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Associations between the *DBH* gene, plasma dopamine β-hydroxylase activity and cognitive measures in Han Chinese patients with schizophrenia

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ABSTRACT

The dopamine (DA) and norepinephrine (NE) systems modulate cognitive function. Dopamine β-hydroxylase (DβH) converts DA to NE, and its activity is under strong genetic control. This study examines the association of plasma DβH (pDβH) activity, *DBH* gene polymorphisms (−1021C>T, rs1611115 and 444G>A, rs1108580) and cognitive deficits in Han Chinese patients with schizophrenia. We assessed pDβH activity and cognitive function using the Verbal Fluency Test (VFT), Trail Making Test (TMT) A-B, Stroop color-word test and Wisconsin Card Sorting Test (WCST) in 200 patients with schizophrenia before and after 8 weeks of antipsychotic treatment (96 patients completed assessments at baseline and post-treatment). We found that rs1611115 was significantly associated with pDβH activity, and there was strong LD between rs1611115 and rs1108580 polymorphisms. Correlation analysis indicated that pDβH activity correlated nominally with improvement in VFT score after 8 weeks antipsychotic treatment. Moreover, there was a significant genotype effect of the rs1108580 on VFT: the VFT score of patients with AA genotype was higher than that of patients with AG/GG genotype either at baseline or the end of 8 weeks after treatment. However, this difference was not observed for rs1611115. Our findings confirm a strong association between genotype at rs1611115 and pDβH activity in Chinese patients with schizophrenia. Our data also suggest the rs1108580 polymorphism may influence some aspects of cognitive function in schizophrenia.

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1. Introduction

Cognitive impairment is a core, stable feature of schizophrenia that limits patient functioning and well-being (Keefe and Harvey, 2012) and associates with poor functional outcome (Fervaha et al., 2014; Kontaxaki et al., 2014). Studies also indicate that cognitive deficits occur prior to the onset of other symptoms of schizophrenia, and generally persist during the course of the illness (Dickerson et al., 2004; Hughes et al., 2003). Almost all individuals diagnosed with schizophrenia have some degree of cognitive impairment (Keefe et al., 2005).

Although the presence and impact of cognitive deficits in individuals with schizophrenia have been widely recognized, the underlying neurobiological mechanisms remain mostly unknown. Metabolic dysfunction in catecholamine neurotransmitters is important in cognitive impairment, and psychopharmacological studies suggest that dopamine receptor antagonists could aggravate the cognitive impairment in patients with schizophrenia (Eisenegger et al., 2014; Nakajima et al., 2013).

Dopamine β-hydroxylase (DβH) is the rate-limiting enzyme for the conversion of dopamine (DA) to norepinephrine (NE) (Condray and Yao, 2011; Friedman et al., 1999), and it may be involved in maintaining the DA/NE balance in the brain. DβH is localized within vesicles of central noradrenergic and adrenergic neurons as well as peripheral noradrenergic (sympathetic) neurons and adrenomedullary neurosecretory cells (Weinshilboum, 1978). Because the DβH enzyme is released from vesicles during sympathetic activity, the enzyme activity and DβH immunoreactive protein can be measured in the serum or plasma (Dunnette and Weinshilboum, 1976; O'Connor et al., 1994, 1983).

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Plasma DβH activity (pDßH) is highly heritable and stable trait. While it varies widely across unrelated individuals (Weinshilboum et al., 1973), it is remarkably stable within individuals (Cubells and Zabetian, 2004). Polymorphic variations at the *DBH* gene, which encodes DβH, have been reported to be associated with cognitive function (Greenwood et al., 2014; Parasuraman et al., 2005). The *DBH* single nucleotide polymorphism, −1021C>T (rs1611115), located in the 5′ upstream region of *DBH*, accounts for 30–50% of the variance in plasma DβH activity across samples from European-American, African-American, and Japanese individuals (Zabetian et al., 2001). In patients with attention-deficit/hyperactivity disorder (ADHD), rs1611115 significantly associated with cognitive function (Kieling et al., 2008). The *ins* allele, which contains the 19-bp sequence absent in the *del* allele and associates with higher plasma pDβH (Cubells et al., 2000), corresponded to poorer performance (Hui et al., 2017, 2013). Taken together, these findings suggest a role of *DBH* gene polymorphisms in cognitive function.

While population genetic data on the *DBH* gene and pDβH activity have been reported in European Americans (Zabetian et al., 2001), African Americans (Tang et al., 2007; Zabetian et al., 2001), Japanese (Zabetian et al., 2001) and Indian South Asians (Bhaduri and Mukhopadhyay, 2008) samples, no such study has yet been conducted in the Han Chinese population. The possible roles of pDβH activity and *DBH* polymorphisms in cognitive function and symptomatic severity in schizophrenia also need to be investigated further, in light of the observations that lower cerebrospinal fluid (CSF) levels of DβH associate with better pre-morbid adjustment in schizophrenia (Sternberg et al., 1983, 1982; van Kammen et al., 1994), and with better response to first-generation anti-psychotic medications (Sternberg et al., 1983, 1982). Therefore, the first aim of the current study was to investigate the association between *DBH* (rs1611115 and rs1108580) and pDβH activity in Han Chinese patients. The second aim was to examine the association among pDβH activity, *DBH* genotype and cognitive function in patients with schizophrenia.

2. Methods

2.1. Subjects

The research subjects included two hundred patients with schizophrenia recruited from Beijing Anding Hospital. All patients met the following inclusion criteria: (1) age 18–60 years, Han Chinese ethnicity; (2) confirmed DSM-IV diagnosis of schizophrenia; and (3) the total scores of PANSS between 60 and 120 at baseline. The exclusion criteria were as follows: had received electroconvulsive therapy within past 60 days; current substance abuse; pregnancy; significant medical conditions including severe cardiovascular, hepatic or renal diseases, and unstable psychiatric condition.

2.2. Study procedure

This study was an assessor-blinded, within-subjects trial evaluating the relationship among *DBH* gene polymorphism, pDβH activity and cognitive function after 8 weeks of treatment with antipsychotic medication during inpatient hospitalization. Each patient was assessed with a series of baseline assessments [demographics, clinical characteristics including the Positive and Negative Syndrome Scale (PANSS), cognitive test, genotype and pDβH activity assay]. Seventy-six patients (38%) had not been treated with antipsychotic medication prior to admission. Following baseline assessment, patients were treated with antipsychotic medication following the clinical judgment of the treatment team; three to 7 days were required for washout if a patient had been previously on an antipsychotic medication; medication doses were adjusted for each patient according to clinical judgment for the first 2 weeks of treatment. The PANSS and cognitive test scores were re-assessed after 8-weeks of antipsychotic treatment in all patients who remained in the hospital after 8 weeks. Re-assessments were not conducted in those discharged prior to 8 weeks.

The ethics committee of Beijing Anding Hospital approved the present study. All subjects provided written informed consent for participation in the study, after the procedure had been fully explained and questions answered.

2.3. Cognitive tests

We assessed cognitive function using the Verbal Fluency Test (VFT), Trail Making Test (TMT) A-B, the Stroop's color-word test and Wisconsin Card Sorting Test (WCST) (Zheng et al., 2015; Xiao et al., 2017). These cognitive tests were implemented before and after 8 weeks of treatment. A total of 89 patients completed all cognitive tests both at baseline and after treatment.

2.4. Genotyping

Venous blood samples were obtained and genomic DNA was extracted from 250 μL whole blood using a DNA direct kit (Promega Corporation, Madison, WI, USA). Genotypes of the selected polymorphisms were performed using the 5′-exonuclease (Taqman®) method. This method is based on detection of allele-specific fluorescence, which is achieved by tethering a fluorophore and a quencher molecule to allele-specific oligonucleotides.

2.5. Plasma DβH activity assay

Samples of heparin anti-coagulated plasma, collected at the time of participant ascertainment, were stored frozen at −80 °C until DβH activity assay. Plasma DβH activity was determined in duplicate 5 μL aliquots of plasma as the rate of conversion of tyramine to octopamine using a method modified from that of Nagatsu et al. (Nagatsu and Udenfriend, 1972). Octopamine was measured by column-switching, reverse phase high performance liquid chromatography (HPLC) system (U3000, Thermo Fisher Scientific, Waltham, MA, USA), using coulometric electrochemical detection, and synephrine as internal standard. DβH activity is reported as μmol of octopamine formed per minute from a solution of tyramine (0.2 mol/L) by 1 L of plasma at 37 °C ($\mu mol \cdot min^{-1} \cdot L^{-1}$).

2.6. Statistical analysis

Data on DβH activity, *DBH* genotype and clinical assessments were first entered in an Excel worksheet and later transferred to IBM SPSS Statistical Software (version 20.0). All statistical analyses were performed in IBM SPSS. Descriptive statistics were used to describe demographic and clinical characteristics of the study sample. We used analyses of variance (ANOVAs) to examine the individual effects of the two SNPs on pDβH activity. Before analysis, we transformed pDβH activity to follow an approximate normal distribution using a square-root transformation (Zabetian et al., 2001). Square-root pDβH was therefore the dependent variable, and genotypes at the *DBH* SNPs were the independent variables. The Hardy-Weinberger Equilibrium test was conducted using an online tool (http://www.had2know.com/academics/hardy-weinberg-equilibrium-calculator-2-alleles.html). LD measures D′ and r^2 were calculated for all possible combinations of the SNPs analyzed using Haploview.

At baseline and 8 weeks after treatment of antipsychotic medications, the scores of cognitive function and PANSS were compared using the paired *t*-test or Wilcoxon matched-pairs signed ranks sum test, respectively. The change in cognitive test scores from baseline (Δ = 8 week score − baseline score) was calculated to evaluate the improvement of cognition in patients. Pearson (normal distribution) or Spearman (non-normal distribution) correlation, as appropriate, was