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SNPs and haplotypes in the S100B gene reveal association with schizophrenia

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Abstract

The S100B gene locates in 21q22.3 and produces neurotrophin mainly in astrocytes of CNS which can act as an extensive marker of glial cell integrity. The synaptic destabilization hypothesis (GGF/SD) suggests that the functional deficiency of growth factors like S100B is involved in the etiology of schizophrenia and the S100B serum concentration is reported to be significantly increased in patients with acute schizophrenia and decreased in chronic schizophrenia patients. To validate the association between S100B and schizophrenia, 384 cases and 401 controls, all Chinese Han subjects, were recruited. Four SNPs V1 (−960C > G), V2 (−111C > T), V3 (2757C > G, rs1051169), and V4 (5748C > T, rs9722) were studied. And haplotype V3–V4 (G–C) showed a significant association with schizophrenia. Our study showed an association between schizophrenia and a possible susceptible haplotype V3–V4 (G–C) which possesses a genetic tendency for increased S100B expression. Our results suggest that S100B could be a susceptible gene for schizophrenia and provide indirect evidence for the GGF/SD hypothesis.

Keywords: S100B; Haplotype; Association; Linkage disequilibrium; Growth factors' deficiency and synaptic destabilization hypothesis of schizophrenia; Sp1

Family, twin, and adoption studies have suggested that genetics play a major role in the transmission of schizophrenia. Despite decades of research on anatomical, physiological, and biochemical changes possibly associated with schizophrenia, insight into the etiology is only fragmentary. Recently, based on a co-localization of loci approach and a large amount of circumstantial evidence, Moises et al. [1] proposed the growth factors' deficiency and synaptic destabilization hypothesis of schizophrenia (GGF/SD). This states that a functional deficiency of glial growth factors and of growth factors produced by glial cells such as neurotrophins and glutamate leads to a weakening of synaptic strength and may cause schizophrenia.

S100 protein is a low molecular weight, EF-hand, Ca^{2+}-binding protein and is found at the highest levels in the human brain, primarily in astrocytes. S100B is the most abundant member of the S100 family in the CNS and is found in the brain at concentrations 30- to 100-fold which are higher than in other tissues. S100B protein has a molecular weight of 21 kDa existing as a homodimer consisting of two β subunits. The human S100B gene is composed of three exons, the first of which specifies the

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5′-untranslated region, while the second and third each encode a single EF-hand, Ca^{2+}-binding domain. The promoter region contains several potential regulatory transcription elements including the cAMP-responsive elements CRE and AP-2, SP1 binding site GC box [2,3].

The gene encoding S100B in humans is located in chromosome 21q22.3 [4]. It is a risk region for Down syndrome, a common form of mental retardation. Down syndrome has been identified as being quite prevalent in schizophrenia patients [5] and Down syndrome patients have been found to have a different spectrum of mental disorders from those without the syndrome.

S100B is a neurotrophin produced mainly by astrocytes and has implications for psychiatry as it represents a biochemical marker of cerebral cellular integrity that can be measured during acute psychotic episodes of schizophrenia. Increased S100B serum concentration has been reported in acute schizophrenia patients and an inverse correlation with illness duration has been identified [6–8]. Rothermundt et al. [7] also found that continuously increased S100B concentration after six weeks of treatment was associated with persistent negative symptomatology (cognitive impairment, affective flattening, and social withdrawal). In contrast, decreased S100B level has been found in chronically ill schizophrenic patients [9]. To date, the possible relationship of schizophrenia with altered transcription of the gene has not been ruled out. All these observations point to the S100B gene as a candidate for susceptibility to schizophrenia and our study was designed to examine the possible association.

Materials and methods

Study participants. Patients with schizophrenia were recruited from subjects with sporadic episodes from psychiatric hospitals in the Shanghai and Jilin provinces; the sample included 384 patients with schizophrenia (mean age 27.2 ± 7.9 years; 50% men). All cases fulfilled the criteria for schizophrenia in the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders. The diagnosis of each patient was confirmed by at least two qualified psychiatrists. The unrelated healthy volunteers consisted of 401 people (mean age 30.2 ± 9.5 years; 49% men) who were recruited from the same areas. All participants were Han Chinese in origin. Written informed consent was obtained from all participants.

Selection of single nucleotide polymorphism. In this study, we carried out systematic screening in all the promoter and exon regions of the S100B gene by re-sequencing. Two steps were performed. First we carried out re-sequencing in 14 pool samples and found five new single nucleotide polymorphisms (SNPs) that did not appear in the NCBI dbSNP and three other SNPs that appeared in dbSNP. We also found a deletion in the promoter. We then examined their frequency in 32 individual samples by the direct sequencing procedure described below. PCR primers for SNP screening and flanking sequences for these SNPs are listed in Appendix. We excluded variations with low allele frequencies and variations in intron, and we therefore selected for further analysis four common SNPs with minor allele frequencies of over 0.1 average covering the gene. We genotyped −960C > G (V1), −111C > T (V2) in promoter, 2757C > G (V3, rs1051169) in exon2, and 5748C > T (V4, rs9722) in 3′-UTR. The location and interval of these SNPs is shown in Fig. 1.

Genotyping of single nucleotide polymorphisms. The SNPs −960C > G and −111C > T were amplified using nested polymerase chain reaction (nested-PCR) in two steps. In step one, we performed long-PCR using the primers: forward: 5′-CTCAGCCCTGTGCT ATTTCC-3′ and reverse: 5′-CGTTCCACCCAAAAGAAAAG-3′. PCR was carried out using 384-well microtitre plates with a final reaction volume of 10 μl containing 9 ng genome DNA and 0.4 U hotstar *Taq* (Qiagen), 0.24 μM of each primer, and 0.2 mM deoxynucleotide triphosphates, in a thermal cycler (GeneAmp 9700, Applied Biosystems). Cycling began with a first stage of 94 °C for 15 min, followed by 18 cycles of 94 °C for 40 s, 65 °C for 40 s (declines 0.5 °C after each cycle), and 72 °C for 2 min and 30 s, then 25 cycles of 94 °C for 40 s, 58 °C for 40 s, and 72 °C for 2 min and 30 s, and was completed with a final stage of 72 °C for 7 min. Long-PCR products were diluted by a factor of 200. Then in step two, we performed nested-PCR in a final reaction volume of 15 μl containing 0.9 μl of the diluted long-PCR product and 1 U *Taq* (Qiagen), 0.24 μM of each primer, and 0.2 mM deoxynucleotide triphosphates, in a thermal cycler (GeneAmp 9700, Applied Biosystems). Cycling began with a first stage of 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 1 min and 30 s, and a final extension period at 72 °C for 10 min. To amplify the single nucleotide polymorphism −960C > G the primers used were forward: as used in long-PCR above, reverse: 5′-TGGACGATGCCTTAGAGATG-3′. To genotype the single nucleotide polymorphism −111C > T the primers used were: forward: 5′-ACAGAACTTCACGCCCAGTG-3′ and reverse: 5′-CAGGGAG GATGGATGAACC-3′. The PCR products were then purified by incubation with 0.15 U Shrimp alkaline phosphatase (Roche, Basel, Switzerland) and 0.75 U exonuclease 1 (New England Biolabs, Beverly, MA, USA) at 37 °C for 45 min, followed by heat inactivation at 80 °C for 15 min. The PCR products were sequenced using an ABI BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City,

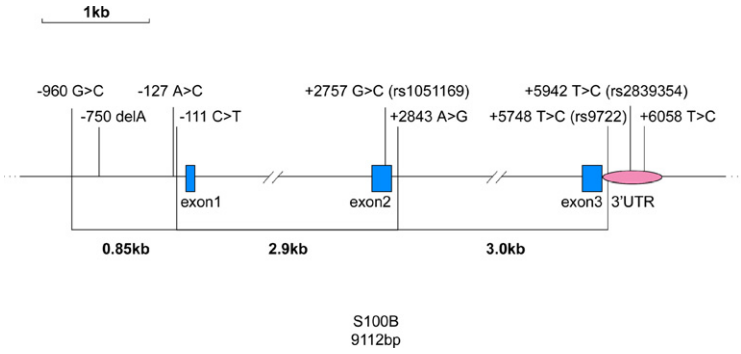

Fig. 1. Structure and SNP distribution of S100B gene. Single nucleotide polymorphisms −960C > G and −111C > T have an interval of 0.85 kbp, −111C > T and rs1051169C > G have an interval of 2.9 kbp, rs1051169C > G, and rs9722C > T have an interval of 3.0 kbp.

CA, USA) on an ABI 3100 DNA sequencer (Applied Biosystems). The PCR primer referred to above was used as the sequencing primer for −960C > G and we designed the sequencing primer 5′-AAAGCTGA CTCCCCACTTCC-3′ for −111C > T. We confirmed all genotypes by repeat sequence assay.

The genotyping of SNP rs1051169C > G and rs9722C > T combines real-time allele-specific PCR kinetic (real-time quantitative) PCR with allele-specific amplification as described by Germer et al. [10]. We genotyped rs1051169C > G and rs9722C > T with SYBR Green I fluorescence in real-time allele-specific PCR on ABI PRISM 7900 Sequence Detection System (Applied Biosystems). We used two separate real-time quantitative PCRs, each of which contained an allele-specific primer of SNP and the same common primer. Heterozygous samples have equal amounts of the two alleles, which should reach a detectable level of fluorescence at the same cycle number, but in homogeneity the cycle number should be different for the two amplification reactions. The PCR primers used in this study were designed by a tetra-primer ARMS-PCR design program. For the rs1051169C > G polymorphism a 105 bp PCR product was amplified using the common primer: 5′-agaggagat ggagaaagtgtcaag-3′, a G allele-specific primer: 5′-acaagctgaagaaatccgaa GtG-3′, and a C allele-specific primer: 5′-acaagctgaagaaatccgaaGtC-3′. Amplification was performed in a 5 μl volume of 2 μl of 2× TaqMan Universal PCR master mix (Applied Biosystems), 10 ng genomic DNA, 0.2 μM allele-specific primer, 0.2 μM common primer, and 0.2× SYBR Green I (Molecular Probe). The PCR cycles began with an UNG digestion stage at 50 °C for 2 min, and an initial denaturation period at 95 °C lasting for 10 min, followed by 55 cycles at 95 °C for 15 s, an annealing phase conducted at 59 °C for 30 s, and a dissociation stage at 95 °C for 15 s. For the rs9722C > T polymorphism a 126 bp PCR product was amplified under the same cycling conditions but with the following primers: a C allele-specific primer: 5′-ccaaacctttcctgtaacaga-TaC, a T allele-specific primer: 5′-ccaaacctttcctgtaacagaTaT-3′, and the common primer: 5′-caatcacaaagcaaatcaagc-3′. To reduce well-to-well variability in PCR conditions, an automated dispenser (Hydra microdispenser, Robbins Scientific) and digital multi-channel pipettes (Thermo Labsystems) were used. To check for genotyping errors, 16 DNA samples were randomly selected from each 96-well plate and re-genotyped. All genotypes were identical to those obtained from the first round of genotyping [11].

Statistic analysis. Allele frequencies were calculated using the excel software for Windows. Deviations from Hardy–Weinberg equilibrium were calculated on http://www.kursus.kvl.dk/shares/vetgen/_Popgen/genetik/applets/kitest.htm. Differences in allele and genotype distributions were calculated using the CLUMP program version 2.2, and OR with 95% confidence intervals on http://www.hutchon.freeserve.co.uk/ConfidOR.htm. Linkage disequilibrium (LD) between two loci, denoted as D' and r^2, were estimated with a two-locus LD calculator (2LD) and EMLD software (http://request.mdacc.tmc.edu/~qhuang/Software/pub.htm). All tests were two tailed and significance was accepted at $P < 0.05$. We constructed haplotypes with the PM program version 1.2 [12]. Differences in allele and haplotype frequency distributions between case and control groups were assessed by the Monte Carlo method using the CLUMP program version 2.2 with at least 10,000 simulations. Haplotypes with frequency <3% were excluded from the Monte Carlo simulation calculation. Odds ratios with 95% confidence intervals were estimated for the effects of high-risk haplotype and calculated by Epi info 2002 software (http://www.cdc.gov/epiinfo/). Power analysis was performed using the G*Power program [11,13].

Results

SNPs and single-locus association analysis

Allele frequencies and single marker analysis are shown in Table 1. The distributions of genotypes of all the four selected markers were in Hardy–Weinberg equilibrium in controls. Among the four markers, only V3 (rs1051169C > G) showed marginal association with schizophrenia ($\chi^2 = 5.89$, $P = 0.02$; OR = 0.78, 95% CI = 0.63–0.95) and no significance was observed in genotypes or allele frequencies for the other three markers between subjects and controls.

Haplotype analysis

To calculate the extent of LD in pairwise combinations of the four SNPs, we calculated D', r^2, and the P value, the normalized linkage disequilibrium (D) statistic for all possible pairs of SNPs. The pairwise D' values are

Table 1
Genotypes and allele frequencies for the four polymorphisms

	Allele[a]					Genotype			
	n	1[b]	2[b]	χ^2 (*P* value)[c]	Odds ratio (95% CI)	11[b]	12[b]	22[b]	χ^2 (*P* value)[c]
V1									
Case	746	550 (0.74)	196 (0.26)	0.52 (0.42)	1.08 (0.86–1.35)	204 (0.55)	142 (0.38)	27 (0.07)	0.93 (0.63)
Control	764	552 (0.72)	212 (0.28)			205 (0.53)	142 (0.37)	35 (0.09)	
V2									
Case	700	524 (0.75)	176 (0.25)	0.53 (0.47)	1.09 (0.86–1.38)	195 (0.56)	134 (0.38)	21 (0.06)	1.80 (0.42)
Control	772	565 (0.73)	207 (0.27)			212 (0.55)	141 (0.37)	33 (0.09)	
V3									
Case	738	371 (0.50)	367 (0.50)	5.89 (**0.02**)	0.78 (0.63–0.95)	107 (0.29)	157 (0.43)	105 (0.28)	7.10 (**0.03**)
Control	728	412 (0.57)	316 (0.43)			121 (0.33)	170 (0.47)	73 (0.20)	
V4									
Case	730	499 (0.68)	231 (0.32)	0.39 (0.54)	1.07 (0.86–1.33)	175 (0.48)	149 (0.41)	41 (0.11)	3.52 (0.17)
Control	748	500 (0.67)	248 (0.33)			161 (0.43)	178 (0.48)	35 (0.09)	

V1, −960C > G; V2, −111C > T; V3, 2757C > G (rs1051169); and V4, 5748C > T (rs9722).

[a] *Note.* Allele 1 and 2 represent the first and second nucleotides given in the name of the SNP, respectively.

[b] *n* (%).

[c] *P* values were tested to evaluate the overall genotype differences between the schizophrenia and control groups. *P* values are given in bold for those <0.05 (significant) or <0.01 (very significant).

Table 2
Pairwise linkage disequilibrium

	V1				V2				V3			
	D	*D′* (SD)	*R*	*P*	*D*	*D′* (SD)	*R*	*P*	*D*	*D′* (SD)	*R*	*P*
V2	0.17	0.91 (0.017)	0.76	<0.0001								
V3	−0.085	0.68 (0.037)	0.15	<0.0001	−0.099	0.83 (0.030)	0.21	<0.0001				
V4	−0.08	0.89 (0.028)	0.14	<0.0001	−0.073	0.86 (0.032)	0.13	<0.0001	0.15	0.86 (0.02)	0.4	<0.0001

shown in Table 2. Strong linkage disequilibrium among the four SNPs was observed (all $D' > 0.7$, $P < 0.0001$).

We constructed six sets of haplotypes. Three were derived from every combination of two adjacent SNPs, two from combinations of every three adjacent SNPs, and one from a combination of all four SNPs (Table 3). We found significant differences between controls and cases in the haplotype combinations of V3–V4 ($\chi^2 = 11.72$, df = 3, global $P = 0.0080$).

Table 3
Global *P* value of haplotypes

Haplotype	χ (df)	Global *P* value
V1–V2–V3–V4	3.24 (3)	0.36
V1–V2–V3	3.26 (2)	0.20
V2–V3–V4	4.26 (3)	0.24
V1–V2	0.32 (2)	0.85
V2–V3	4.28 (2)	0.12
V3–V4	11.72 (3)	**0.0080**

All global *P* values with haplotype frequencies were calculated using CLUMP version 2.2. Bold font indicates significantly associated statistics.

The data show that haplotype V3–V4 (G–C) is much more frequent in cases than in controls ($\chi^2 = 7.84$, df = 1, $P = 0.0051$, OR = 1.50, 95% CI = 1.12–2.00) and that haplotype V2–V3 (C–G) is more frequent in cases than in controls ($\chi^2 = 4.25$, df = 1, $P = 0.039$, OR = 1.25, 95% CI = 1.01–1.56). Another haplotype V2–V3–V4 (C–G–C) also shows association with schizophrenia ($\chi^2 = 3.95$, df = 1, $P = 0.047$, OR = 1.35, 95% CI = 0.99–1.85) (Table 4).

Using a G*Power analysis program based on Cohen's method [14], we established that when an effect size in-

Table 4
Haplotype frequencies between controls and cases

Haplotype	Case	Control	*P* value	OR (95% CI)
V1–V2	681	733		
CC	495 (0.72)	525 (0.70)	0.66	1.05 (0.83–1.34)
GC	22 (0.03)	27 (0.04)	0.64	0.87 (0.47–1.60)
GT	164 (0.24)	181 (0.24)	0.79	0.97 (0.75–1.24)
V2–V3	657	689		
CC	186 (0.28)	219 (0.31)	0.16	0.85 (0.67–1.08)
CG	320 (0.47)	297 (0.42)	**0.039**	**1.25 (1.01–1.56)**
TC	151 (0.22)	173 (0.25)	0.36	0.89 (0.69–1.15)
V3–V4	708	698		
CC	344 (0.49)	370 (0.53)	0.097	0.84 (0.68–1.04)
CT	11 (0.016)	22 (0.032)	0.048	0.48 (0.22–1.06)
GC	139 (0.20)	98 (0.14)	**0.0051**	**1.50 (1.12–2.00)**
GT	214 (0.30)	208 (0.30)	0.86	1.02 (0.81–1.29)
V1–V2–V3	621	642		
CCC	180 (0.27)	208 (0.31)	0.19	0.85 (0.67–1.09)
CCG	298 (0.45)	276 (0.41)	0.075	1.22 (0.97–1.54)
GTC	143 (0.22)	158 (0.23)	0.51	0.92 (0.70–1.20)
V2–V3–V4	630	643		
CCC	174 (0.27)	190 (0.28)	0.45	0.91 (0.71–1.17)
CGC	115 (0.18)	91 (0.13)	**0.047**	**1.35 (0.99–1.85)**
CGT	194 (0.30)	199 (0.29)	0.95	0.99 (0.78–1.27)
TCC	147 (0.23)	163 (0.24)	0.40	0.90 (0.69–1.17)
V1–V2–V3–V4	598	598		
CCCC	169 (0.26)	180 (0.28)	0.48	0.91 (0.71–1.18)
CCGC	95 (0.15)	74 (0.11)	0.081	1.34 (0.95–1.88)
CCGT	192 (0.30)	193 (0.30)	0.95	0.99 (0.77–1.27)
GTCC	142 (0.22)	151 (0.23)	0.55	0.92 (0.70–1.21)

Individual haplotype *P* values and OR with 95% confidence intervals (95% CI) were calculated using Epi info 2002 software (Centers for Disease Control and Prevention, Atlanta, GA, USA). Bold font indicates significantly associated haplotypes and statistics. Haplotypes with frequency <3% are not shown and are excluded from the Monte Carlo simulation calculation.

dex of 0.1 (corresponding to a "weak" gene effect) was used, then sample size had a 80.01% power in detecting a significant ($\alpha < 0.05$) genotype association and a 97.74% power in detecting a similar allele and haplotype association. Using an effect size index of 0.2 (corresponding to "weak to moderate" gene effect), our sample size had a 99.99% power in detecting a significant ($\alpha < 0.05$) haplotype association and 100% power in detecting allele and haplotype association.

Discussion

In this study, we investigated the relationship between four polymorphisms in the S100B gene and schizophrenia in a Chinese Han population. Although we found no significant differences in the frequencies of genotype and allele of −960C > G (V1), −111C > T (V2), 2757C > G (V3, rs1051169), and 5748C > T (V4, rs9722) between schizophrenic patients and control groups, we did find evidence of association of haplotype rs1051169–rs9722 (V3–V4) with schizophrenia. In fact, most haplotype differences of the rs1051169–rs9722 (V3–V4) between patient and control groups arose from the haplotype V3–V4 (G–C) (Table 4). Haplotype V3–V4 (G–C) shows much more frequency in cases than in controls. It is hypothesized that an individual carrying V3–V4 (G–C) haplotype possesses a genetic tendency for increased S100B expression if combined with other genetic and environmental factors so that it may be related to susceptibility to schizophrenia [15].

Although we did not detect a significant difference of polymorphisms −960C > G ($P = 0.42$), −111C > T ($P = 0.47$) which are located in the promoter region as between the case and control groups, attention should be paid to these polymorphisms because the promoter region contains several potential regulatory transcription elements including the cAMP-responsive elements CRE, AP-2, R-CCAAT, AATAA, SP1 binding site GC box, and the potential regulatory element SPE (S100 protein element) [2,3]. If at least some of them are functional for binding, then the generated duplication may affect the transcriptional regulation of the gene. From the Genome database (http://www.ifti.org/Tfsitescan), we found a putative binding site for Sp1 in the promoter of the S100B gene. The DNA sequences recognized by Sp1 have been reported to be the GC box sequence that contains the CCCCG**C**C. Interestingly, the base "**C**" in the GC box was the locus of V2 (S100B −111C > T). The Sp1 may be responsible for increasing the rate of transcription through binding to the S100B −111C > T polymorphism [16]. Although we did not detect a significant difference ($P = 0.47$) in S100B −111C > T between the case and control groups, attention should be paid to this polymorphism, and replicate experiments with larger samples are needed to detect the difference.

Several genes involved in Down syndrome and in Alzheimer may possibly be involved in the etiology of schizophrenia, and indirectly cause the association indicated by linkage disequilibrium in this study. Down syndrome would appear to be significantly increased in patients with schizophrenia [5]. Patients with Down syndrome were found to have a different spectrum of mental disorders from those without the syndrome. S100B gene has been mapped to 21q22.3, which is a risk region for Down syndrome. Although genes DSCR, SLC5A3, and DYRK1A are all implicated in the etiology of Down syndrome, we cannot exclude their role in schizophrenia because they are adjacent to the S100B gene, located in 21q22.3, 21q22.2, and 21q22.1, respectively, and because of the correlation between the phenotype of Down syndrome and that of schizophrenia [17–19]. Further studies are needed to prove their function in schizophrenia. Besides, gene APP which has been implicated in Alzheimer is also located in 21q22.1. Although several studies indicate that no mutation of the APP gene is positively associated with schizophrenia [20,21], we should be careful about coming to any firm conclusion before robust replicated research has been done.

Our study is the first to report an association of the S100B gene with schizophrenia. What we have established in this work is an association between schizophrenia and a possible susceptible haplotype V3–V4 (G–C) that possesses a genetic tendency for increased S100B expression. Our work provides evidence that the S100B gene is positively associated with schizophrenia in the Chinese Han population and provides indirect backup evidence for a relationship between schizophrenia and GGF/SD. The haplotype association observed in this study provides an indicator for further studies on the relation between the S100B gene and schizophrenia as well as providing evidence for the GGF/SD hypothesis. However, as case–control studies are susceptible to positive and negative artifacts from unknown population stratifications, it will be necessary to validate or replicate our association results in other independent large-size ethnic groups.

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Appendix

PCR primers for SNP screening of the S100B gene and summary of the identified SNPs[a]

Primer name	Primers	Primer pair	Product size (bp)	Polymorphisms	Minor allele frequency	Location in gene	dbSNP	Flanking sequences
(1) Promoter-long-fw	5′-CTCAGCCCTGTGCTATTTCC-3′	(1)(8)[b]	1236					
(2) Promoter-1-re	5′-TGGACGATGCCTTAGAGATG-3′	(1)(2)	430	S100B −960C > G[c]	0.31	Promoter	NO	ggagaaggacG/Ctgatgctgg
(3) Promoter-2-fw	5′-TGTTCAGAGGCTCCAGTCG-3′	(1)(2)	430	S100B −750delA[c]	0.08	Promoter	—	aggcttcagcAaaggggcac
(4) Promoter-2-re	5′-TTCCAAGTACAGGCTGAGCA-3′	(5)(6)	452	S100B −127A > C	0.06	Promoter	NO	ggcctggcagA/Ccctgccaccc
(5) Promoter-3-fw	5′-ACAGAACTTCACGCCCAGTG-3′	(5)(6)	452	S100B −111C > T[c]	0.35	Promoter	NO	caccccgccC/Ttcggctccca
(6) Promoter-3-re	5′-CAGGGAGGATGGATGAACC-3′							
(7) Exon1-fw	5′-TTTTCTTCCTCAGCCCATGT-3′							
(8) Promoter-long-re	5′-CGTTCCACCCAAAAGAAAAG-3′							
(9) Exon2-fw	5′-TCTGGGTTGAGGTCTGTATTGA-3′	(9)(10)	389	S100B +2757C > G (leu-leu)[c]	0.46	Exon2	YES (rs1051169)	aatccgaactG/ Caaggagctca
(10) Exon2-re	5′-CCACACTGAGGGTTCCGTTA-3′	(9)(10)	389	S100B +2843A > G	0.24	Intron2	NO	tcctcttaaaA/Gtccatctgta
(11) Exon3-long-fw	5′-ACCCCCAGAGATTTCTGCTT-3′	(11)(15)[b]	913					
(12) Exon3-1-re	5′-CACAAAGCAAATCAAGCTTCC-3′	(11)(12)	464	S100B +5748C > T[c]	0.26	3′UTR	YES (rs9722)	gtaacagagaT/ Cggtcatgcaa
(13) Exon3-2-fw	5′-GCAGCCAAACCTTTCCTGTA-3′	(13)(14)	457	S100B +5942G > T	0.03	3′UTR	YES (rs2839354)	actggccccaG/Tggactcttgt
(14) Exon3-2-re	5′-GGCCAACCAGCTGTTATCTG-3′	(13)(14)	457	S100B +6058C > T	0.08	3′UTR	NO	cagaaattacC/Tccggggcacc
(15) Exon3-long-re	5′-TGCGAGTTCTGATGGAGTTG-3′							

[a] *Note.* SNPs are named according to the nomenclature recommended by Antonarakis [22]. For promoter SNPs, the A of the ATG of the initiator Met codon is denoted nucleotide +1. The nucleotide 5′ to +1 is numbered −1. Exon SNPs are numbered according to their position in the coding sequence. Intron SNPs are designated by IVS (intervening sequence), positive numbers start from the G of the donor site GT.

[b] Primer pairs amplified for long PCR product which act as the template of the following nested PCR.

[c] Polymorphism selected as having the following association analysis with schizophrenia.

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