Acta Neuropsychiatrica 2009: 21: 186–190
All rights reserved
DOI: 10.1111/j.1601-5215.2009.00401.x

© 2009 John Wiley & Sons A/S
ACTA NEUROPSYCHIATRICA

Lack of association between BDNF Val66Met gene polymorphism and late-onset depression in a Chinese Han population

You J, Yuan Y, Shi Y, Zhang Z, Zhang X, Li H. Lack of association between BDNF Val66Met gene polymorphism and late-onset depression in a Chinese Han population.

Background: Val66Met polymorphism in the brain-derived neurotrophic factor (BDNF) gene has been suggested to be associated with major depressive disorder (MDD). There were a few reports of the relationship between the variant and late-onset depression (LOD) in Chinese Han population.
Objective: To investigate the relationship among BDNF Val66Met gene variants, BDNF plasma level and LOD.
Methods: Chinese Han patients with LOD ($n = 99$) and control subjects ($n = 110$) were assessed for BDNF Val66Met gene polymorphism. BDNF plasma level was tested only in LOD.
Results: There were no significant differences in genotypes and allele frequencies between cases and controls ($p = 0.744$ and $p = 0.845$, respectively). Plasma BDNF level also did not show significant differences in three genotypes in LOD ($p = 0.860$).
Conclusion: The Val66Met polymorphism in BDNF gene may not confer susceptibility to LOD in Chinese Han population.

Jiayong You[1], Yonggui Yuan[1,3], Yanyan Shi[1], Zhijun Zhang[2], Xiangrong Zhang[1], Hailin Li[1]

[1]Department of Psychiatry, Nanjing Brain Hospital affiliated to Nanjing Medical University, Nanjing, 210029, China; [2]Department of Neuropsychiatry, Affiliated ZhongDa Hospital of Southeast University, Nanjing, 210009, China; and [3]School of Clinical Medicine, Southeast University, Nanjing, 210009, China

Keywords: brain-derived neurotrophic factor (BDNF); gene; late-onset depression

Yonggui Yuan, Department of Psychiatry, Nanjing Brain Hospital affiliated to Nanjing Medical University, Guangzhou Road 264#, Nanjing 210029, PR China.
Tel: +86 25 83700011-6209;
Fax: +86 25 8370 9025;
E-mail: yygylh2000@sina.com.cn

Zhijun Zhang, Department of Neurology, Affiliated ZhongDa Hospital of Southeast University. DingJiaQiao Road 87#, Nanjing 210009, PR China
E-mail: zhijunzhang838@yahoo.com.cn

Introduction

The etiology of major depressive disorder (MDD) remains unknown, despite many different etiologic hypotheses have been produced. A number of studies have suggested that neurodegenerative mechanisms are involved in MDD. Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophic superfamily and is responsible for promoting and modifying growth, development and survival of neurons (1). Lower BDNF mRNA levels have been reported in the hippocampus and anterior cingulate cortex from MDD patients (2), and reduced serum (plasma) BDNF levels were also reported in some MDD patients (3,4). Some other studies further suggested that serum (plasma) BDNF levels were elevated in the MDD patients after treated by antidepressant drugs (5). Thus, the BDNF gene located on chromosome 11p13 is a putative candidate gene for MDD taking aspects of the neurodevelopmental, neurodegenerative hypotheses. The single nucleotide polymorphism (SNP) rs6265, which causes a valine-to-methionine substitution at codon 66 (Val66Met) in the BDNF gene, has been shown to affect intracellular trafficking and activity-dependent secretion of BDNF (6).

Previous studies testing associations between BDNF Val66Met gene variants and MDD have produced contradictory results. Ribeiro et al. (7) reported individuals homozygous for major allele (GG) had an increased chance of being depressed in Mexican-Americans (OR = 1.795% CI = 1.17 − 2.47). Two other genetic association studies, both

https://doi.org/10.1111/j.1601-5215.2009.00401.x Published online by Cambridge University Press

in Chinese populations studies by Tsai et al. (8) and Hong et al. (9) failed to establish BDNF Val66Met gene as a risk factor for MDD. Schumacher et al. (10) performed one large genetic case-control study for BDNF, including 1097 controls and 465 patients with major depressive disorder from a German population. They also did not find the positive association between BDNF Val66Met gene and MDD, but haplotypes G-174bp-A, G-170bp-A and C-174bp-G emerged as important discriminators between the patients and controls ($p = 0.00065$). In this study, we examined BDNF Val66Met gene in health controls and late-onset depressed Chinese Han populations to find whether specified gene type increase in late-onset depression (LOD) and to detect the relationship between the gene and BDNF plasma level.

Materials and methods

Subjects

A total of 99 patients (29 men and 70 women, mean age 69.69 ± 5.38) were recruited from the Affiliated Brain Hospital of Nanjing Medical University, China, between December 2005 and June 2007. All the patients met criteria for a current major depressive episode according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth edition (DSM-IV) (11). The age of first episode for all the patients was 60 years or older, and the patients had scores of $\geqslant$17 on the 17-item Hamilton Depression Scale (HAM-D) (12). Exclusion criteria included medical illnesses, epilepsy, substance abuse disorders, including alcohol, active agents within the last year, schizophrenia, delusional disorder, psychotic disorders not elsewhere classified, bipolar disorder or anti-social personality disorder diagnosed by DSM-IV.

The 110 normal controls (33 men and 77 women, mean age 69.45 ± 5.14) according to the exclusion criteria were recruited from community population in Nanjing. Inclusion criteria for the control subjects were physical health and no history of mental disorder, neurological disease or drug abuse, and no undergoing psychotropic treatment. All controls had a score of $\leqslant$7 in the HAM-D. The sex and age between cases and controls are well matched which show no significant differences ($p > 0.05$).

The study was approved by the Ethics Committee of the Nanjing Medical University. All subjects participated after giving informed consent.

Measure of plasma BDNF level

Fasting venous blood samples (5 ml) from the patients and normal controls were collected in anticoagulant tubes between 7:30 a.m. and 8:00 a.m. After centrifugation (centrifugation at 2500 × g for 8 min), the samples were stored at −80°C until assayed.

BDNF concentrations were measured using the BDNF Emax Immunoassay System kit (Promega; Madison, WI, USA) according to the manufacturer's instructions. To minimise the assay variance, plasma BDNF was measured in all subjects on the same day. Briefly, anti-BDNF monoclonal antibody was coated into 96-well plates being used to capture the neurotrophins in the plasma. The captured BDNF bound specifically BDNF polyclonal antibody (pAb), which were washed and incubated with anti-IgY antibody conjugated to horseradish peroxidase to detect the amount of specifically bound pAb. The plates were incubated with a chromogenic substrate to produce a colour reaction. The amount of BDNF in the test solution is proportional to the colour generated in the redox reaction. The standard curve and the samples were analysed in triplicate and showed a direct relation between optical density and BDNF concentration. The intra-assay coefficient of variation was less than 6%.

Genotyping of BDNF Val66Met

Genomic DNA was extracted from 300 μl ethylenediamine tetraacetic acid (EDTA)- anticoagulated venous blood using Puregene DNA Purification Kit (Gentra, Minnesota, USA) according to the manufacturer's recommendations. Polymerase chain reaction-based restriction fragment length polymorphism (PCR-RFLP) assay was performed to genotyping the DNA sequence variants (196 G/A) of the BDNF gene. The primer sequences used for analysis of 196 G/A were the sense primer 5′-ACTCTGGAGAGCGTGAAT-3′ and the antisense primer 5′-ATACTGTCACACACGCTC-3′. The amplification conditions were initiated at 94°C for 5 min, followed by 35 cycles consisting of denaturation at 94°C for 30 s, annealing at 60°C for 30 s and extension at 72°C for 30 s, with a final extension step of 5 min at 72°C. The PCR product was digested with NlaIII (Fermentas, Lithuania) at 37°C for 10 h. The products were electrophoresed on 12% polyacrylamide gel at 200 V for 2 h. The presence of 168 and 75 bp bands indicated the existence of A allele, the presence of 243 bp band indicated the existence of G allele, while the presence of 75, 168, and 243 bp indicated AG heterozygote.

Statistical analysis

The data were analysed using the SPSS for Window11.0 software. The categorical data were analysed using the chi-squared test or the Fisher's exact

https://doi.org/10.1111/j.1601-5215.2009.00401.x Published online by Cambridge University Press

test, if necessary. Continuous variables were analysed using the Student's t-test for the comparison among the groups. The criterion for significance was set at $p < 0.05$ for all the tests.

Results

The data of BDNF genotypes and alleles were given in LOD patients and controls (Table 1). The Val66Met-genotype distributions for these two groups were in Hardy–Weinberg equilibrium. The genotype and allele distributions in the LOD patients did not show significant differences from those in the control subjects ($p = 0.744$ and 0.845, respectively). We also examined the association between BDNF genotypes and quantitative measures of the LOD, including BDNF plasma level, duration of illness, age-of-onset, HAM-D and Hamilton Anxiety Scale (HAM-A) (13) at baseline. Table 2 showed that no significant difference was observed among the three BDNF genotype groups of the LOD. Additionally, no significant effect was shown for BDNF genotype in terms of suicidal history and psychotic symptoms for the LOD patients ($p = 0.688$ and 0.199, respectively).

Discussion

The main finding of the present study was that there was no association of BDNF Val66Met polymorphism with LOD. To our knowledge, it is firstly reported that Val66Met genotype has no association with BDNF plasma level in LOD. Increasing evidence suggested that BDNF serum or plasma level decreased at baseline and increased after treated by antidepressant or electro-convulsive treatment (ECT) in depressive patients (14,15). Egan et al. (6) showed that depolarisation-induced secretion was reduced in 66Met BDNF-transfected neurons compared with 66Val BDNF analogues. Thus, subjects carrying Met66Met may have lower BDNF activity, which leads to higher risk for the development of MDD (16). Nevertheless, BDNF is secreted through both constitutive and regulated pathways; the latter is a key step to control of synaptic plasticity. However, the BDNF Val66Met variant only affects activity-dependent secretion of BDNF but not the constitutive pathway. Consequently, this provides plausible explanations for BDNF concentrations in blood being unaffected by the genotype (17).

It should also be noted that the relation between plasma and brain BDNF levels is not very clear. One study reported that BDNF could cross the blood-brain barrier freely from the brain to the blood (18). Piccinni et al. (15) reported that the plasma BDNF levels increased to the values found in control subjects in parallel with the clinical improvement, while antidepressant treatments did not induce any change in serum BDNF levels, which remained lower than those found in the control group at all assessment times. Thereafter, they suggest, while plasma BDNF would behave as a state-dependent marker, serum BDNF might represent a trait of illness. Similarly, considering the interactions among

Table 1. BDNF Val66Met genotype distributions and allele frequency in 99 late-onset depression patients and 110 normal controls

	Genotypes, n (%)				Allele frequency		
Group	Val/Val	Val/Met	Met/Met	p	Val	Met	p
Patients	25(25.3)	48(48.5)	26(26.3)	0.744	49.5	50.5	0.845
Controls	26(23.6)	59(53.6)	25(22.7)		50.5	49.5	

Table 2. Demographic data and clinical characteristics of the three BDNF-genotype groups within the late-onset depression patients

	Val/Val ($n = 25$)	Val/Met ($n = 48$)	Met/Met ($n = 26$)	F/χ^2	P
Age, years (SD)	69.24 ± 4.88	70.83 ± 5.92	68.00 ± 4.36	2.532	0.085
Gender, F/M	17/8	38/10	15/11	3.873	0.144
Age-of-onset, years (SD)	65.48 ± 4.44	67.58 ± 6.23	65.08 ± 4.02	2.382	0.098
Episodes (times)	3.08 ± 1.86	2.88 ± 1.94	3.07 ± 2.35	0.124	0.884
Duration of illness (years)	3.80 ± 4.36	3.25 ± 3.90	2.98 ± 3.54	0.294	0.746
History of psychosis, with/without	6/19	12/36	7/19	0.061	0.970
HAM-D score (SD)	27.80 ± 9.37	26.80 ± 6.57	31.25 ± 10.94	1.408	0.252
HAM-A score (SD)	20.82 ± 7.80	21.72 ± 5.76	24.39 ± 5.33	2.115	0.127
Suicidal attempt, with/without	10/15	24/24	13/13	0.749	0.688
Psychotic symptoms, with/without	5/20	8/40	1/25	3.229	0.199
BDNF (pg/ml)	900.42 ± 214.95	883.92 ± 134.49	903.97 ± 165.21	0.151	0.860

SD, standard deviation.

https://doi.org/10.1111/j.1601-5215.2009.00401.x Published online by Cambridge University Press

plasma, platelets and the blood-brain barrier, plasma BDNF levels may reflect brain BDNF levels (19).

Several genetic association studies have been carried out in MDD, bipolar affective disorder, Alzheimer's disease and schizophrenia. The majority of these studies have produced negative results (8,9,10,17). As all etiopathogenisis of these diseases are unclear, the heritability of diseases may derive from a number of genes influenced by environmental factor, each with a small effect. So the effect of only one gene SNP may be tinier. The previously ambiguous findings of association studies between the BDNF gene Val66Met polymorphisms and MDD or some other psychiatric diseases are not unusual for genetic analyses of complex genetic diseases. Given the fact that the positive association between BDNF gene Val66Met polymorphism and bipolar disorder has only been shown for a Caucasian population but not for a Japanese or our Chinese sample, it appears likely that this association is ethnicity dependent (20). Shimizu et al. (21) had similar findings and pointed that the frequency of healthy individuals who carried Val/Val gene type was significantly decreased in Japanese (33.8%) than in Italians (48.7%) or in Americans (68.4%). So they presumed that ethnic difference of the polymorphism frequencies might contribute to inconsistent results in genetic association study. In our study, to reduce the risk of ethnic biases, all the cases and control subjects were of Chinese Han population. Hwang et al. (22) pointed that the positive associations between BDNF Val66Met genetic polymorphism and depression might have been due to the chance or a stratification effect in the sample collection. Although our study did not find positive association in LOD, the possibility could not be ruled out that other polymorphisms of the BDNF gene are involved in the pathogenesis of MDD. On the basis of the findings of animal, pharmacological and clinical studies, which mainly focused on depression, BDNF has been proposed as a susceptibility locus for MDD. Szeszko et al. (23) reported subjects with BDNF Val/Val homozygotes had a larger hippocampal formation than Val/Met heterozygotes. Decreased hippocampal volume had more chance in the development of depression especially geriatric depression (24).

BDNF gene Val66Met polymorphism only reflected the role of a single locus. Haplotype-based studies might be considered advantageous as they use combined genetic information from several markers. Schumacher et al. (10) investigated a possible relationship among three genetic variants at the BDNF locus [SNPs rs988748 and rs6265 (Val66Met), and a dinucleotide repeat (GT) n] in large samples. Although there were no single-marker associations, haplotypes were differentially distributed between case and control subjects at a global significance of $p = 0.0092$. Thus, to determine whether or not the BDNF gene is associated with MDD, further studies on more polymorphic sites within and close to the gene in larger, independent samples are required.

Our current study found no association between BDNF genotype, age-of-onset of depression, duration of illness, psychotic symptoms and suicidal attempt history in LOD. Depression and anxiety severity, as evaluated by HAM-D and HAM-A, also did not differ significantly among the three BDNF genotype groups. These findings implied that the BDNF Val66Met genetic polymorphism did not play a major role in these clinical manifestations of LOD. The results were consistent with the study conducted in Taiwan patients with major depression (9). Shimizu et al. (25) also reported that their results which did not support BDNF Val66Met genetic polymorphism may be implicated in the putative common pathophisiology of depression in panic disorder. Further work is continuing to explore more BDNF gene locus and elucidates the relationship in LOD.

Conclusion

This study indicated that allele and genotype association with BDNF Val66Met polymorphisms did not show significant differences between the patients and controls. The BDNF plasma level had no association with genotype in Chinese Han patients, suggesting that the SNP was not a robust genetic risk factor for LOD.

Acknowledgements

This study was supported by grants from Key Program of Medical Development of Nanjing (No: ZKX07018 Yonggui Yuan).

Declaration of Interests

The authors declare they have no biomedical financial or potential conflicts of interest.

References

1. Duman RS, Monteggia LM. A neurotrophic model for stress-related mood disorders. Biol Psychiatry 2006;**59**: 1116–1127.
2. Dwivedi Y, Rizavi HS, Conley RR et al. Altered gene expression of brain-derived neurotrophic factor and receptor tyrosine kinase B in postmortem brain of suicide subjects. Arch Gen Psychiatry 2003;**60**:804–815.
3. Shimizu E, Hashimoto K, Okamura N et al. Alterations of serum levels of brain derived neurotrophic factor (BDNF) in depressed patients with or without antidepressants. Biol Psychiatry 2003;**54**:70–75.

https://doi.org/10.1111/j.1601-5215.2009.00401.x Published online by Cambridge University Press

4. Karege F, Bondolfi G, Gervasoni N et al. Low brain-derived neurotrophic factor (BDNF) levels in serum of depressed patients probably results from lowered platelet BDNF release unrelated to platelet reactivity. Biol Psychiatry 2005;**57**:1068–1072.
5. Karege F, Perret G, Bondolfi G et al. Decreased serum brain-derived neurotrophic factor levels in major depressed patients. Psychiatry Res 2002;**109**:143–148.
6. Egan MF, Kojima M, Callicott JH et al. The BDNF val66met polymorphism affects activity dependent secretion of BDNF and human memory and hippocampal function. Cell 2003;**112**:257–269.
7. Ribeiro L, Busnello JV, Cantor RM et al. The brain-derived neurotrophic factor rs6265 (Val66Met) polymorphism and depression in Mexican-Americans. Neuroreport 2007;**18**:1291–1293.
8. Tsai SJ, Cheng CY, Yu YW et al. Association study of a brain-derived neurotrophic-factor genetic polymorphism and major depressive disorders, symptomatology, and antidepressant response. Am J Med Genet B Neuropsychiatr Genet 2003;**123**:19–22.
9. Hong CJ, Huo SJ, Yen FC et al. Association study of a brain-derived neurotrophic-factor genetic polymorphism and mood disorders, age of onset and suicidal behavior. Neuropsychobiology 2003;**48**:186–189.
10. Schumacher J, Jamra RA, Becker T et al. Evidence for a relationship between genetic variants at the brainderived neurotrophic factor (BDNF) locus and major depression. Biol Psychiatry 2005;**58**:307–314.
11. American Psychiatric Association. Diagnostic and statistical manual of mental disorders, 4th edn. Washington, DC: American Psychiatric Press, 1994.
12. Hamilton M. A rating scale for depression. J Neurol Neurosurg Psychiatry 1960;**23**:56–62.
13. Hamilton M. The assessment of anxiety states by rating. Br J Med Psychol 1959;**32**:50–55.
14. Huang TL, Lee CT, Liu YL. Serum brain-derived neurotrophic factor levels in patients with major depression: effects of antidepressants. J Psychiatr Res 2008;**42**:521–525.
15. Piccinni A, Marazziti D, Catena M et al. Plasma and serum brain-derived neurotrophic factor (BDNF) in depressed patients during 1 year of antidepressant treatments. J Affect Disord 2008;**105**:279–283.
16. Gervasoni N, Aubry JM, Bondolfi G et al. Partial normalization of serum brain-derived neurotrophic factor in remitted patients after a major depressive episode. Neuropsychobiology 2005;**51**:234–238.
17. Yu H, Zhang ZJ, Shi YM et al. The decreased serum BDNF concentrations of amnestic mild cognitive impairment is unrelated to the Val66Met polymorphism in Chinese Han. J Clin Psychiatry 2008;**69**:1104–1111.
18. Pan W, Banks WA, Fasold MB et al. Transport of brain-derived neurotrophic factor across the blood-brain barrier. Neuropharmacology 1998;**37**:1553–1561.
19. Lee HY, Kim YK. Plasma brain-derived neurotrophic factor as a peripheral marker for the action mechanism of antidepressants. Neuropsychobiology 2008;**57**:194–199.
20. Kaija H, Sami A, Martti H et al. Brain-derived neurotrophic factor (BDNF) polymorphisms G196A and C270T are not associated with response to electroconvulsive therapy in major depressive disorder. Eur Arch Psychiatry Clin Neurosci 2007;**257**:31–35.
21. Shimizu E, Hashimoto K, Iyo M. Ethnic difference of the BDNF 196G/A (val66met) polymorphism frequencies: the possibility to explain ethnic mental traits. Am J Med Genet B Neuropsychiatr Genet 2004;**126B**:122–123.
22. Hwang JP, Tsai SJ, Hong CJ et al. The Val66Met polymorphism of the brain-derived neurotrophic-factor gene is associated with geriatric depression. Neurobiol Aging 2006;**27**:1834–1837.
23. Szeszko PR, Lipsky R, Mentschel C et al. Brain-derived neurotrophic factor Val66met polymorphism and volume of the hippocampal formation. Mol Psychiatry 2005;**10**: 631–636.
24. Hickie I, Naismith S, Ward PB et al. Reduced hippocampal volumes and memory loss in patients with early and late-onset depression. Br J Psychiatry 2005;**186**: 197–202.
25. Shimizu E, Hashimoto K, Koizumi H et al. No association of the brain-derived neurotrophic factor (BDNF) gene polymorphisms with panic disorder. Prog Neuropsychopharmacol Biol Psychiatry 2005;**29**:708–712.

https://doi.org/10.1111/j.1601-5215.2009.00401.x Published online by Cambridge University Press