Association Between *DβH 5′*-Insertion/Deletion Polymorphism and Cognition in Patients With Chronic Schizophrenia

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ABSTRACT

Background: Cognitive deficits have been identified as a core feature of patients with schizophrenia. Many genes associated with the dopamine and norepinephrine systems are related to the cognitive deficits of patients with schizophrenia. Dopamine β-hydroxylase (DβH) is a key enzyme that converts dopamine to norepinephrine and for which activity and levels are under strong genetic control.

Objective: To examine whether the *DβH 5′*-insertion/deletion (Ins/Del) polymorphism influences cognitive function in patients with chronic schizophrenia.

Method: The presence of the *DβH 5′*-Ins/Del polymorphism was determined in 733 patients with chronic schizophrenia (diagnosed according to *DSM-IV*) and 544 healthy controls using a case-control design. We assessed all of the patients' psychopathology using the Positive and Negative Syndrome Scale (PANSS) and cognition in 540 patients with chronic schizophrenia and 297 healthy controls using the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS). This study was conducted between 2008 and 2011.

Results: The allelic and genotypic frequencies of the *DβH 5′*-Ins/Del polymorphism were not significantly different between patients with schizophrenia and healthy controls (both *P* values > .05). However, the cognitive test scores were significantly lower in patients than in the healthy controls for all scales (all *P* values < .05), except visuospatial/constructional (*P* > .05). The attention score significantly differed according to the genotypic group (*P* < .05) in patients but not in the healthy controls (*P* > .05). In patients with chronic schizophrenia, the mean ± SD attention score was lower in the *DβH 5′*-Del/Del genotype (65.7 ± 16.8) than in the *DβH 5′*-Ins/Del genotype (71.4 ± 18.0; *P* = .007) and the *DβH 5′*-Ins/Ins genotype (70.8 ± 17.1; *P* = .02).

Conclusions: This study found that patients with chronic schizophrenia had poorer cognitive function than the healthy controls in all examined cognitive domains except for visuospatial/constructional. No significant association was found between the *DβH 5′*-Ins/Del polymorphism and patients with chronic schizophrenia. However, the *DβH 5′*-Del/Del genotype may be specific to attentional decrements in patients with chronic schizophrenia.

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Cognitive deficits are a core feature of psychotic disorders, especially in patients with schizophrenia.[1] Studies have suggested that the cognitive impairments of schizophrenia occur in several cognitive domains, such as attention, learning, language, memory, executive functioning, and cognitive processing speed.[2,3] Such cognitive impairments occur before other psychiatric symptoms appear and generally persist throughout the disease,[4,5] which may influence treatment, rehabilitation, quality of life, and even employment for patients with schizophrenia.[6,7] Moreover, both familial and twin studies have shown that genetic factors account for approximately 50% of the variability in memory ability, 70% of the variability in verbal reasoning, and 79% of the variability in abstract reasoning.[8–10] These studies have provided evidence that genetic variants may play an important role in the cognitive deficits of patients with schizophrenia.

Dopamine β-hydroxylase (DβH) is the enzyme that catalyzes the conversion of dopamine to norepinephrine in adrenergic and noradrenergic neurons in the central nervous system.[11] Inhibiting DβH activity increases dopamine levels and decreases norepinephrine levels, both of which are involved in cognitive dysfunction in patients with schizophrenia.[1,12,13] A study[14] found an inverse relationship between plasma DβH (pDβH) activity and cognitive deficits in patients with schizophrenia. Also, a recent study[15] found that the *DβH* knockout mice were unable to synthesize norepinephrine, which was related to the cognitive dysfunction. DβH in the prefrontal cortex (PFC) may be associated with the immediate memory deficits that have been reported in patients with schizophrenia.[16–18] Moreover, DβH has been shown to be involved in mood regulation and attention impairment.[19] Furthermore, dopamine and norepinephrine are important neurotransmitters that have been linked to cognitive function.[20–23] Dopamine transmission dysfunction in the dopamine D_1 receptors in the PFC may contribute to cognitive deficits in schizophrenia.[24] DβH activity and levels are under strong genetic control.[25] The above studies show that cognitive function can be influenced by the *DβH* gene that encodes the rate-limiting enzyme for the conversion of dopamine to norepinephrine in patients with schizophrenia.[1,13]

Clinical Points

- The allelic and genotypic frequencies of the *DβH 5′*-Ins/Del polymorphism were not significantly different between 733 patients with chronic schizophrenia and 544 healthy controls.
- Patients with chronic schizophrenia had poorer cognitive function than did the healthy controls in all examined cognitive domains, except for visuospatial/constructional.
- The *DβH 5′*-Del/Del genotype may be specific to attentional decrements in patients with chronic schizophrenia.

Several studies have shown that the variants of the *DβH* gene are associated with cognitive impairments. For example, it has been found that the *DβH*5′-1021C>T polymorphism is a functional site, which significantly influences global executive function in children and adolescents with attention-deficit/hyperactivity disorder (ADHD), attention bias for neural facial expression in a young Han Chinese population, and sustained attention in a healthy population.[26–28] Additionally, several other variants in the *DβH* gene have been associated with sustained attention impairment in ADHD and spatial working memory in a healthy population.[19,29] Several studies reported that the *DβH* genetic variants were associated with susceptibility for schizophrenia. For example, one recent study[25] found the first direct evidence for the linkage between the *DβH* gene and pDβH, which suggested that the variants of the *DβH* gene contributed strongly to the regulation of pDβH in schizophrenia. The *DβH* gene is localized to chromosome 9q34,[30] which has reported modest positive linkage scores with schizophrenia in two family studies.[31,32] Moreover, a recent study[3] has showed that the *DβH* variant was associated with the development of first-episode schizophrenia. Taken together, these findings suggest that the *DβH* genetic variants may have effects on susceptibility to schizophrenia and may contribute to cognitive function. However, the most recent meta-analysis results[33] provided no evidence of association between *DβH* polymorphisms and patients with schizophrenia. Therefore, the association between the *DβH* gene and susceptibility for schizophrenia deserves further investigation.

The 4.5 kilobases upstream of the 5′ flank transcriptional starting site in the *DβH* gene contain a 19 nucleotide functional insertion-deletion (Ins/Del) polymorphism named *DβH 5′*-Ins/Del polymorphism, which is located on chromosome 9q34. The *DβH 5′*-Ins/Del polymorphism has been shown to be strongly associated with DβH activity.[34] One study[35] has found that the pDβH activity of individuals with the *DβH 5′*-Del/Del genotype was only half the level of individuals with the *DβH 5′*-Ins/Ins genotype. Some previous studies[27,36–38] have found that the *DβH 5′*-Ins/Del polymorphism was associated with cognitive function in elderly women, the average reaction response time in Han Chinese females, the attention bias for negative faces in younger Han Chinese population, and post-error slowing in young white healthy adults. Taken together, these results suggest that the *DβH 5′*-Ins/Del polymorphism is a functional site and may be involved in cognitive function in patients with schizophrenia. Moreover, a recent study[3] has found that the *DβH 5′*-Del allelic number contributes to immediate memory impairment in first-episode schizophrenia. However, no studies have examined the *DβH 5′*-Ins/Del polymorphism in relation to cognitive function in patients with chronic schizophrenia. The aim of this study is to examine whether the *DβH 5′*-Ins/Del polymorphism influences cognitive function in patients with chronic schizophrenia.

METHOD

Subjects

Patients with chronic schizophrenia (n = 733; 624 men, 109 women) were recruited between 2008 and 2011 from the inpatient unit of Beijing HuiLongGuan Hospital, including 10 male inpatient wards and 2 female inpatient wards, because limited resources prevented us from continuing to recruit patients from another 5 female inpatient wards. Inclusion criteria were patients who were aged 20–75 years, had a confirmed *DSM-IV* diagnosis of schizophrenia, had at least 5 years of illness, had been receiving a stable dose of oral antipsychotics for at least 12 months before entry into this study, and provided written informed consent and were able to take part in the psychopathology assessment. Diagnoses were made for each patient by 2 independent experienced psychiatrists and confirmed by the Structured Clinical Interview for *DSM-IV* Axis I Disorders-Patient Edition (SCID-I/P).[39] Moreover, all patients were of the chronic type in the inpatient unit. Their mean ± SD age was 48.0 ± 9.5 years, and their mean number of education years was 8.9 ± 4.2. They had been ill for a mean of 24.7 ± 9.2 years and had received current antipsychotic treatment for a mean of 40.9 ± 51.0 months. Since admission, all patients received dietetically balanced hospital meals, which were occasionally supplemented by gifts (usually fruit). Patients had the opportunity to exercise for about an hour per day. Antipsychotic drug treatment was mainly monotherapy, with the most common medications consisting of clozapine, risperidone, perphenazine, sulpiride, chlorpromazine, and haloperidol. The mean daily dose of antipsychotics in chlorpromazine equivalents was 401.7 ± 180.9 mg/d for each patient using the standard guidelines.[40]

Healthy controls (n = 544; 241 men, 303 women) were recruited from the local community through advertisement. Their mean age was 45.7 ± 12.6 years and mean number of education years was 8.4 ± 3.2. Healthy controls and patients had a similar socioeconomic status and similar dietary patterns. Current mental status and personal or family history of mental disorders were assessed using unstructured interviews. None of the healthy controls presented a personal or family history of psychiatric disorders.

All subjects were Han Chinese recruited at the same time from the Beijing area. Complete medical history, physical

examination, and laboratory tests were obtained from patients and control subjects. All subjects were in good physical health. Any subjects with any abnormalities whatsoever were excluded, including cardiovascular disease, cerebrovascular disease, infections, cancer, unstable diabetes, uncontrolled hypertension, and pregnancy. Neither patients with chronic schizophrenia nor healthy controls were experiencing drug or alcohol abuse/dependence, which was determined by the laboratory urine tests. This study was approved by the institutional review board of HuiLongGuan Hospital, and written informed consent was obtained from all subjects.

Clinical Measures

A detailed questionnaire including a complete medical history, physical examination, and medical and psychological conditions was obtained from patients with chronic schizophrenia and healthy controls. Additional information was collected from available medical records.

Cognitive function was assessed using the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS, form A).[41] The RBANS comprises 12 subtests that are used to calculate 5 age-adjusted index scores and a total score. The test indices are immediate memory, attention, language, visuospatial/constructional, and delayed memory. The RBANS was previously translated into Chinese by our group, and its clinical validity and test-retest reliability were established in healthy controls and patients with schizophrenia.[42] The total and 5 index scores reported in this present study are standard scores.

All patients were tested with the RBANS while they were in a stable state. Since this is a sample of chronic patients, the average duration of hospitalization ranged from 3 to approximately 6 months. The cognitive performance was generally tested in the second or third month of their hospitalization while they did not display acutely psychotic symptoms or deterioration of function.

Four experienced psychiatrists (who were blind to the clinical status of patients with chronic schizophrenia), assessed the severity of the patients' psychopathology using the Positive and Negative Syndrome Scale (PANSS).[43] The PANSS is composed of 3 areas (positive symptoms, negative symptoms, and general psychopathology) and a total PANSS score.[43] The attending psychiatrists were simultaneously trained in the use of the PANSS before this study was initiated. After training, repeated assessments showed that the interobserver correlation coefficient was maintained at greater than 0.80 for the PANSS total score.

Genotyping

The genotypes of *DβH 5′*-Ins/Del were identified as reported in our previous study.[3] In each experiment, an individual known to be heterozygous for the *DβH 5′*-Ins/Del polymorphism was included as a positive control to ensure amplification of both alleles. A reagent control without DNA served as a negative control. Two investigators independently analyzed Ins/Del polymorphism patterns to confirm genotype assignment. The results were scored with investigators blinded to case-control status. If the 2 investigators' genotype assignments did not agree, the samples were repeated. Also, genotyping error checks were conducted by regenotyping within a subsample (n = 50), and reproducibility was routinely > 0.99.

Statistical Analysis

Deviations from the Hardy-Weinberg equilibrium (HWE) were assessed using the HWSIM program.[44] *DβH 5′*-Ins/Del allelic and genotypic frequencies were compared between patients and healthy controls using χ^2 tests. Group differences were compared using Student *t* tests or 1-way analysis of variance (ANOVA) for continuous variables and χ^2 for categorical variables. Post hoc comparisons between subgroups were made using Fisher least significant difference procedure. When ANOVA identified significant differences in cognitive scores according to the genotypic group in patients or healthy controls, the effects of gender, age, education, age at onset, duration of illness, antipsychotic types, medication doses, and duration of current antipsychotic treatment were tested by adding these variables to the analysis model as covariates.

For the main models, analyses of covariance were constructed. The *DβH* genotypes were the independent variables, and the cognitive scores shown by the RBANS total and 5 index scores were the dependent variables. Sex, age, and education were the covariates. In each model, we tested the main effects of diagnosis, genotype, and genotype × diagnosis. Bonferroni corrections were applied to each test to adjust for multiple testing. SPSS version 17.0 was used to perform the statistical analysis. Data were presented as mean ± SD, and all *P* values were 2-tailed, with the significance level set at .05.

The power of sample was calculated with QUANTO software, which has been developed at the University of Southern California (http://biostats.usc.edu/software),[45] with known risk allelic frequencies, and a schizophrenia population prevalence of 1%. Log additive, recessive, and dominant models were examined. The significance level was set at a *P* value of .05.

RESULTS

The demographic characteristics are summarized in Table 1. The patients with chronic schizophrenia and the healthy controls differed significantly in gender, age, and education (all *P* values < .05). The distribution of the *DβH* genotypes was consistent with HWE in both patients with chronic schizophrenia and healthy controls (both *P* values > .05). There were no significant differences in the *DβH* allelic and genotypic distributions between patients and healthy controls ($\chi^2_1 = 2.97$, $P = .09$; $\chi^2_2 = 5.08$, $P = .08$; respectively). We still did not find allelic and genotypic differences between the 2 groups after adjusting for gender and age (both *P* values > .05).

RBANS data were available for 540 patients with chronic schizophrenia and 297 healthy controls. RBANS total and index scores along with the effects of the *DβH*

5′-Ins/Del polymorphism on the RBANS total and index scores are summarized in Table 2. Cognitive test scores were significantly lower in patients with chronic schizophrenia than in healthy controls on all scales (all P values < .01) except for the visuospatial/constructional (P > .05) after adjusting for age, sex, and education. The *DβH* 5′-Ins/Del polymorphism had a significant genotypic effect on the attention index in patients with chronic schizophrenia (F = 3.00, P < .05) after adjusting for age, sex, education age at onset, duration of illness, antipsychotic types, medication doses, and duration of current antipsychotic treatment. However, the significant effect of this polymorphism on the attention score of RBANS in patients with chronic schizophrenia did not pass the Bonferroni corrections (P > .05). Patients with chronic schizophrenia who were Del homozygous had significantly lower attention scores than patients who were either Ins heterozygous or Ins homozygous (P = .007 and P = .02, respectively; Bonferroni corrected: both P values < .05, respectively), but not in the healthy controls (P > .05). Moreover, there were no significant genotype and genotype × diagnosis effects for immediate memory, language, visuospatial/construction, delayed memory, and RBANS total score in all subjects (P > .05).

In addition, the sample size for the RBANS data is somewhat reversed considering the approximate 2:1 ratio of patients to controls. Considering unmatched patient and control studies, roughly equal numbers of patients to controls is most efficient; however, literature suggests an up to 1:4 patient-control ratio for matched case-control studies to find valid associations with regard to power.[46] Further statistical analysis of a 1-to-1 matching of patients to controls was carried out, thereby minimally reducing the sample to 250 patients and 250 controls. This eliminated the gender, age, and education difference, but the primary results on the RBANS were unchanged. Cognitive test scores were significantly lower in patients with chronic schizophrenia than in healthy controls on all scales (all P values < .01), except for the visuospatial/constructional (P > .05). Interestingly, the *DβH* 5′-Ins/Del polymorphism had a more significant genotypic effect on the attention index in patients with increased P value (F = 3.68, P < .01; Bonferroni corrected: P > .05), but not in the controls (P > .05). Furthermore, a 4-to-1 matching of patients to controls was also carried out, thereby minimally reducing the sample size to 60 patients and 240 controls in a third analysis. The primary results on the RBANS were unchanged, but the effect of the *DβH* polymorphism on cognitive performance disappeared because of too few samples (date not shown).

This sample had 0.99 statistical power to detect this polymorphism associated with chronic patients through dominant, recessive, or log-additive inheritance, with a genetic effect of 2.0 (α = .05, 2-tailed test).

Table 1. Demographic Characteristics, Clinical Data, and *DβH* 5′-Ins/Del Allelic and Genotypic Distributions in Patients With Chronic Schizophrenia and Healthy Controls

Variable	Chronic Schizophrenia (n = 733)	Healthy Controls (n = 544)	Statistic	*P* Value
Sex, n			240.45	< .01
Men	624	241		
Women	109	303		
Age, mean ± SD, y	48.0 ± 9.5	45.7 ± 12.6	13.02	< .01
Education, mean ± SD, y	8.9 ± 4.2	8.4 ± 3.2	4.27	.04
DβH 5′-Ins/Del allelic frequency, n (%)				
Del	621 (42.4)	424 (39.0)	2.97	.09
Ins	845 (57.6)	664 (61.0)		
DβH 5′-Ins/Del genotypic frequency, n (%)				
Del/Del	131 (17.9)	93 (17.1)	5.08	.08
Ins/Del	359 (49.0)	238 (43.8)		
Ins/Ins	243 (33.2)	213 (39.2)		
Age at onset, mean ± SD, y	23.4 ± 5.4			
Duration of illness, mean ± SD, y	24.7 ± 9.2			
Atypical antipsychotic, n	565			
Typical antipsychotic, n	168			
Daily antipsychotic dose (chlorpromazine equivalents) mean ± SD, mg/d	401.7 ± 180.9			
Duration of current antipsychotic treatment, mean ± SD, mo	40.9 ± 51.0			
PANSS score, mean ± SD				
Positive symptoms	12.8 ± 5.4			
Negative symptoms	23.0 ± 8.2			
General psychopathology	26.0 ± 6.2			
Total score	61.8 ± 15.1			

Abbreviations: DβH 5′ = dopamine β-hydroxylase-5′, Del = deletion, Ins = insertion, PANSS = Positive and Negative Syndrome Scale.

Table 2. Comparisons Among the RBANS Total and Index Scores (mean ± SD) by Diagnostic and Genotypic Groupings

	Chronic Schizophrenia[a]			Healthy Controls			Diagnosis		Genotype		Diagnosis × Genotype	
RBANS score	Del/Del (n = 95)	Ins/Del (n = 271)	Ins/Ins (n = 174)	Del/Del (n = 49)	Ins/Del (n = 124)	Ins/Ins (n = 124)	*F*	*P* Value	*F*	*P* Value	*F*	*P* Value
Immediate memory	59.0 ± 16.5	58.5 ± 15.8	57.9 ± 16.3	74.0 ± 16.4	71.9 ± 18.4	73.3 ± 16.9	72.00	< .01	0.32	.72	0.27	.76
Attention	65.7 ± 16.8	71.4 ± 18.0[b]	70.8 ± 17.1[c]	87.2 ± 21.8	83.4 ± 19.7	84.5 ± 19.7	74.79	< .01	0.46	.63	2.25	.11
Language	79.2 ± 14.6	81.7 ± 15.7	82.6 ± 15.0	94.2 ± 13.4	92.9 ± 13.8	93.1 ± 12.8	83.11	< .01	0.56	.57	0.77	.46
Visuospatial/ constructional	74.9 ± 17.0	77.0 ± 18.2	78.0 ± 17.6	79.3 ± 15.6	77.0 ± 14.1	78.0 ± 15.7	0.00	.998	0.43	.65	0.60	.55
Delayed memory	67.4 ± 18.9	66.6 ± 19.7	67.3 ± 19.0	86.5 ± 16.3	83.7 ± 15.9	85.4 ± 13.8	104.76	< .01	0.73	.48	0.02	.99
Total score	62.5 ± 13.8	64.7 ± 15.0	64.8 ± 14.3	79.6 ± 15.8	76.9 ± 15.0	77.7 ± 14.2	97.83	< .01	0.30	.74	0.70	.50

[a] A significant genotypic effect on the attention index in patients with chronic schizophrenia: P < .05.
[b] P < .01 for comparison of the attention index between chronic patients with Del/Del and those with Ins/Del.
[c] P < .05 for comparison of the attention index between chronic patients with Del/Del and those with Ins/Ins.
Abbreviations: Del = deletion, Ins = insertion, RBANS = Repeatable Battery for the Assessment of Neuropsychological Status.

DISCUSSION

To our knowledge, this report is the first to investigate the relationship between the *DβH* 5′-Ins/Del polymorphism and cognitive deficits in patients with chronic schizophrenia. We found significantly poorer cognitive function in almost all cognitive domains except the visuospatial/constructional domain in patients with chronic schizophrenia than in the healthy controls. There was no significant association between patients with chronic schizophrenia and the *DβH* 5′-Ins/Del polymorphism. However, the *DβH* 5′-Del/Del genotype may be specific to attentional decrements compared to *DβH* 5′-Ins/Del or *DβH* 5′-Ins/Ins in patients with chronic schizophrenia.

Our finding that the Del allele frequency of the *DβH* 5′-Ins/Del polymorphism was 39.0% in healthy controls (Table 1) was consistent with one study[47] using a healthy Han Chinese population. Previous biochemical studies[48–50] have shown that schizophrenia and other psychiatric disorders are associated with low pDβH activity. However, we found that the *DβH* 5′-Ins/Del polymorphism may not directly contribute to the susceptibility to patients with chronic schizophrenia. This finding is consistent with previous studies[47,51,52] reporting no significant association between the *DβH* 5′-Ins/Del polymorphism and patients with chronic schizophrenia in white, Korean, and Chinese ethnic groups. Moreover, several previous studies[50,53] have found that schizophrenia is not associated with other *DβH* gene polymorphisms, including Ala304Ser and 444G/A. In addition, an earlier study[54] found no significant association between the *DβH* 5′-Ins/Del polymorphism and schizophrenia. These results suggest that some of the *DβH* gene polymorphisms, especially the *DβH* 5′-Ins/Del polymorphism, do not play a role in susceptibility to patients with chronic schizophrenia. However, one study[25] which found the first direct evidence for the linkage between the *DβH* gene and pDβH, suggested that the *DβH* 5′-1021C>T polymorphism, rs1611122, and rs6271 on the *DβH* gene contribute strongly to the genetic regulation of pDβH in schizophrenia. Thus, further studies investigating other *DβH* gene polymorphisms and haplotypes associated with chronic schizophrenia are warranted.

This study found that patients with chronic schizophrenia had more significant cognitive deficits than did healthy controls in almost all cognitive domains except the visuospatial/constructional domain. This result was consistent with previous studies.[1,3] However, some studies have had inconsistent results. For example, our present study found that patients with chronic schizophrenia appear to have impaired attention and normal visuospatial/constructional function, whereas other studies[4,22] have reported that patients with schizophrenia exhibit normal attention and impaired visuospatial/constructional function. These inconsistent findings of cognitive deficits in patients with schizophrenia may be due to multiple and complex factors such as age, gender, education, smoking, body mass index, duration of illness, antipsychotic drugs, treatment duration, and genetic background. Therefore, the underlying psychopathological mechanisms of cognitive deficits in patients with chronic schizophrenia are still not completely understood and deserve further investigation in the future.

This study was the first to find that the *DβH* 5′-Del/Del genotype was related to poorer attention than those with the *DβH* 5′-Ins/Del or *DβH* 5′-Ins/Ins in patients with chronic schizophrenia. The underlying mechanism responsible for the *DβH* 5′-Del/Del genotypic influence on attention could reflect its lower transcriptional activity and the resulting decreased production of norepinephrine and altered balance of dopamine and norepinephrine in the relevant brain regions. This is consistent with Alzheimer's disease, poorer cognitive function in elderly women, and first-episode schizophrenia.[3,36,55] Moreover, a recent study[27] found that the interaction between the *DβH* 5′-Ins/Del polymorphism and the repeated sequence of monoamine oxidase A significantly influenced attention bias for negative expressions. The same study also found that the *DβH* 5′-1021C>T polymorphism was significantly associated with attention bias for neural facial expression in a young Han Chinese population. Two other studies[19,28] showed that variants in the *DβH* gene were associated with sustained attention deficits in children with ADHD and in healthy controls. *DβH* converts dopamine to norepinephrine, which are both involved in attention regulation.[21,23] In addition, some studies[56–58] have found that the dopamine agonist amphetamine may improve sustained attention in healthy controls and rats, and it may ameliorate sustained attention impairments in individuals with ADHD. Noradrenergic drugs such as clonidine may influence the alerting component of sustained attention in humans and rats.[20,59] These results suggest that the *DβH* 5′-Ins/Del polymorphism, which influences the activity and levels of this hydroxylase, may contribute to attention deficits in patients with chronic schizophrenia. In support of this hypothesis, a previous study[14] found that lower pDβH activity influences cognitive impairments in schizophrenia.

Our present study has several limitations. First, due to limited resources and difficulties in recruiting the large case-control sample in our current study, the patients and controls were not matched for age, sex, and education. Although these differences were adjusted for in the analyses, the bias in the statistical analysis may be caused by these unmatched characteristics in this study. Hence, our findings in this study will need to be replicated in a larger and matched sample before a firm conclusion can be drawn. Second, few female patients with schizophrenia were recruited in our present study due to limited resources, showing that the selection of the sample was skewed. Therefore, because our samples were unmatched for sex, the sample is nonrepresentative, which might lead to bias in the statistical analysis. A replication study with a larger sample size and a balance of both sexes would remedy this limitation. Third, in our present study, genotyping was performed using amplified fragment length polymorphism (AFLP) analyses, essentially because we did not have a microsatellite-based technique in our hospital while this study was carried out. AFLP is a rather crude way

to perform genotyping considering current technologies. Hence, a misclassification of genotypes is possible in spite of our quality controls. It is highly possible that we do see only false-positive results. However, such misclassification would typically bias the results toward no effect. A replication study with a microsatellite-based genotyping technique in a different population would help to address this limitation. Fourth, although we found that there was no significant susceptibility inference by the *DβH 5′*-Ins/Del to develop schizophrenia, the allele and genotype frequencies between patients and controls approached significance ($P = .09$ and $P = .08$, respectively). It is highly possible that we do see only false-negative results, which might be due to ill-effective patient-control matching. Unmatched patient and control studies, especially the significant differences in gender, age, and education between patient and control groups, might lead to bias in the statistical analysis due to the imbalance in the ratio of patients to controls in our current study. Fifth, all patients with schizophrenia in the present study were chronically hospitalized, with long-term duration of illness, and treated with heterogeneous antipsychotics and doses, which clearly were different from the healthy controls and might be responsible for the differences in cognitive performances between them. Moreover, generalizing our study is limited by our sample of chronically hospitalized patients, who had more severe psychopathology and a longer duration of illness than typical psychotic outpatients. Sixth, although the RBANS is becoming a widely used screening instrument in neuropsychological assessment, it also has some limitations. For example, it is unable to evaluate all of the cognitive functions that may be altered in patients, such as motor abilities or executive functioning. Moreover, the RBANS has not been used widely in China, and the applicability and the potential use of the RBANS in Chinese individuals and schizophrenic patients need to be confirmed further. Finally, the *DβH* genotypes can be regarded as trait-dependent features, while the cognitive performance is state dependent. Moreover, many factors, such as decreased attention, uncooperativeness, or the psychosis itself, may have contributed to the cognitive impairment in schizophrenia. Therefore, a comparison of genotypes with the cognitive performance at any given time could be misleading. Hence, our findings of association between the *DβH 5′*-Ins/Del polymorphism and cognitive impairments in chronic patients with schizophrenia should be explained with caution. Those clinical factors related to cognitive performance in schizophrenia should be considered.

In summary, we found that the *DβH 5′*-Ins/Del polymorphism may not play a role in susceptibility to patients with chronic schizophrenia. However, patients with chronic schizophrenia had more significant cognitive impairments than the healthy controls in all cognitive domains, except for the visuospatial/constructional domain. Moreover, the *DβH 5′*-Ins/Del polymorphism might play a role in attention impairment in patients with chronic schizophrenia; however, it is worthy of mentioning the following factors before making this conclusion. First, a number of other variants (eg, *DβH 5′*-1021C>T) that are also functional and could in fact be what is contributing to the significant association between *DβH 5′*-Ins/Del polymorphism and attention impairment found in this study should be considered. Unfortunately, we have not assessed these variants yet. Second, the functional polymorphism including *DβH 5′*-Ins/Del, *DβH 5′*-1021C>T, and other variants may influence cognitive performance, especially on attention via their adjusted effects on pDβH levels. However, the pDβH levels were not assayed in our present study. Taken together, no pDβH levels, together with the absence of screening for other *DβH* variants that may be present, the possibility of erroneous genotyping (or rather ill-effective validation of genotyping performed), and the sample size discrepancies between cases and controls would not enable us to make a firm conclusion that the *DβH 5′*-Ins/Del polymorphism is associated with cognitive deficit in patients with chronic schizophrenia. Further, the relatively high frequency of the polymorphism in the healthy controls implies, even at face value, that, if involved in cognitive deficit, this finding must be in an interactive or multiple-hit capacity. Hence, this finding that a particular *DβH 5′* Ins/Del polymorphism is associated with attention deficit within chronic schizophrenia patients remains preliminary because of these limitations and requires replication in larger samples with patient-control matching in different ethnicities using the most advanced genotyping techniques.

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