant association (*P* = 0.023), which, however, could not withstand the Bonferroni correction. Two other SNPs,

Table 3
Effect sizes (beta) across genotypes for each SNP in schizophrenia patients.

	rs2839349		rs9722		rs881827 ^a		rs1051169		rs2839357		rs3788266	
	Beta	P value	Beta	P value	Beta	P value	Beta	P value	Beta	P value	Beta	P value
Mental rotation-RT												
0°	-65.6	0.471	295.0	0.0016**	-185.0	0.051	296.8	0.0008**	244.7	0.009*	-166.1	0.088
60°	-6.3	0.946	230.2	0.016*	-174.7	0.068	269.7	0.003**	142.8	0.137	-134.8	0.175
120°	-89.3	0.566	324.2	0.043*	-164.9	0.307	269.2	0.078	234.2	0.146	-130.9	0.432
180°	151.7	0.536	182.0	0.473	-287.0	0.263	315.4	0.190	126.0	0.620	-68.8	0.793
240°	-208.1	0.250	493.3	0.0080**	-222.1	0.239	319.1	0.072	414.2	0.027*	-189.5	0.329
300°	-32.4	0.760	243.0	0.026*	-170.8	0.120	166.5	0.110	192.0	0.080	-26.9	0.813
Block design												
Raw score	0.512	0.481	-1.703	0.023*	1.177	0.120	-1.289	0.070	-1.305	0.082	0.236	0.762

^a Gender as the covariate.

* *P* value <0.05.

** *P* value <0.0083.

Table 4
Post hoc analysis for the differences in performances across genotypes in schizophrenia patients.

	Genotype1	Genotype2	Genotype3	Post-hoc
Mental rotation-RT				
rs9722	AA	AG	GG	
RT-0°	2272.3 ± 1640.7	1599.4 ± 1174.2	1496.9 ± 860.5	AA > AG = GG
RT-60°	2344.0 ± 1362.9	1860.3 ± 1306.4	1759.3 ± 903.0	AA > GG, GG = AG
RT-120°	3285.0 ± 2496.8	2645.8 ± 2067.9	2482.1 ± 1588.7	AA > GG, GG = AG
RT-240°	3842.0 ± 3195.8	2632.2 ± 2476.8	2503.8 ± 1614.0	AA > AG = GG
RT-300°	2604.2 ± 1302.0	2035.6 ± 1631.1	1958.4 ± 952.2	AA > GG, GG = AG
rs1051169	GG	GC	CC	
RT-0°	2141.6 ± 1773.3	1536.6 ± 858.2	1465.0 ± 879.2	GG > GC = CC
RT-60°	2239.6 ± 1518.2	1861.7 ± 1133.5	1671.0 ± 833.0	GG > GC, GC = CC
rs2839357	GG	GA	AA	
RT-0°	2173.9 ± 1646.4	1595.5 ± 1156.0	1520.3 ± 890.1	GG > GA = AA
RT-240°	3700.5 ± 3253.8	2618.9 ± 2448.5	2543.8 ± 1632.3	GG > GA = AA
Block design				
rs9722	AA	AG	GG	
Raw score	24.9 ± 9.4	27.3 ± 8.5	28.7 ± 8.9	GG > AA, GG = AG

Table 5
Effect sizes (beta) of each haplotype in schizophrenia patients.

	rs9722-rs1051169-rs2839357						rs9722-rs1051169					
	AGG		GGA		GCA		AG		GG		GC	
	Beta	P value	Beta	P value	Beta	P value	Beta	P value	Beta	P value	Beta	P value
Mental rotation-RT												
0°	272.0	0.004**	104.2	0.481	-287.0	0.001**	295.0	0.002**	79.8	0.584	-296.8	0.0008**
60°	176.9	0.069	187.4	0.213	-250.3	0.006**	230.2	0.016*	163.3	0.271	-269.7	0.003**
120°	306.4	0.060	-11.9	0.963	-231.5	0.129	324.2	0.043*	-63.5	0.799	-269.2	0.010*
180°	185.0	0.473	473.1	0.234	-289.0	0.229	182.0	0.719	399.8	0.308	-315.4	0.190
240°	499.6	0.008**	-302.3	0.304	-271.4	0.126	493.3	0.008**	-336.5	0.245	-319.1	0.072
300°	226.9	0.041*	-116.1	0.500	-151.7	0.145	243.0	0.026*	-141.2	0.405	-166.5	0.110
Block design												
Raw score	-1.6	0.034*	0.7	0.531	1.0	0.147	-1.7	0.023*	0.7	0.568	1.3	0.070

* P value < 0.05.

** P value < 0.0083.

rs1051169 ($P=0.070$) and rs2839357 ($P=0.082$), only showed a tendency toward association (see Tables 3 and 4).

We next performed a haplotypic analysis for two haplotype combinations, namely rs9722-rs1051169-rs2839357 and rs9722-rs1051169. No demographic characteristics showed differences among these haplotypes (all P values > 0.05). For rs9722-rs1051169, there were three haplotypes, AG, GG and GC, with frequencies of 30.1%, 58.7% and 11.2% respectively. The AG haplotype showed a positive association with mean RTs at all degrees except 180° (all P value < 0.05), and the GC haplotype showed a negative association at 0° ($P=0.0008$) and 60° ($P=0.0029$). For the block design task, the AG haplotype showed a negative association with raw scores ($P=0.023$) while the GC haplotype showed a tendency toward a positive association ($P=0.070$). After the Bonferroni correction, the association between AG and the mean RTs at 0° ($P=0.002$) and 240° ($P=0.008$) and the association

between GC and the mean RTs at 0° and 60° were still significant. For rs9722-rs1051169-rs2839357, we observed five haplotypes, of which three were common. The frequencies for these three haplotypes were 0.289 (AGG), 0.108 (GGA) and 0.576 (GCA). The AGG haplotype showed a positive association with the mean RTs at 0° ($P=0.004$), 240° ($P=0.008$), and 300° ($P=0.041$) and also showed a negative association with the raw score on the block design task ($P=0.034$). The GCA haplotype showed a negative association with the mean RTs at 0° ($P=0.001$) and 60° ($P=0.006$). Only the association between the AGG haplotype and the mean RTs at 300° as well as the raw score of the block design task could not withstand the Bonferroni correction (see Table 5).

In healthy volunteers, none of the demographic characteristics showed any differences among the three genotypes of each SNP (see supplementary Table S3) and no association was found between any task performance and any SNP (see Table 6).

Table 6
Association analysis in healthy controls for each SNP.

	rs2839349		rs9722		rs881827		rs1051169		rs2839357		rs3788266	
	Beta	P	Beta	P	Beta	P	Beta	P	Beta	P	Beta	P
Mental rotation- RT												
0°	0.8	0.980	3.3	0.927	-4.5	0.904	-6.8	0.838	14.8	0.686	23.5	0.514
60°	-31.5	0.558	63.2	0.279	-24.2	0.689	10.0	0.852	53.3	0.364	16.1	0.781
120°	-9.0	0.915	143.9	0.116	-138.5	0.144	122.3	0.146	152.5	0.097	13.9	0.879
180°	13.0	0.919	-34.5	0.804	19.3	0.893	-13.5	0.916	37.6	0.788	62.6	0.648
240°	43.2	0.640	-18.4	0.855	-32.7	0.753	-30.7	0.739	12.2	0.904	114.7	0.248
300°	-37.2	0.605	19.9	0.800	29.2	0.719	0.120	0.999	32.8	0.676	-1.3	0.987
Block design												
Raw score	0.421	0.622	-0.766	0.409	0.391	0.683	-1.051	0.216	-1.252	0.178	0.543	0.553

Table 7
MANOVA analysis results with main effects of genotype, group and their interaction.

	Main effect				Interaction	
	Group		Genotype		Group* genotype	
	F	P value	F	P value	F	P value
rs9722						
RT-0°	58.452	<0.001*	4.532	0.011*	2.999	0.051
RT-60°	51.374	<0.001*	4.191	0.016*	0.675	0.510
RT-120°	43.547	<0.001*	3.616	0.028*	0.350	0.705
RT-240°	43.812	<0.001*	3.742	0.024*	2.004	0.136
RT-300°	54.570	<0.001*	3.517	0.047*	0.918	0.400
rs1051169						
RT-0°	71.037	<0.001*	5.285	0.005*	4.364	0.013*
RT-60°	70.993	<0.001*	3.511	0.031*	2.305	0.101
rs2839357						
RT-0°	54.125	<0.001*	3.381	0.035*	1.913	0.149
RT-240°	41.166	<0.001*	3.008	0.050*	1.281	0.469
Block design rs9722						
Raw score	72.540	<0.001*	3.114	0.045*	0.448	0.639

* P value <0.05.

Combining the patients and healthy volunteers, a MANOVA further showed that the main effects of rs9722 for the mean RTs at all degrees except 180°, as well as the raw scores in the block design task, were significant. However, the interaction between rs9722 and groups only showed a tendency toward significance for the mean RT at 0° ($F=2.999$, $P=0.051$). For rs1051169, the main effects were significant for the mean RTs at 0° ($F=5.285$, $P=0.005$) and 60° ($F=3.511$, $P=0.031$). The interaction between rs1051169 and the groups was significant for the mean RTs at 0° ($F=4.364$, $P=0.013$) (see Table 7). Moreover, the main effects of the genotypes of rs2839357 were significant for the mean RTs at 0° ($F=3.381$, $P=0.035$) and 240° ($F=3.008$, $P=0.050$); however, the interaction between groups and genotypes was not significant (see Table 7).

4. Discussion

In this study, we investigated the association between SNPs of the S100B gene and spatial ability in both schizophrenia patients and controls. We used two classic tasks, the mental rotation task and the block design task, to assess the spatial ability of both groups. For the mental rotation task, we found that the A allele of rs9722, the G allele of rs1051169 and the haplotype composed of these two alleles were associated with elevated mean RTs among patients. For the block design task, we also obtained a significant association at rs9722 with the A allele being associated with lower raw scores in the patient group, although this result could not withstand the Bonferroni correction. These results indicate that the above-mentioned alleles or haplotypes are risk factors for poor spatial ability in schizophrenia patients.

Note that both the A allele of rs9722 and the G allele of rs1051169 have also been suggested as risk factors for elevated S100B levels in serum and/or the frontal cortex [12]. Additionally, elevated S100B levels have been shown to be associated with poor cognitive function in schizophrenia patients [19]. By directly linking the above alleles to spatial disability in schizophrenia patients, the present study helped to complete the picture of the S100B gene and schizophrenia. We can tentatively conclude that the risk alleles and haplotypes of SNPs (especially rs9722, rs1051169) seem to produce elevated S100B levels, which can lead to poor cognitive function and the onset of schizophrenia [14]. Rs9722 may be one of the most probable functional variants because it is located in the 3' untranslated region, which contains important elements, such as microRNA target sites [30], AU-rich elements [33] and so on, for the regulation of gene expression. Obviously, other SNPs,

which are in the vicinity of rs9722 and rs1051169 and which show a strong linkage with rs9722 and rs1051169 may turn out to be the actual causal variants for the association observed in this current study. This region, therefore, needs further study, including resequencing the S100B gene, in order to validate previous and our results. One SNP we might rule out is rs2839357. Although we found that it was significantly or marginally associated with the mean RT of the mental rotation task and with the raw score of the block design task in patients, these associations did not survive the Bonferroni correction. Furthermore, from the haplotypic analysis results, we found that a combination that included rs2839357 (rs9722–rs1051169–rs2839357) did not demonstrate a stronger association than did the combination that did not include rs2839357 (rs9722–rs1051169). Therefore, we believe that the significant results for rs2839357 were most likely due to its strong linkage with rs9722 or rs1051169 or a yet-to-be-determined SNP in the vicinity.

Our conclusion about the role of the S100B gene should be considered as tentative however for two reasons. First, our study did not replicate an association between the S100B gene and schizophrenia, perhaps due to our relatively small sample size for an epidemiological genetic study and/or our imperfectly matched control group. Second, we need to consider the involvement of other psychopathological and pathophysiological processes that may interact with the functions of the S100B gene. In a recent genetic study of healthy volunteers, for example, Beste et al. [4] found that a functional SNP in the promoter region of the 5-HT1A gene may affect attentional processes during mental rotation. Given that 5-HT1A is an important regulator of S100B [7], it is plausible that there may be an interaction between 5-HT1A and S100B on mental rotation.

In the controls, we found no significant association for any SNP even before the Bonferroni correction. Considering that several significant association results were found in the patient group, but not in the controls, it seems possible that the role of the S100B gene polymorphisms was different in the patients from that in the controls. In fact, some animal studies have also provided evidences for this postulate. In animal studies, different levels of S100B played different roles in cognitive functioning. Too high levels of S100B have been demonstrated to be harmful [8]; whereas S100B levels that are near normal physiological levels may be protective [10,15,18]. So, the relationship between S100B and cognitive function in patients (with high levels of S100B) and controls (with normal physiological levels of S100B) may be different. We should of course treat this speculation cautiously because, as mentioned above, the cases and controls were not well-matched in terms of age, sex, educational level, and IQ in this study and because there was only one statistically significant interaction between group and genotype for a single SNP (rs1051169) and a single cognitive measurement (the mean RT at 0°).

In this study, we obtained similar association results on both the mental rotation task and the block design task; however, the associations from the mental rotation task were stronger than those from the block design task. As we have described in the introduction, the mental rotation task and the block design task seem to assess two partially independent aspects of spatial ability. So, these results may suggest that spatial intuition (assessed by the mental rotation task) is more sensitive than spatial construction (assessed by the block design task) to the effect of S100B gene polymorphisms. Moreover, we also found significantly worse performances on both of the spatial ability tasks in the patients compared with the normal controls. The data suggest that both types of tasks satisfied the first criterion of an endophenotype.

In conclusion, we found an important role for S100B gene polymorphisms in the spatial ability of schizophrenia patients. These findings, on the one hand, can help us better understand the rela-

tionship between S100B and schizophrenia and, on the other hand, encourage further studies using spatial disability as an endophenotype of schizophrenia.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.bbr.2010.11.004](https://doi.org/10.1016/j.bbr.2010.11.004).

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