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BDNF Polymorphisms Are Associated With Cognitive Performance in Schizophrenia Patients Versus Healthy Controls

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ABSTRACT

Background: Accumulating evidence has shown that brain-derived neurotrophic factor (BDNF) may be involved in the pathogenesis of schizophrenia. Moreover, *BDNF* genetic variants, especially the Val66Met polymorphism, may influence specific aspects of human cognition. This study aimed to investigate the potential association of *BDNF* gene polymorphisms with susceptibility to schizophrenia and cognitive impairments in patients with schizophrenia in a Han Chinese population.

Methods: Four polymorphisms (rs6265, rs12273539, rs10835210, and rs2030324) of the *BDNF* gene were analyzed in a case-control study of 1,887 Han Chinese individuals (844 patients meeting *DSM-IV* diagnosis of schizophrenia and 1,043 healthy controls). Cognitive function was measured using the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) in 598 patients and 434 controls. The current study was conducted from 2008 to 2011.

Results: Significant differences in the genotype and allele frequencies between patients and controls were observed only for rs10835210 (both $P < .05$). Further, we found that the rs10835210 polymorphism had a significant effect on language performance only in schizophrenia ($P < .05$). However, *BDNF* rs12273539 played a stronger role in cognitive performance among both patients and healthy controls, especially on attention ($P < .001$) and the RBANS total score ($P < .01$).

Conclusions: These findings suggest the role of these *BDNF* gene variants in susceptibility to schizophrenia and in some aspects of cognitive function.

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Brain-derived neurotrophic factor (BDNF) is a neurotrophic protein that is vital in neurodevelopment as well as in modulating activity-dependent synaptic plasticity among mature neurons, particularly in the hippocampus and neocortex.[1,2] For example, BDNF appears to play an important role in both early long-term potentiation and late-phase long-term potentiation within hippocampal neurons,[3] which constitute a cellular model of learning and memory.[1] Inhibition of BDNF signaling by gene knockout or antisense RNA impairs spatial learning and memory.[4] Recent studies indicate that a single nucleotide polymorphism (rs6265) producing a valine (Val) to methionine (Met) substitution at codon 66 (Val66Met) alters the intracellular trafficking and activity-dependent secretion of mature BDNF and affects hippocampal function and memory performance in humans.[5,6] The schizophrenia patients who are Met allele carriers have lower hippocampal function while performing a declarative memory task than schizophrenia patients who are not Met allele carriers.[6] These Met allele patients also have reduced hippocampal and prefrontal gray matter.[7] Ho et al[8] found that the *BDNF* 66Met variant correlates with poor medial temporal lobe-related memory performance. Our recent study[9] also showed an association between the *BDNF* Met variant and poor visuospatial/constructional performance in both schizophrenia patients and healthy controls. Taken together, the convergence of these findings suggests that the *BDNF* genetic variant, especially the Val66Met polymorphism, may influence specific aspects of human cognition.

BDNF could be implicated in the neurodevelopmental abnormalities found in the brains of individuals with schizophrenia.[10] The majority of studies report decreased serum BDNF levels in treated and first-episode schizophrenia patients,[11–16] although some authors failed to replicate these findings in either medicated or unmedicated patients.[17] Some studies reported that the *BDNF* Val66Met polymorphism was associated with susceptibility for schizophrenia.[18–20] However, several studies did not replicate the result.[21–23] A few studies have examined the association between *BDNF* haplotypes and schizophrenia. For example, a case-control study carried out in a large sample of Chinese individuals found a highly statistically significant association between a common 4-locus haplotype A-274-C-T for rs6265–(GT)n–rs2030324–rs2883187 and schizophrenia.[24] However, the subsequent 2 studies[25,26] did not confirm that *BDNF* haplotypes were associated with schizophrenia.

To date, no study has tested for the effect of *BDNF* haplotype on cognition in both individuals with schizophrenia and healthy control subjects. Our goal in the current study was to expand upon previous findings implicating *BDNF* as a candidate gene for schizophrenia and neurocognitive function by assessing a number of polymorphisms at the *BDNF* locus. To do this, we analyzed, in a population of patients with schizophrenia and healthy subjects, the presence of one of most commonly studied *BDNF* single nucleotide polymorphisms (SNPs)—namely, Val66Met (rs6265)—as well as that of 3 other tag SNPs located in the gene (rs12273539, rs2030324, and rs10835210), which have been previously suggested to impact different psychiatric disorders and/or serum levels of BDNF.[27–31] For example, a

Clinical Points

- Four *BDNF* gene polymorphisms (Val66Met [rs6265], rs12273539, rs10835210, and rs2030324) were genotyped in 844 schizophrenia patients and 1,043 controls.
- Significant differences in the genotype and allele frequencies between patients and controls were observed only for rs10835210.
- The rs10835210 polymorphism had a significant effect on language performance only in patients, while rs12273539 played a stronger role in attention among all subjects.

previous study showed that the rs12273539 polymorphism and the haplotype including the allele of this polymorphism were associated with major depressive disorder.[28] Another study reported a significant association between rs2030324 and schizophrenia.[29] Recently, rs10835210 was found to be associated with bipolar disorder, schizophrenia,[30] and attention-deficit/hyperactivity disorder.[31] Hence, we chose these 4 polymorphisms and hypothesized that the *BDNF* genotype and haplotype may play a role in the cognitive impairments in patients with schizophrenia and healthy controls.

MATERIALS AND METHODS

Subjects

Using a cross-sectional naturalistic design, 844 inpatients (male/female = 663/181) were recruited from Beijing HuiLongGuan Psychiatric hospital and HeBei Province Veterans Psychiatric Hospital in BaoDing city, 50 miles from Beijing. The recruitment criteria included the following: (1) age of 25–75 years; (2) Han Chinese ethnicity; (3) confirmed *DSM-IV* diagnosis of schizophrenia; (4) at least 5 years of illness; (5) receipt of stable doses of oral antipsychotic drugs for at least 6 months; and (6) written informed consent and ability to take part in neuropsychological assessment. Diagnoses were made for each patient by 2 independent experienced psychiatrists and confirmed by the Structured Clinical Interview for *DSM-IV* (SCID).[32] Their clinical subtypes were paranoid (n = 277, 32.8%), undifferentiated (n = 459, 54.4%); disorganized (n = 66, 7.8%), residual (n = 39, 4.6%), and catatonic (n=3, 0.36%). The patients had a mean (SD) age of 47.8 (9.7) years and a mean (SD) education of 12.1 (3.6) years. They had schizophrenia for a mean of 24.3 (9.8) years with 9.9 (9.3) years of hospitalization. Antipsychotic drug treatment consisted mainly of monotherapy with clozapine (n = 396), risperidone (n = 178), chlorpromazine (n = 62), sulpiride (n = 44), perphenazine (n = 41), quetiapine (n = 34), aripiprazole (n = 25), haloperidol (n = 20), loxapine (n = 14), and other typical antipsychotics (n = 15) or other atypical antipsychotics (n = 15). Mean (SD) antipsychotic dose (in chlorpromazine equivalents) was 466 (429) mg/d.

The resident registration files provided a random sample of control subjects (aged 25–75 years) who lived in the Haidian District of Beijing, and we sent each subject a letter explaining the purpose of the study. Of the 1,500 eligible subjects, 1,097 completed the baseline interview (participation rate: 73%). Local officials and health centers arranged for the interviews and measurements to take place at the center office at times convenient to the participants. A research psychiatrist assessed current mental status and personal or family history of any mental disorder in controls by unstructured clinical interviews. Fifty-four subjects were excluded due to Axis I disorders, including anxiety disorder (n = 20), depression (n = 12), alcohol/drug abuse (n = 15), and psychiatric disorders (n = 7).

We obtained a complete medical history from and conducted physical examination and laboratory tests for patients and control subjects. Any subjects with test abnormalities or medical illnesses were excluded. Neither patients with schizophrenia nor control subjects suffered from drug or alcohol abuse/dependence. All subjects were Han Chinese, and they gave written informed consent for participating after the study had been fully explained. The Institutional Review Board (IRB) of Beijing HuiLongGuan hospital approved this study. The study was conducted from 2008 to 2011.

Clinical Symptom Assessment

Four psychiatrists who were blind to clinical status assessed the patients' psychopathology with the Positive and Negative Syndrome Scale (PANSS)[33] on the day of the blood sampling. To ensure consistency and reliability of ratings across the study, these 4 psychiatrists, who had worked at least 5 years in clinical practice, simultaneously attended a training session on using the PANSS before the start of the study. After training, they maintained an interrater correlation coefficient greater than 0.8 for the PANSS total score.

Cognitive Testing

We assessed cognitive functioning for all subjects using the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS, Form A).[34] The RBANS comprises 12 subtests that are used to calculate 5 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 the RBANS into Chinese and established the clinical validity and test-retest reliability of the Chinese version among controls and patients with schizophrenia.[35]

Genotyping

Genomic DNA was extracted using standard procedures. We used a restriction fragment length polymorphism (RFLP) method for genotyping the 4 tag SNPs as previously described.[36] Genotyping was duplicated by 2 investigators independently for accuracy and carried out with the investigators blind to the clinical status. If the 2 investigators' genotype assignments did not agree, the samples were repeated. Also, genotyping

Table 1. Demographic Characteristics in Patients With Schizophrenia and Healthy Controls[a]

Variable	Schizophrenia (n = 844)	Healthy Controls (n = 1,043)	Statistic[b] (*P* Value)
Sex, male/female, n	663/181	586/457	194.3 (< .001)
Age, y	47.8 (9.7)	44.9 (13.6)	19.7 (< .001)
Education, y	8.9 (2.7)	9.5 (3.3)	11.7 (< .001)
Body mass index, kg/m^2	24.5 (3.9)	25.0 (3.8)	4.21 (< .05)
Smoking, n (%)			145.5 (< .001)
Smoker	541 (64)	366 (36)	
Nonsmoker	303 (36)	650 (64)	
Age at onset, y	23.5 (5.4)	…	…
No. of hospitalizations	4.2 (2.6)	…	…
Antipsychotic types, n (%)			…
Typical	182 (22)	…	
Atypical	663 (78)	…	
Antipsychotic dose, mg/d[c]	466 (429)	…	…
PANSS			…
Positive symptom subscore	11.7 (5.2)	…	
Negative symptom subscore	22.8 (8.4)	…	
General psychopathology subscore	25.2 (6.4)	…	
Total score	59.8 (15.8)	…	

[a]Data are reported as mean (SD) unless otherwise noted.
[b]χ^2 used for categorical variables and 1-way analysis of variance used for continuous variables.
[c]Antipsychotic dose reported in chlorpromazine equivalents.
Abbreviation: PANSS = Positive and Negative Syndrome Scale.
Symbol: … = not applicable.

error checks were conducted by re-genotyping within a subsample (n = 50), and reproducibility was routinely > 0.99.

Statistical Analysis

Differences in clinical characteristics between patients and controls or between genotype groups were analyzed using χ^2 for categorical variables and 1-way analysis of variance for continuous variables or 1-way analysis of covariance to adjust for clinical confounding factors.

Deviation from the Hardy-Weinberg equilibrium (HWE) was tested separately in cases and controls using χ^2 goodness-of-fit test. The difference in the allele and genotype frequencies for the *BDNF* gene polymorphisms between patients and healthy controls was analyzed using the χ^2 test.

We tested associations between the *BDNF* genotypes and the cognitive measures using a general factorial design. For the main models, the *BDNF* genotypes and diagnosis (cases vs controls) were entered as fixed effects. Scores for each cognitive domain and the total scores on the RBANS were entered as the dependent variables, with sex, age, education, smoking, and body mass index (BMI) included as covariates. 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. Stepwise multiple regression analysis used RBANS total or index scores as dependent variables with *BDNF* genotype group as the independent variable, and covariates in these stepwise forward entry models included age, gender, education, BMI, smoking, duration of illness, age at onset, PANSS score, and antipsychotic medication dosage, type (typical vs atypical antipsychotics), and duration. Haploview 4.2 (Daly Laboratory Inc, Cambridge, Massachusetts) was used to compute haplotype frequency and haplotype association. The quantitative trait test was performed using UNPHASED 3.1.5 (MRC Biostatistics Unit, University of Cambridge, Cambridge, United Kingdom), which examined the association of gene polymorphisms and cognitive performance. Bonferroni corrections were applied to each test to adjust for multiple testing.

The PASW Statistics 18.0 software (SPSS Inc, Chicago, Illinois) was used to conduct all statistical analysis. Data were presented as mean (SD). All *P* values were 2-tailed at the significance level of < .05.

RESULTS

Subject Characteristics

Table 1 showed significant differences in gender, age, education, BMI, and smoking between cases and controls (all $P < .05$), which were adjusted in the following analyses.

Allele and Genotype Frequencies of 4 *BDNF* SNPs Between Patients and Controls

The genotype and allele frequencies of 4 SNPs located in the *BDNF* gene are summarized in Table 2. No deviation from HWE was detected in the cases or controls (all $P > .05$). Significant differences in the genotype and allele frequencies between patients and controls were observed for rs10835210 (genotype $\chi^2 = 7.46$, $P = .024$, allele $\chi^2 = 7.26$, $P = .007$). The frequency of the A allele (minor allele) of rs10835210 was higher in patients than in controls. There was no allelic or genotypic association between the other 3 SNPs and schizophrenia (Table 2). Because significant differences were noted in the gender, age, education, BMI and smoking between patients and controls, a logistic regression analysis was performed for these 4 SNPs. We found that the A allele

Table 2. Genotypic and Allelic Distributions for SNPs in the *BDNF* Gene Between Patients With Schizophrenia and Controls[a]

Marker		Transcript			Genotype					Allele			
SNP ID	Chromosome position	M/m	Position	Group	M/M	M/m	m/m	χ^2	*P*	M	m	χ^2	*P*
rs6265	27679916	G/A	Coding	Schizophrenia	211 (0.25)	456 (0.54)	177 (0.21)	1.87	.39	878 (0.52)	810 (0.48)	0.10	.75
				Control	271 (0.26)	532 (0.51)	240 (0.23)			1,074 (0.51)	1,012 (0.49)		
rs12273539	27683311	T/C	Intron 8	Schizophrenia	582 (0.69)	236 (0.28)	25 (0.03)	2.46	.29	1,400 (0.83)	286 (0.17)	0.002	.96
				Control	709 (0.68)	313 (0.30)	21 (0.02)			1,731 (0.83)	355 (0.17)		
rs10835210	27695910	C/A	Intron 5	Schizophrenia	397 (0.47)	371 (0.44)	76 (0.09)	7.46	**.024**	1,165 (0.69)	523 (0.31)	7.26	**.007**
				Control	553 (0.53)	417 (0.40)	73 (0.07)			1,523 (0.73)	563 (0.27)		
rs2030324	27726915	C/T	Intron 1	Schizophrenia	279 (0.33)	430 (0.51)	135 (0.16)	0.42	.81	988 (0.59)	700 (0.41)	0.09	.76
				Control	344 (0.33)	542 (0.52)	156 (0.15)			1,230 (0.59)	854 (0.41)		

[a]Values are shown as n (%). *P* values < .05 are in boldface type.
Abbreviations: *BDNF* = brain-derived neurotrophic factor, m = minor allele, M = major allele, SNP = single-nucleotide polymorphism.

Table 3. Comparisons of Total and Index Scores on the RBANS by Diagnostic and Genotype Groupings[a]

	Schizophrenia			Healthy Controls				
Genotype	M/M (n = 414)	M/m (n = 165)	m/m (n = 19)	M/M (n = 290)	M/m (n = 135)	m/m (n = 9)	Genotype, *F* (*P* value)	Genotype × Diagnosis, *F* (*P* value)
rs12273539								
Attention	69.4 (17.4)	74.0 (17.6)	69.4 (20.7)	85.5 (20.4)	91.2 (19.1)	90.2 (17.8)	**8.39 (< .001)[b]**	0.51 (.61)
Language	81.3 (15.6)	82.2 (15.0)	82.7 (16.4)	92.9 (13.4)	97.1 (12.1)	84.2 (16.6)	**4.47 (.012)**	**3.10 (.045)**
RBANS total	64.0 (14.5)	66.6 (15.7)	64.9 (15.1)	78.9 (15.3)	83.0 (14.0)	72.9 (10.2)	**7.58 (.001)[b]**	1.29 (.28)
	M/M (n = 276)	M/m (n = 266)	m/m (n = 48)	M/M (n = 229)	M/m (n = 178)	m/m (n = 24)		
rs10835210								
Language	82.3 (15.6)	81.1 (15.1)	78.2 (15.1)	94.6 (13.3)	93.0 (13.0)	94.5 (12.2)	**3.39 (.034)**	0.65 (.52)

[a]Values are shown as mean (SD). *P* values < .05 are in boldface type.
[b]The differences still remain significant after the Bonferroni corrections.
Abbreviations: m = minor allele, M = major allele, RBANS = Repeatable Battery for the Assessment of Neuropsychological Status.

of rs10835210 was significantly more frequent in cases than in controls and continued to be significantly associated with schizophrenia (both $P < .05$).

Genotype Effects on Cognitive Performance

RBANS data were available from 598 patients and 434 healthy controls. Cognitive test scores were significantly lower in schizophrenia than control subjects on the total scores and all indexes (all $P < .001$) except for visuospatial/constructional index ($P > .05$).

As shown in Table 3, the 3 genotypes of *BDNF* rs12273539 significantly differed on the RBANS total score ($F_{2,1031} = 7.58$, $P = .001$), attention ($F_{2,1031} = 8.39$, $P < .001$), and language ($F_{2,1031} = 4.47$, $P = .012$) in all subjects. The differences remained significant for attention and RBANS total score after Bonferroni correction (both $P < .01$). Post hoc Bonferroni test showed that only the C/T versus C/C differences in attention ($P < .001$) and total score ($P = .003$) were significant.

The 3 genotypes for *BDNF* rs10835210 significantly differed on the language scale ($F_{2,1020} = 3.39$, $P = .034$). Post hoc Bonferroni test showed a trend toward a significant difference between CC and AA homozygous genotypes ($P = .06$). Using multiple regression, inclusion of heterozygotes produced a significant linear correlation between the number of A variant alleles (eg, 0, 1, or 2 alleles) and the language index score within the patients with schizophrenia ($t = -2.24$, $P = .03$), but not in the controls ($P = .42$). The *BDNF* rs10835210 genotype accounted for 3.7% of the variance in language performance in patients with schizophrenia. These results suggest a weak effect of the variant A allele of the *BDNF* rs10835210 gene polymorphism in language impairment in schizophrenia.

Haplotype Effects on Cognitive Performance

Since there was no association of all the haplotypes and the RBANS index and total scores after Bonferroni corrections in controls, we reported the haplotype analysis results only in patients with schizophrenia.

As shown in Table 4, the sliding window haplotype analysis revealed that the haplotypes were associated with attention, language, and RBANS total score. After the Bonferroni correction, we showed a significant association between rs12273539-rs10835210 or rs6265-rs12273539-rs10835210 individually and attention (both corrected $P < .05$). Furthermore, attention performance was worse in patients carrying rs6265(Met)-rs12273539(T)-rs10835210(A) ($P = .002$; Bonferroni-corrected $P = .012$) and rs12273539(T)-rs10835210(A) ($P = .004$; Bonferroni-corrected $P = .016$).

Similarly, after the Bonferroni correction, we showed a significant association between rs6265-rs2030324 or rs6265-rs12273539-rs2030324 individually and language (both corrected $P < .05$). Furthermore, the language index was worse in subjects carrying rs6265(Met)-rs2030324(T) ($P = .003$; Bonferroni-corrected $P = .012$) and rs6265(Met)-rs12273539(T)-rs2030324(T) ($P = .005$; Bonferroni-corrected $P = .025$).

Table 4. The Quantitative Trait Test for Association of Haplotype With Cognitive Functioning in Schizophrenia

Cognition Measure/Haplotype[a]	Frequency, %	χ^2	*P*	Corrected *P*[b]
Attention				
rs6265(M)-rs12273539(M)	15.4	4.19	.041	
rs12273539(M)-rs10835210(M)	2.0	8.50	.004	.016
rs12273539(M)-rs2030324(M)	14.1	3.85	.049	
rs6265(m)-rs12273539(m)-rs10835210(m)	2.7	9.24	.002	.012
Language				
rs6265(m)-rs2030324(M)	3.9	8.67	.003	.012
rs12273539(m)-rs10835210(M)	28.9	3.97	.046	
rs6265(M)-rs12273539(m)-rs10835210(M)	26.8	5.69	.017	
rs6265(m)-rs12273539(M)-rs2030324(M)	2.8	7.89	.005	.025
rs6265(M)-rs12273539(m)-rs10835210(M)-rs2030324(M)	23.3	4.99	.02	
Total score				
rs6265(m)-rs2030324(M)	3.9	6.11	.013	
rs12273539(M)-rs10835210(M)	2.1	6.55	.011	
rs6265(m)-rs12273539(m)-rs2030324(M)	2.9	4.89	.027	

[a]rs6265: M = G = Val, m = A = Met; rs12273539: M = C, m = T; rs10835210: M = C, m = A; rs2030324: M = C, m = T.
[b]Bonferroni-corrected *P* value. Only those differences that remained significant after Bonferroni correction are listed in this column.
Abbreviations: m = minor allele, M = major allele.

DISCUSSION

Two major findings evolved from the present study: (1) The *BDNF* gene rs10835210 polymorphism may contribute to the susceptibility to schizophrenia in a Chinese Han population, and (2) the *BDNF* gene variants may play a role in some aspects of cognitive function in either schizophrenia patients or healthy controls, especially rs10835210 variant in language only in schizophrenia patients and rs12273539 variant in attention and total cognitive performance in both patients and healthy controls.

BDNF Gene Polymorphisms and Susceptibility to Schizophrenia

To our knowledge, this is the first report of association between the *BDNF* gene polymorphism rs10835210 and schizophrenia. Our result showed that the frequency of mutational allele A was higher in patients than in healthy controls, suggesting that allele A may be a risk factor for schizophrenia. This finding is in agreement with a similar association for bipolar disorder and schizophrenia in Koreans, showing that the rs10835210 AA and CA genotype frequencies were higher in bipolar disorder and schizophrenia patients than in healthy control subjects and subjects with major depression.[30] Interestingly, a recent study reported a significant correlation between the rs10835210 polymorphism and attention deficit hyperactivity disorder.[31] Taken together, these results suggest that *BDNF* rs10835210 may play a role in psychiatric disorders.

This rs10835210 polymorphism is an intronic variant located 16 kb away from Val66Met (rs6265); however, whether this polymorphism affects BDNF levels or activity is still unknown. Although the precise biochemical effect of the rs10835210 polymorphism is presently unknown, the fact that other SNPs in the intronic regions of *BDNF* might regulate the level of serum[27] suggests that the possible regulation by rs10835210 of the serum BDNF level could be an underlying mechanism for the observed association with schizophrenia. However, this is only our speculation; whether this *BDNF* polymorphism can affect circulating levels of the protein warrants further investigation.

Recently, the psychiatric genetics community has achieved remarkable progress in elucidating the genetic basis of schizophrenia due to large-scale genome projects, technological innovations, and the accrual of large sample sets. A number of genome-wide association studies (GWAS) have identified a spectrum of common and rare genetic risk variants for schizophrenia.[37] However, a recent study that has evaluated the hypothesis-driven candidate gene literature for schizophrenia with respect to a large GWAS dataset[38] did not confirm *BDNF* gene to contribute susceptibility for schizophrenia. By contemporary standards, a typical candidate gene study for schizophrenia requires sample sizes of ~11,000 cases plus controls for a single marker, 17,500 subjects for 10 markers, and 24,000 subjects for 100 markers.[38] Hence, our current sample size has poor statistical power, and our finding of association between the *BDNF* gene rs10835210 polymorphism and schizophrenia in a Chinese Han population may be the result of false positives. Larger samples in non-Chinese populations are required to definitively evaluate our finding.

BDNF Gene Polymorphisms and Cognition in Both Schizophrenia Patients and Controls

We found that the heterozygous carriers of rs12273539, regardless of whether they were healthy controls or patients with schizophrenia, consistently performed better than their T/T mutant homozygous counterparts on the attention index and RBANS total scores. These results suggested that the *BDNF* gene polymorphism rs12273539 may play a role in human cognition in both schizophrenia patients and healthy control subjects, especially attention performance in a Chinese Han population. Further haplotype analyses showed that the attention index performance was worse in patients carrying either rs6265(Met)-rs12273539(T)-rs10835210(A) or rs12273539(T)-rs10835210(A), which

provides further support that the rs12273539 mutant allele T may be associated with lower attention performance.

The underlying mechanisms that are responsible for the influence of rs12273539 on the cognitive performance, especially on attention, are unknown. We still do not know whether this *BDNF* rs12273539 variant is functional or not, since there are no in vitro or knock-in animal models that have assessed this issue. Interestingly, a previous study in a Chinese population showed that the rs12273539 variant was not associated with schizophrenia; however, a 3-marker haplotype containing the minor allele of 12273539 was significantly more frequent in patients than in controls. Furthermore, the haplotype containing the major allele of 12273539 was significantly associated with negative symptoms.[39] Taken together, these findings provide evidence for a role of the rs12273539 variant in the psychopathology of schizophrenia in a Chinese sample.

Interestingly, unlike *BDNF* rs12273539, which has similar effects in attention for both schizophrenia patients and healthy control subjects, rs10835210 seems to play a different role in language performance in schizophrenia patients and controls. For example, we found a significant linear correlation between the number of A variant alleles and the language index score only in patients. Further haplotype analysis also showed a significant association between rs6265-rs10835210 or rs6265-rs12273539-rs10835210 individually and language in schizophrenia. Moreover, the language index performance was worse in subjects carrying either rs6265(Met)-rs10835210(A) or rs6265(Met)-rs12273539(T)-rs10835210(A), which includes the mutant A allele of rs10835210. These results support the association of rs10835210 mutant allele A with lower language performance only in schizophrenia. However, the functional effects of the *BDNF* rs10835210 variant and the mechanisms by which this variant produced different effects on cognitive function in patients with schizophrenia than in healthy controls are unclear and deserve further investigation.

As cited previously, the *BDNF* Met allele carriers have shown significant deficiencies in episodic memory[5,6] and visuospatial/constructional performance[9] in both healthy controls and schizophrenia patients. However, unexpectedly, we did not confirm the role for this polymorphism in cognitive function among healthy controls or individuals with schizophrenia in our present study. Although the effects of the *BDNF* Val66Met polymorphism on cognition have been extensively documented in schizophrenia patients and healthy carriers, the results are inconsistent.[40,41] There are several explanations for this inconsistency. First, significant differences in *BDNF* Val66Met genotype frequencies were observed in different populations. For example, the Met allele frequency in the healthy comparison subjects (0.49) in our present study was similar to that found in earlier studies involving Han Chinese (0.43–0.52),[21,24] but was notably different from that found in white comparison populations (about 0.20),[18,19] suggesting that *BNDF* genetic variants may differ in frequency and/or effect on cognition among different ethnic groups. Second, the assessments of cognition are subject to change depending on patient type (acute vs chronic illness, active phase vs remission, or outpatient vs inpatient), duration of illness, treatment history (past and present), and the different cognitive tests, suggesting that the assessment of cognitive function is state dependent. The differences in severity of cognitive deficits might be responsible for this inconsistency. Therefore, a comparison of genotypes with the cognition at a given time could not be accurate. It may be difficult to accurately measure the effects of *BDNF* genotype on cognition in schizophrenia patients with the common cross-sectional design. This is especially true if the Val66Met polymorphism produces only a small effect, and maybe another contributing factor of the reported variance between studies could play an important role in this discrepancy.[40,41]

Several limitations of this study should be noted. First, in our present study, the patient and the healthy control groups had a significant gender difference, which may have led to bias in the statistical analysis due to the imbalance in the unmatched sex of our samples. Second, despite a relatively large sample in our current study, the study is still small given today's standard due to the anticipated small effect sizes of gene variants. Hence, our sample size provided only poor statistical power, and it is possible that we do see false-positive results in the present study and our findings need to be considered cautiously. A replication study would be needed to include a large sample size. Moreover, we had genotyped only 4 polymorphisms in the present study, but there are more polymorphisms in the *BNDF* gene than these chosen 4 variants. Hence, the coverage of genetic variation is too limited considering the total *BDNF* gene variants include at least 50 polymorphisms. Therefore, it would be much better to use GWAS in larger samples to capture true positive results found in our present study. To date, there has been no GWAS to explore the genetic effects on the cognitive impairments in schizophrenia, which deserves further investigation. Third, the RBANS is a brief test battery and is unable to evaluate all of the cognitive functions that may be altered in patients, such as motor abilities or executive functioning. It has been proven that age and level of education would account for a statistically significant portion of the variance across performance on the RBANS and its individual indices.[42,43] In our current study, the average age of our patients was comparatively high and the education level low, which may affect the actual cognitive performance in schizophrenia patients. Fourth, 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, as well as maybe worse cognitive performance. Moreover, heterogeneous antipsychotics and doses are limitations, especially the uncommonly high proportion of the patients treated with clozapine in this study. Therefore, our results need to be replicated in a large group of first-episode, never-medicated patients with schizophrenia from different ethnic populations.

In summary, we found a potential genetic association of *BDNF* with risk for schizophrenia, especially the mutant

A allele of the *BDNF* gene polymorphism rs10835210. Further, the *BDNF* rs10835210 genotype is specifically associated with language performance in schizophrenia. Also, *BDNF* rs12273539 may play a stronger role in cognitive performances in both schizophrenia patients and healthy controls, especially on attention and total cognitive performance as measured by the RBANS. The importance of our current BDNF research in schizophrenia is 2-fold, serving to direct further research regarding the role of the BDNF system in the pathogenesis and psychopathology of schizophrenia and to highlight novel avenues for the potential treatment of schizophrenia itself as well as its cognitive dysfunction via the BDNF system as a viable therapeutic target.[41] However, the findings in our present study remain preliminary due to the confounds of chronic illness and medication exposure in our patient subjects, limited sample size and low statistical power, and poor coverage of genetic variations in *BDNF* and thus require replication in larger samples of first-episode, never-medicated patients with schizophrenia from different ethnic populations.

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Drug names: aripiprazole (Abilify); clozapine (Clozaril, FazaClo, and others); quetiapine (Seroquel and others); risperidone (Risperdal and others).

No trade: chlorpromazine; haloperidol; loxapine; perphenazine, sulpiride.

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