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Association between the brain-derived neurotrophic factor (*BDNF*) gene and Schizophrenia in the Chinese population

Qing-Ying Chen [a,b], Qi Chen [b,c], Guo-Yin Feng [d], Chun-Ling Wan [b,c,*], Klaus Lindpaintner [e], Li-Jun Wang [f], Zheng-Xiong Chen [g], Zhen-Song Gao [g], Ji-Sheng Tang [h], Xing-Wang Li [b,c], Lin He [b,c,*]

[a] *Institute for Nutritional Sciences, Shanghai Institute of Biological Sciences, Chinese Academy of Sciences, 319 Yue Yang Road, Shanghai 200031, PR China*
[b] *Bio-X Center, Shanghai Jiaotong University, PO Box 501, Hao Ran Building, 1954 Hua Shan Road, Shanghai 200030, PR China*
[c] *Institute for Nutritional Sciences, SIBS, Chinese Academy of Sciences, 294 Taiyuan Road, Shanghai 200031, PR China*
[d] *Shanghai Institute of Mental Health, 600 South Wan Ping Road, Shanghai 200030, PR China*
[e] *Roche (China) Ltd, 1100 Long Dong Avenue, Pudong New Area, Shanghai 201203, PR China*
[f] *Shenyang Tiexi Institute of Mental Health, Liaoning, China*
[g] *Shantou Institute of Mental Health, Guangdong, China*
[h] *Shandong Institute of Mental Health, Shandong, China*

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Abstract

Brain-derived neurotrophic factor (*BDNF*) belongs to a family of the neurotrophin which plays important roles in the development of the brain. *BDNF* has been suggested as a factor that increases the risk of schizophrenia. In this study, we genotyped three single nucleotide polymorphisms (SNPs) in the *BDNF* gene using a set sample of Han Chinese subjects consisting of 560 schizophrenes and 576 controls. No significant differences were found for either the genotype or allele distribution of analyzed polymorphisms, nor was any gender-specific association found. Thus, our data suggest that the *BDNF* gene may not be an important factor in susceptibility to schizophrenia.

Keywords: *BDNF*; Case-control; Association; Schizophrenia; Chinese

Schizophrenia is a heterogenous disorder involving genetic, biologic and environmental factors. Although schizophrenia afflicts approximately 1% of the population throughout the world, the ultimate biological cause of the disorder remains elusive. Lines of evidence suggest that neurodevelopmental abnormalities of specific brain areas, including disturbances of neuron migration, alteration in neural plasticity and changes in synaptic connection, are important factors in the pathogenesis of schizophrenia [2,3,25].

The brain-derived neurotrophic factor (*BDNF*), the gene encoded on human chromosome 11p13, is a member of the superfamily of the neurotrophin which plays a critical role in promoting and modifying growth, differentiation, and survival of neurons in the central nervous system (CNS). As the most abundant of the neurotrophins in the brain, BDNF is important for guiding the neurons of CNS during their development and maintaining their survival in adulthood [48].

BDNF is found to be involved in the maintenance of long-term potentiation (LTP), a cellular mechanism of learning and memory, and participates in modulating the synthesis, metabolism and release of neurotransmitters, and therefore having a role in regulating synaptic plasticity [1,13,26,34,36].

Animal experiments have revealed that *BDNF* is broadly distributed in the central nervous system and is enriched in the hippocampal formation, cerebral cortex and limbic areas [12,21,32]. During forebrain development in the rat, *BDNF* mRNA expression has the highest levels in the hippocampus and the lowest in the striatum [10,21,49]. Furthermore, *BDNF* mRNA is variously expressed in the subfields of the hippocampus, with low expression in pyramidal cells in CA1, moderate expression in CA2 and high expression in CA3 [7].

* Corresponding author. Tel.: +86 21 62822491; fax: +86 21 62822491.
E-mail address: helin@sjtu.edu.cn (L. He).

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The function and distribution of *BDNF* in the CNS raise the possibility that this neurotrophin is relevant to schizophrenia and a number of anatomical and clinical studies have been done to assess the potential contribution of BDNF to the pathophysiology of the disorder. Takahashi et al. reported that higher BDNF levels were detected specifically in the anterior cingulate cortex and hippocampus of schizophrenic patients, when compared with controls [46]. In their postmortem brain study, Durany et al. reported that BDNF concentrations were significantly increased in the cerebral cortex and decreased significantly in the hippocampus of schizophrenic patients [9]. Recently, two postmortem studies have reported decreased levels of *BDNF* mRNA and protein in the prefrontal cortex of subjects with schizophrenia [20,55]. In addition, BDNF levels were significantly reduced in the serum of schizophrenic patients but not in their whole blood [33,50]. On the other hand, several other studies have failed to detect altered serum BDNF levels in schizophrenic patients [23,41].

Lines of genetic association studies have been carried out in order to determine the possible correlation between polymorphisms in the *BDNF* gene and schizophrenia. Proschel et al. [35] identified a GT dinucleotide repeat in the human gene for *BDNF* in 1992. In a French Caucasian population, Krebs found an excess of the 172–176 bp alleles of the GT repeat polymorphism in patients with late onset, in neuroleptic-responding patients and in non-substance-abusing patients. However, some studies have given negative results [37,54]. A single nucleotide substitution (C270T) polymorphism, located in the 5′-noncoding region of the *BDNF* gene, was found to be associated with schizophrenia in a Caucasian population [45]. Nanko et al. found the frequency of the T allele of the C270T polymorphism to be significantly increased in Japanese patients compared with controls [29]. However, some recent studies have failed to confirm these findings. Galderisi et al. and Szczepankiewicz found no differences in allele and genotype distribution between patients and controls [16,44]. In addition, Hong et al. reported a trend ($p = 0.055$) between genetic predisposition and a nonsynonymous mutation rs6265 in 93 schizophrenic patients [22].

All these data make the *BDNF* gene a good candidate for association study of schizophrenia. Given the importance of independent observation of association findings in genetically complex diseases such as schizophrenia, we set out to investigate the role of *BDNF* in the etiology of schizophrenia in an independent sample of schizophrenic patients and controls from China. Three SNP polymorphisms—rs3750934, rs6265 and ro_000011924 (from Roche database) were genotyped in 560 Chinese patients and 576 Chinese control individuals.

All subjects were Han Chinese in origin. A total of 560 unrelated schizophrenic patients (53.4% male) with a mean age 37.3 ± 13.6 were recruited from the Liaoning, Guangdong and Shandong provinces of China. Consensus diagnosis of each patient was made by two independent psychiatrists according to the DSM-IV (Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition) [14] criteria for schizophrenia. Five hundred and seventy-six unrelated healthy subjects (51.9% male) with a mean age 33.2 ± 10.5 from the same geographical region were used as controls. All were interviewed to exclude any history of psychiatric disorders. The study was approved by local psychiatry research ethics committees and informed consent was obtained from all subjects.

We selected three SNPs—rs3750934, rs6265 from dbSNP (http://www.ncbi.nlm.nih.gov) and ro_000011924 from the Roche database, spanning around 1047 bp in the *BDNF* gene. rs3750934 is in the intron near the coding sequence (CDS) of the *BDNF* gene. rs6265 is located in the CDS of the gene, resulting in an amino acid substitution from valine to methionine. ro_000011924 is in the penultimate exon of the gene. Genomic DNA was extracted from venous blood collected from subjects by a standard phenol extraction procedure.

All SNPs were genotyped by allele-specific PCR, in which primers were designed to specifically amplify the reference allele or its variant in separate PCR reactions [18]. The assay used in this study combines kinetic (real-time) PCR with allele-specific amplification [17]. The primers sequences used for three SNPs were as follows: For rs3750934: 5′-ACAATCAGATGGGCCACAC/T-3′ (allele-specific primer) and 5′-GGCTTTCTTTCACCGGGATG-3′ (common primer); for rs6265: 5′-CATCCAACAGCTCTTCTATCAC/T-3′ (allele-specific primer) and 5′-CTTGACATCATTGGCTGACAC-3′ (common primer); for ro_000011924: 5′-CACAACTTAAAAAGTCTGCATTA/G-3′ (allele-specific primer) and 5′-ACGGCAACAAACCACAACA-3′ (common primer). For real-time PCR, two PCR reactions were performed for each sample, with 10 ng genomic DNA, 0.05 μl $^{\Delta}$Z05 enzyme (Roche company), 0.2 μM allele-specific primer, 0.2 μM common primer and 0.2 × SYBR® Green I (Molecular Probe, Inc.) in a total volume of 25 μl. To reduce well-to-well variability in PCR reaction conditions, an automated dispenser (Hydra® microdispenser, Robbins Scientific) and digital multichannel pipettes (Thermo Labsystems) were used. Kinetic PCR reactions were performed on an ABIPRISM 7900 Sequence Detection System (Applied Biosystems). After an initial 2 min incubation step at 50 °C to activate the AmpErase® uracil-N-glycosylase (UNG) and a step of 12 min at 95 °C to deactivate UNG and activate AmpliTaq Gold® enzyme, 50 cycles consisting of 15 s at 95 °C and 30 s at annealing temperature were performed, followed by a final stage of dissociation to check the PCR product. Allele calling was manually performed as in previous research in our laboratory [47].

Allele frequencies in different groups of subjects were compared using the CLUMP program (version 1.9) [39] with 10,000 stimulations. The *p*-values reported are two-tailed and significance was accepted at $p < 0.05$. A *p*-value of 0.05 was considered significant in tests for Hardy–Weinberg equilibrium. The standardized measure of linkage disequilibrium (LD), denoted as D′, was estimated with software 2LD [57]. Haploypte frequencies were estimated by EHPLUS, which performs model-free analysis and permutation tests of allelic association based on EH [56]. The odds ratio and 95% confidence interval were calculated on the web http://202.120.7.14/analysis/myAnalysis.php [40].

In our case-control analysis, 560 schizophrenics were genotyped and compared with a set of 576 controls. Table 1 gives the allele and genotype frequencies of the three markers. Genotypic