ROLE OF BDNF VAL66MET FUNCTIONAL POLYMORPHISM IN TEMPORAL LOBE EPILEPSY

Ning Shen,1∗ Xilin Zhu,1∗ Hua Lin,2 Jingyun Li,1 Liping Li,2 Fenghe Niu,1 Aihua Liu,2 Xiaopan Wu,1 Yuping Wang,2 and Ying Liu1

1National Laboratory of Medical Molecular Biology, Institute of Basic Medical Science, Chinese Academy of Medical Science; School of Basic Medicine, Peking Union Medical College, Beijing, P. R. China; 2Department of Neurology, Xuanwu Hospital, Capital Medical University, Beijing, P. R. China

Various studies suggested that brain-derived neurotrophic factor (BDNF) gene polymorphisms contributed to the development of many neurological disorders. However, whether BDNF Val66Met polymorphism is associated with epilepsy remains controversial. In our study, we tried to investigate the effects of this functional polymorphism on the occurrence of temporal lobe epilepsy (TLE) and its clinical phenotypes. Case-control studies were employed to study the association between BDNF Val66Met polymorphism and TLE, as well as its clinical phenotypes, and magnetic resonance imaging examinations and voxel-based morphometry analyses were carried out for further study. Our results showed that the frequency of Met allele was found to be lower in the TLE patients compared with the control subjects (43.9% vs. 48.6%, P = 0.012, OR = 1.21, 95% CI = 1.04–1.41), and the frequency of Met66 allele carriers in the TLE with hippocampal sclerosis was significantly lower than those non-carriers (20.5% vs. 29.1%, P = 0.040). However, we failed to find the difference between different genotypes and hippocampal asymmetry. Our findings suggested that BDNF Val66Met polymorphism might be correlated with epileptogenesis, and Met66 allele might play a protective role against the occurrence of TLE.

KEYWORDS: association, BDNF, endophenotype, polymorphism, temporal lobe epilepsy (TLE)

INTRODUCTION

Temporal lobe epilepsy (TLE), the most common type of human focal epilepsy, is a complex neurological disorder with heterogeneous clinical manifestations. Genetic factors, together with environmental factors such as brain trauma and febrile seizure, may contribute to the occurrence of TLE.

Brain-derived neurotrophic factor (BDNF), which is an important neurotrophic protein in the central nervous system (CNS), may play a vital role in the survival, differentiation and development of the neuron [1]. Studies have shown that upregulation of BDNF mRNA and protein might contribute to epileptogenesis [2–4], and overexpression of BDNF in the hippocampus could lead to long-term effects on hyperexcitability of the hippocampal network through seizure activity as well as other insults [5]. However, a recent study has found that BDNF overexpression reduced neuroinflammation in early epilepsy and had a protective effect on the progress of the disease [6]. BDNF Val66Met, a single-nucleotide polymorphism (SNP) at nucleotide 196 (G/A) of BDNF gene coding sequence, can cause an amino acid substitution at the codon 66 of pro-BDNF sequence. BDNF secretion and hippocampal function could be influenced by Val66Met polymorphism [7], which was found to be associated with many neurological disorders [8–11]. The association between BDNF polymorphism and TLE was first reported by a Japanese group [12]; however, the following studies conducted in other groups failed to replicate the results [13,14]. Therefore, the relationship between BDNF
Val66Met polymorphism and epileptogenesis merits further genetic and biological investigation.

The discrepancy of the previous genetic association studies may be caused by clinical phenotype heterogeneity of the patients with TLE; therefore, we took the endophenotype which is more believable to reflect the biological characteristics of the disease for further investments in our study. TLE patients with hippocampal sclerosis (TLE-HS+) have an obvious pathologic feature with neuronal loss and reactive astrogliosis. Histopathological studies found that seizures were induced by abnormal neuronal activity, which were caused by hippocampal sclerosis through the proliferation of astrocytes, that is to say, hippocampal sclerosis is the cause rather than the result of epileptogenesis [15]. Immunohistochemical experiences found that hippocampus with evidence of sclerosis showed different patterns of immunostaining and neuronal densities of hippocampal subfields, and there was also a distinctive difference in the onset age, neuronal densities and electrophysiological characteristics [16]. Taken together, these results strongly suggested that there were different pathogeneses between TLE-HS+ and TLE-HS−. HS could be diagnosed through magnetic resonance imaging (MRI) by increased hippocampal T2 signal. Voxel-based morphometry (VBM), a morphometric method which can quantify the density and volume of brain tissue, is able to simply and automatically assess the morphology of a whole brain even with small-scaled differences [17,18]. VBM analysis of brain MRI could be used to evaluate the extent of hippocampal atrophy and structural abnormalities in hippocampus subfields in TLE patients [19–21]. The progress of hippocampal lesions could be reflected by asymmetry index (AI) of hippocampus, which can be useful for clinical diagnosis [22,23]. Therefore, we believe that it is accurate to estimate the influence of genetic factors by using AI to measure the progress of hippocampal sclerosis.

The aim of our study was to investigate whether BDNF Val66Met polymorphism was associated with the occurrence of TLE as well as its clinical phenotypes, and explore preliminarily the effects of BDNF Val66Met on the epileptogenesis on brain imaging. Our results suggested that BDNF Val66Met polymorphism was associated with the occurrence of TLE, and Met66 allele might be a protective factor against the seizures. However, we failed to find out the effects of Val66Met polymorphism on hippocampal asymmetry. Our investigation might lay a genetic foundation for the molecular mechanism of TLE and provide a molecular biological evidence for clinical diagnosis, treatment and prognosis.

Methods

Subjects

Four hundred ninety-nine TLE patients and 1181 healthy control subjects of Chinese Han population origin were recruited. All TLE patients were receiving treatment from March 2004 to April 2013 in the Department of Neurology, Xuanwu Hospital, Capital Medical University. Inclusion criteria were based on “Classification of Epilepsies and Epileptic Syndromes” by Commission on Classification and Terminology of the International League Against Epilepsy (ILAE, 1989). The TLE diagnosis was carried out by comprehensive evaluation of seizure symptoms, MRI and video electroencephalography (EEG) showing typical ictal activity or interictal discharges. Patients with MRI evidence of brain tumor, vascular injury, brain parasite and those with obvious familial temporal lobe epilepsy pattern in three generations of relatives were excluded. The control subjects were recruited in a health examination conducted in Peking Union Medical College Hospital. The patients group with a mean age of 32.6 consisted of 249 males and 250 females, and the control group with the age of 33.6 consisted of 602 males and 579 females. All subjects provided a written informed consent. This study was approved by the Ethics Committee of Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences.

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes by standard procedures. The Val66Met was genotyped by TaqMan SNP Genotyping assay. The primers and probes were designed and synthesized by GeneCore Bio Technologies (Shanghai, China): 5′-CTGCG TCCTATTGT TTTTCTTCA TT-3′ (forward prime), 5′-GGCTTGACATCATTGGCTGA C-3′ (reverse prime), FAM-CTTCTATCATGTTG TTTGCA- MGB (probe 1) and HEX-CTTCTATCATGTTG TTTGCA- MGB (probe 2). Amplification reactions were carried out in a volume of 10 ml by using Bio-Rad iQ5 Multi-color Real-Time PCR Detection system (Bio-Rad), and reaction conditions were as follows: 95 °C for 10 min, followed by 40 cycles of 92 °C for 15 s and 60 °C for 1 min. Sanger sequencing was used to confirm the genotyping results in 5% randomly selected samples.

MRI examination and data processing

MRI was performed on a 1.5T GE system in 29 TLE patients and 29 healthy controls, and TLE patients were
divided into two groups based on HS or not. Images were acquired using a three-dimensional T1 fast spoiled gradient echo sequence (matrix size = 256 × 192, NEX = 1.00, field of view = 24 cm, thickness = 1.4 mm, prep time = 350 ms, flip angle = 15°). Segmentation, spatial normalization and smoothing were conducted on the whole brain and hippocampus using the VBM analyses with FIRST software, and the left and right hippocampal volumes and intracranial volume were measured separately. Standard hippocampal volume was obtained after the intracranial volume standardized verified using linear regression. Asymmetry of hippocampal volume was characterized by calculation of an asymmetry index (AI) defined as follows:

\[
AI = \frac{|R - L|}{\frac{1}{2}(R + L)}
\]

where \(R\) is the right hippocampal volume, and \(L\) is the left hippocampal volume [22].

**Statistical analyses**

\(\chi^2\) goodness-of-fit test was applied to access the genotypic distributions of SNPs for Hardy–Weinberg equilibrium (HWE). Differences in allele and genotype frequencies between groups were performed with 2 × 2 and 2 × 3 contingency tables, respectively. Comparison of differences in the value of AI was made using one-way analysis of variance. The Bonferroni correction was applied to correct a \(P\) value in multiple comparisons. \(P < 0.05\) (two-sided) was considered as statistical significance. All the above statistical analyses were conducted using SPSS 17.0 (SPSS Inc., Chicago, IL).

**Results**

There were no significant differences in age and gender ratio between TLE patients and healthy control groups \((P > 0.05)\). Genotype distribution in both TLE and healthy control subjects were consistent with HWE according to \(\chi^2\) goodness-of-fit test \((P > 0.05)\). Table 1 shows that the frequency of Met allele in the TLE subjects was lower than the control subjects \((43.9\%\) vs. \(48.6\%, P = 0.012, OR = 1.21, 95\% CI = 1.04–1.41)\), and there were statistical differences in genotype distribution between the TLE and healthy control subjects \((P = 0.029)\).

To further study the association between BDNF Val66Met polymorphism and the clinical phenotypes, the TLE patients were divided into two groups according to whether they carried Met66 allele or not. The results showed that the frequency of Met66 allele carriers in the TLE with HS was significantly lower compared with the non-carriers \((20.5\%\) vs. \(29.1\%, P = 0.040)\). The clinical phenotype analyses were summarized in Table 2.

The clinical characteristics of 12 TLE patients with unilateral HS \((\text{TLE-HS+})\), 17 TLE patients without HS \((\text{TLE-HS-})\) and 29 healthy controls were summarized in Table 3, and the intracranial volume as well as hippocampal volume measured by VBM analyses was also showed in this table. The TLE-HS+ group showed a robust increase than the other two groups by comparing the value of hippocampal AI \((P < 0.001)\); however,
Table 3. Clinical characteristics of subjects with hippocampal volume measurement.

| Clinical characteristic | TLE-HS(+) (n = 12) | TLE-HS(−) (n = 17) | Healthy control (n = 29) | P       |
|------------------------|--------------------|--------------------|-------------------------|---------|
| Age, year (mean, SD) *  | 31.4 ± 10.2        | 31.2 ± 13.0        | 30.4 ± 11.1             | 0.93    |
| Onset age (mean, SD) *  | 17.8 ± 13.9        | 22.8 ± 13.5        | –                       | 0.33    |
| Male (%)                | 8 (66.7)           | 11 (64.7)          | 14 (48.3)               | 0.41    |
| Family history of epilepsy (%) | 0 (1)            | 2 (11.8)          | –                       | 0.22    |
| Antecedent FC (%)       | 2 (16.7)           | 6 (35.3)           | –                       | 0.27    |
| Intracranial volume (cm³) * | 1117.4 ± 153.4    | 1214.2 ± 886.2    | 1279.0 ± 180.6          | 0.01    |
| Left hippocampal volume (mm³) ** | 5405.6 ± 673.2 | 5631.7 ± 604.2 | 5744.5 ± 547.4          | 0.26    |
| Right hippocampal volume (mm³) ** | 5202.3 ± 730.9  | 5602.2 ± 629.6    | 5924.0 ± 497.1          | 0.03    |
| AI*                    | 0.1903 ± 0.1094    | 0.0586 ± 0.0541    | 0.0674 ± 0.0481         | <0.001  |

*SD: standard deviation
**Normalized hippocampal volume

we did not find that BDNF Val66Met exerted its effects on hippocampal asymmetry (see Figure 1).

Discussion

In our study, we analyzed the association between BDNF Val66Met polymorphism and TLE from aspects of gene, genotype–phenotype correlation and brain imaging. Association studies together with clinical phenotype analyses suggested that Met66 allele might be a protective factor for the occurrence of TLE. However, we failed to find the effects of BDNF Val66Met polymorphism on the hippocampal asymmetry.

Several lines of evidence showed that BDNF might play a vital role in the pathological process of epilepsy. Heterozygous BDNF knockdown mice showed a reduced susceptibility to seizures [24]. On the contrary, BDNF overexpression could facilitate the seizures and increase the excitability of neurons [25]. Activity-dependent secretion of BDNF could be reduced by BDNF Val66Met polymorphism through interfering BDNF protein packaging and transport in cortical neuron [7]. Several previous studies have demonstrated that there was an association between BDNF Val66Met polymorphism and epileptogenesis. Kanemoto et al. first reported the association between BDNF polymorphism and partial epilepsy in a Japanese population in 2003, and Louhivuori et al. also found that Val66Met polymorphism was associated with epilepsy in Fragile X syndrome [12,26]. However, Lohoff et al. failed to replicate the results of the Japanese group, and Bragatti et al. found that there was no major clinical impact of this polymorphism on TLE [13,14]. The recent study reported that BDNF Val66Met polymorphism might reduce the risk of seizures in Rett syndrome [27], and Met66 allele might play a protective effect in regional gray matter (GM) volumes in patients with sclerosis, major depression as well as healthy people [11,28,29], which was consistent with our results. Therefore, taken our results with the previous findings together, we speculated that BDNF might exert a positive role on the occurrence of TLE, and BDNF Met66 allele might inhibit seizure activity by reducing the level of brain BDNF.

Figure 1. The asymmetry index (AI) of hippocampus in TLE-HS(+), TLE-HS(−) and controls: (A) The AI levels were significantly higher in TLE-HS(+) group than in two another groups (TLE-HS+: n = 12, TLE-HS−: n = 17, control: n = 29); (B) BDNF Val66Met polymorphism had no effects on hippocampal asymmetry.
through affecting mature protein secretion, but the protection mechanism of Met66 allele still needed further study.

Hippocampal function could be influenced by BDNF Val66Met polymorphism, showing poor memory, declined intellectual evaluation and decreased hippocampal volume [7]. Our clinical phenotype analyses also suggested that this SNP might be associated with the occurrence of HS and influence hippocampal function. Therefore, we individually compared all the TLE-HS+ with the health control subjects, and we noticed that the frequency of Met allele was found to be lower in the TLE-HS+ patients compared with the control subjects (40.1% vs. 48.6%, \( P = 0.012, \ OR = 0.71, 95\% \ CI = 0.54–0.93\)), and there were statistical differences in genotype distribution between the TLE-HS+ and healthy control subjects (\( P = 0.029 \)) (see Supplementary S1), which further suggested that Met66 might be a protective factor involved in the pathogenesis of TLE-HS. Moreover, hippocampal lesions are not only the major pathological features, but also one of the evidences of clinical diagnosis for TLE patients. To investigate the relationship between BDNF Val66Met and hippocampal function, we measured the severity of hippocampal lesions by calculating AI value and then analyzed the relationship between Val66Met polymorphism and the value of AI; however, we found that all types of BDNF polymorphism had a high AI value of hippocampal in the TLE+HS, which indicated that the progression of hippocampal lesion might affect hippocampal asymmetry, but BDNF Val66Met polymorphism did not. Here are the possible reasons. First, VBM analyses were conducted only in 29 TLE patients and 29 healthy controls, which greatly limited the reliability of our data and reduced statistical power, so that we failed to detect the effects sensitively. Second, several studies suggested that the epistatic effects between Val66Met allele and 5-HTTLPR might be related to the hippocampal function [30,31], which might diminish or cover the contribution of single locus. Third, we used AI as a clinical measurable indicator to analyze the impairment of hippocampus in our study. Although AI analysis was more sensitive and specific than previous methods of analysis, further studies will be required to establish that whether AI can be used for describing the progress of epileptogenesis and instructing clinical diagnosis.

In conclusion, our results suggested that BDNF Val66Met polymorphism might be associated with epileptogenesis, and Met66 allele might play a protective role against the occurrence of TLE. Unfortunately, VBM analysis failed to find the relationship between BDNF Val66Met polymorphism and epileptogenesis. Our findings enriched the understanding of sporadic TLE susceptibility genes, and our data laid a foundation for the research works of other neurological diseases.

The positive results of our research could be explained from the perspective of biological function, which had an instructive significance for the functional research of BDNF Val66Met polymorphism. Furthermore, our investment could provide a molecular biological evidence for the clinically seizure risk prediction, diagnosis, individual treatment and prognosis. However, it is difficult to elucidate the biological roles of BDNF Val66Met based on our relatively superficial investigation; therefore, further investigations with a larger number of samples need to be carried out to shed light on the underlying mechanism.

Acknowledgments

We are grateful to all of the subjects who kindly agreed to participate in this study. This work was strongly supported by grant from The 12th Five-Year Plan “major drug discovery” science and technology major projects which is entitled as “innovative drug research and development technology platform construction and new drug development of public resources”, project number: 2011ZX11307.

Declaration of Interest

No conflict of interest.

References

1. Huang EJ, Reichardt LF. Neurotrophins: roles in neuronal development and function. Ann Rev Neurosci 2001;24:677–736.
2. Mathern GW, Babb TL, Leite JP, et al. The pathogenic and progressive features of chronic human hippocampal epilepsy. Epilepsy Res 1996;26(1):151–61.
3. Takahashi M, Hayashi S, Kakita A, et al. Patients with temporal lobe epilepsy show an increase in brain-derived neurotrophic factor protein and its correlation with neuropeptide Y. Brain Res. 1999;818(2):579–82.
4. Murray KD, Isackson PJ, Esken TA, et al. Altered mRNA expression for brain-derived neurotrophic factor and type II calcium/calmodulin-dependent protein kinase in the hippocampus of patients with intractable temporal lobe epilepsy. J Comp Neurol 2000;418(4):411–22.
5. Binder DK, Croll SD, Gall CM, Scharfman HE, BDNF and epilepsy: too much of a good thing? Trends Neurosci. 2001;24(1):47–53.
6. Bovolenta R, Zucchini S, Paradiso B, et al. Hippocampal FGF-2 and BDNF overexpression attenuates epileptogenesis-associated neuroinflammation and reduces spontaneous recurrent seizures. J Neuroinflamm 2010;7:1–6.
7. Egan MF, Koijima M, Callcott JH, et al. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 2003;112(2):257–69.
8. Hall D, Dhilla A, Charalambous A, et al. Sequence variants of the brain-derived neurotrophic factor (BDNF) gene are strongly associated with obsessive-compulsive disorder. Am J Hum Genet 2003;73(2):370–6.
9. Geller B, Badner JA, Tillman R, et al. Linkage disequilibrium of the brain-derived neurotrophic factor Val66Met polymorphism in children with a prepubertal and early adolescent bipolar disorder phenotype. Am J Psychiatry 2004;161(9):1698–700.

10. Neves-Pereira M, Cheung JK, Pasdar A, et al. BDNF gene is a risk factor for schizophrenia in a Scottish population. Mol Psychiatry 2005;10(2):208–12.

11. Chen SL, Lee SY, Chang YH, et al. The BDNF Val66Met polymorphism and plasma brain-derived neurotrophic factor levels in Han Chinese patients with bipolar disorder and schizophrenia. Prog Neuro-psychopharmacol Biol Psychiatry 2014;51:99–104.

12. Kanemoto K, Kawasaki J, Tarao Y, et al. Association of partial epilepsy with brain-derived neurotrophic factor (BDNF) gene polymorphisms. Epilepsy Res 2003;53(3):255–8.

13. Lohoff FW, Ferraro TN, Dahl JP, et al. Lack of association between variations in the brain-derived neurotrophic factor (BDNF) gene and temporal lobe epilepsy. Epilepsy Res 2005;66(1–3):59–62.

14. Bragatti JA, Schenkel LC, Torres CM, et al. No major clinical impact of Val66Met BDNF gene polymorphism on temporal lobe epilepsy. Epilepsy Res 2010;88(2–3):108–11.

15. de Lanerolle NC, Lee TS. New facets of the neuropathology and molecular profile of human temporal lobe epilepsy. Epilepsy Behav 2005;7(2):190–203.

16. de Lanerolle NC, Kim JH, Williamson A, et al. A retrospective analysis of hippocampal pathology in human temporal lobe epilepsy: evidence for distinctive patient subcategories. Epilepsia 2003;44(5):677–87.

17. Bonilha L, Rorden C, Castellano G, et al. Voxel-based morphometry reveals gray matter network atrophy in refractory medial temporal lobe epilepsy. Arch Neurol 2004;61(9):1379–84.

18. Bernasconi N, Duchesne S, Janke A, et al. Whole-brain voxel-based statistical analysis of gray matter and white matter in temporal lobe epilepsy. NeuroImage 2004;23(2):717–23.

19. Takaya S, Ikeda A, Mitsueda-Ono T, et al. Temporal lobe epilepsy with amygdala enlargement: a morphologic and functional study. J Neuroimaging 2014;24(1):54–62.

20. Li J, Zhang Z, Shang H. A meta-analysis of voxel-based morphometry studies on unilateral refractory temporal lobe epilepsy. Epilepsy Res 2012;98(2–3):97–103.

21. Keller SS, Roberts N. Voxel-based morphometry of temporal lobe epilepsy: an introduction and review of the literature. Epilepsia 2008;49(5):741–57.

22. Scott RC, King MD, Gadian DG, et al. Hippocampal abnormalities after prolonged febrile convulsion: a longitudinal MRI study. Brain 2003;126(Pt 11):2551–7.

23. Yasuda CL, Morita ME, Alessio A, et al. Relationship between environmental factors and gray matter atrophy in refractory MTL. Neurology 2010;74(13):1062–8.

24. Chang Q, Khare G, Dani V, et al. The disease progression of MeCP2 mutant mice is affected by the level of BDNF expression. Neuron 2006;49(3):341–8.

25. Croll SD, Suri C, Compton DL, et al. Brain-derived neurotrophic factor transgenic mice exhibit passive avoidance deficits, increased seizure severity and in vitro hyperexcitability in the hippocampus and entorhinal cortex. Neuroscience 1999;93(4):1491–506.

26. Louhivuori V, Arvio M, Soronen P, et al. The Val66Met polymorphism in the BDNF gene is associated with epilepsy in fragile X syndrome. Epilepsy Res 2009;85(1):114–7.

27. Nectoux J, Bahi-Buisson N, Guellec I, et al. The p.Val66Met polymorphism in the BDNF gene protects against early seizures in Rett syndrome. Neurology 2008;70(22 Pt 2):2145–51.

28. Ramasamy DP, Ramanathan M, Cox JL, et al. Effect of Met66 allele of the BDNF rs6265 SNP on regional gray matter volumes in patients with multiple sclerosis: a voxel-based morphometry study. Pathophysiology 2011;18(1):53–60.

29. Gonul AS, Kitis O, Eker MC, et al. Association of the brain-derived neurotrophic factor Val66Met polymorphism with hippocampus volumes in drug-free depressed patients. World J Biol Psychiatry 2011;12(2):110–8.

30. Kaufman J, Yang BZ, Douglas-Palumberi H, et al. Brain-derived neurotrophic factor-5-HTTLPR gene interactions and environmental modifiers of depression in children. Biol Psychiatry 2006;59(8):673–80.

31. Hunnerkopf R, Strobel A, Gutknecht L, et al. Interaction between BDNF Val66Met and dopamine transporter gene variation influences anxiety-related traits. Neuropsychopharmacology 2007;32(12):2592–60.

Supplementary material available online
Supplementary Table S1