Progress in Neuro-Psychopharmacology & Biological Psychiatry 34 (2010) 930–933

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

The study of BDNF Val66Met polymorphism in Chinese schizophrenic patients

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ARTICLE INFO

Article history:
Received 6 January 2010
Received in revised form 14 April 2010
Accepted 20 April 2010
Available online 24 April 2010

Keywords:
Age of onset
Association
Brain-derived neurotrophic factor
Polymorphism
Schizophrenia

ABSTRACT

Accumulating evidence showed that brain-derived neurotrophic factor (BDNF) may be involved in the pathophysiology of schizophrenia. Recent studies have reported that the Val66Met polymorphism of the BDNF gene may be associated with susceptibility for schizophrenia and age of onset of this disease, with mix results. In the present study, the BDNF Val66Met gene polymorphism was examined in 387 inpatients (259 men and 128 women) meeting the DSM-IV criteria for schizophrenia and unrelated 365 healthy controls (255 men and 110 women). The schizophrenia symptomatology was assessed by the Positive and Negative Syndrome Scale (PANSS). Age of onset was defined as the age at which the psychotic symptoms first appeared. Our results showed that genotype frequency distributions and allelic frequencies did not differ between patients and controls. No interaction was found between sex and genotypes. Analysis of covariance (ANCOVA) showed a significance of the BDNF Val66Met genotypes on the age of onset ($F=3.76$, $p<0.02$), after adjusting sex, age and duration of illness. Furthermore, ANCOVA showed that the significance of the BDNFVal66Met genotypes on age of onset was increased comparing the Val66Met heterozygotes with the combination of Val66Val and Met66Met homozygotes ($F=5.85$, $p<0.01$). Our results suggest that the BDNF Val66Met polymorphism may not contribute directly to the susceptibility to schizophrenia, but to the onset of the disease. Furthermore, our results show the heterozygous effect of the BDNF Val66Met gene on the clinical variability of schizophrenia phenotype.

1. Introduction

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family of growth factors, not only regulates cell survival, proliferation, and synaptic growth in the developing central nervous system (CNS), but also is a critical element in modulating synaptic changes and brain plasticity (Poo, 2001; Tyler et al., 2002; Egan et al., 2003). Converging lines of evidence suggest that BDNF could be implicated in the neurodevelopmental abnormalities found in schizophrenia brain (Nawa et al., 2000). Recent studies indicate that a single nucleotide polymorphism (rs6265) producing a nonconservative amino acid substitution (valine to methionine) in the 5′-precusor peptide (pro-BDNF) at codon 66 (Val66Met) has been shown to dramatically alter the intracellular trafficking and packaging of pro-BDNF and, thus, regulate secretion of the mature peptide (Egan et al., 2003). Two studies reported that the Val66Met polymorphism of the BDNF gene was associated with susceptibility for schizophrenia (Neves-Pereira et al., 2005; Rosa et al., 2006). However, several studies did not replicate the result (Naoe et al., 2007; Xu et al., 2007; Varnas et al., 2008). The most recent meta-analysis results provided no evidence of association between Val66Met polymorphism and schizophrenia, and large heterogeneity between studies (Kanazawa et al., 2007; Zintzaras, 2007).

Two recent studies in Japanese (Numata et al., 2006) and African–American cohort (Chao et al., 2008) show that the BDNF Val66Met

Abbreviations: ANOVA, one-way analysis of variance; ANCOA, analysis of covariance; BDNF, brain-derived neurotrophic factor; CNS, central nervous system; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders; LSD, least significant difference; OR, odds ratio; PANSS, Positive and Negative Syndrome Scale; TD, tardive dyskinesia.

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doi:10.1016/j.pnpbp.2010.04.019

polymorphism is associated with age of onset of schizophrenia; however, other studies in Caucasian populations have not observed a similar association (Gourion et al., 2005; Naoe et al., 2007). It was thought important to replicate the work with a different ethnic population to rule out a false positive result from ethnic differences in the frequency distribution of the DNA marker or from the population structure. The present work was therefore undertaken to investigate whether the BDNF Val66Met polymorphism was associated with susceptibility for schizophrenia and onset of this disease in ethnically homogeneous samples of Chinese Han population.

2. Materials and methods

2.1. Subjects

Three hundred and eighty-seven physically healthy patients (male/female = 259/128) who met DSM-IV for schizophrenia and unrelated 365 healthy controls (255 men and 110 women) were included in the study. All schizophrenic patients were recruited from among the inpatients of Beijing Hui-Long-Guan Hospital, a Beijing City owned psychiatric hospital. Diagnoses were made for each patient by two independent experienced psychiatrists. All schizophrenic patients were of the chronic type, with duration of illness for at least 5 years, with a mean duration of illness of 23.8 ± 9.4 years.

All patients had been receiving stable dose of oral neuroleptic medications for at least 12 months prior to entry into the study.

Normal controls were recruited from the local community, and matched for age and gender. Current mental status and personal or family history of any mental disorder was assessed by a clinical psychiatrist. None of the healthy control subjects presented a personal or family history of psychiatric disorder.

All subjects were Han Chinese being recruited at the same period from Beijing area.

A complete medical history and physical examination were obtained from all subjects.

Any subjects with physical abnormalities were excluded. Neither the schizophrenic patients nor the control subjects suffered from drug or alcohol abuse/dependence. All subjects gave signed, informed consent to participate in the study, which was approved by the Institutional Review Board, Beijing HuiLongGuan Hospital.

2.2. Clinical assessment

The patient's psychopathology was assessed on the day of the blood sampling by four psychiatrists who were blind to the clinical status with the PANSS. To ensure consistency and reliability of rating across the study, these four psychiatrists who had worked at least 5 years in clinical practice simultaneously attended a training session in the use of the PANSS before the start of the study. After training, a correlation coefficient greater than 0.8 was maintained for the PANSS total score by repeated assessments during the course of the study.

Age of onset was defined as the age at which the psychotic symptoms first appeared, according to medical case notes and interviews with the patients and their family members.

2.3. BDNF genotyping

DNA was extracted using standard protocols. The genotypes of the BDNF Val66Met polymorphisms were identified as reported earlier (Neves-Pereira et al., 2002). Briefly, a 113-bp segment was amplified by PCR, using the following primers: 5′-GAGGCTTGACATCATTGGCT-30 and 50-CGTGTACAAGTCTGCGTCCT- 3′. The Val66Met polymorphism was differentiated by Eco721 restriction enzyme. The fragments were separated on a 3.5% agarose gel at 100 V, and fragments were visualized with ethidium bromide. The uncut product size was 113 bp (allele A), and allele G comprised the cut bands of 78 and 35 bp. Genotyping was duplicated and carried out blind to the clinical status.

2.4. Statistical analysis

The Hardy–Weinberg equilibrium in schizophrenia and normal controls was tested by using the χ^2 test for goodness of fit. The χ^2 tests and Fisher's exact test, if necessary, were performed to assess the difference in genotype and allele frequencies in schizophrenia and normal controls. Odds ratio (OR) and their 95% confidence intervals were calculated to evaluate the effects of different genotypes. Between-group differences in continuous variables were evaluated using the Student's *t*-test or one-way analysis of variance (ANOVA), followed by the Fisher's least significant difference (LSD) multiple range test for between-group comparison. Analysis of covariance (ANCOVA) was further constructed with the BDNF Val66Met genotypes as the independent variables, and the age of onset as dependent variables, with sex, age, education, and duration of illness as the covariates. Bonferroni corrections were applied to adjust for multiple testing. The significance criterion was set at $p<0.05$.

3. Results

Table 1 shows the characteristics of the subjects in the schizophrenia and normal controls. There were no significant differences in sex, age, education and BMI between two groups (all $p>0.05$). Allele frequencies, genotype distributions and the statistical analysis are shown in Table 2. Genotype distributions had no deviation from Hardy–Weinberg equilibrium in both groups ($p>0.05$). There was no significant difference in frequency of alleles of BDNF in schizophrenia and normal controls ($\chi^2=0.09$, $df=1$, $p=0.77$), with an OR of 1.03 (95% CI (0.84–1.26). Genotype distribution was also not significantly different between patients and the control population ($\chi^2=0.10$, $df=2$, $p=0.95$). No interaction was found between sex and genotypes either for the whole group or when the normal controls and patients were examined separately (all $p>0.05$).

Table 3 shows the relationships between BDNF Val66Met genotype and clinical variables in schizophrenia patients. ANCOVA showed a significance of the BDNF Val66Met genotypes on the age of onset ($F=3.76$, $p<0.02$), after adjusting sex, age, education and duration of illness. In addition, in the present study, no sex difference in age of onset was observed ($p>0.05$), possibly due to a limited sample size.

BDNF Val66Met genotype accounted for 14% of the variance (adjusted $R^2=0.14$). Furthermore, ANCOVA showed that the significance of the BDNFVal66Met genotypes on age of onset was increased comparing the Val66Met heterozygotes with the combination of Val66Val and Met66Met homozygotes ($F=5.85$, $p<0.01$). However, there was no significant difference in other variables, including the clinical symptoms shown on PANSS, although there was a trend toward the significant difference in negative symptom subscore of PANSS ($p<0.07$).

4. Discussion

Our results demonstrate that the BDNF Val66Met polymorphism may not contribute directly to the susceptibility to schizophrenia. This

Table 1
Characteristics of schizophrenia and normal controls.

	Schizophrenia ($n=387$)	Normal controls ($n=365$)
Sex (male/female)	259/128	255/110
Age (years)	50.5 ± 9.9	49.8 ± 9.3
Education (years)	9.6 ± 5.6	10.2 ± 5.8
Body mass index (kg/m^2)	24.8 ± 4.9	25.1 ± 5.2