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A Study on the Association between Patients with Post-Traumatic Stress Disorder in Li and Han Ethnic Groups in Hainan Province and DNA Methylation of Brain-Derived Neurotrophic Factor Genes

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Abstract

Objective: To explore the pathogenesis of PTSD in the brain-derived neurotrophic factor (BDNF) gene methylation of patients with posttraumatic stress disorder (Posttraumatic Stress Disorder, PTSD) in Hainan Province, the relationship between the influence of BDNF gene methylation and the influence of PTSD. **Methods:** A case-control study method was adopted, strictly in accordance with DSM-IV and PTSD diagnosis, and 150 Li PTSD patients matched with gender and age of 300 Han PTSD patients were selected as the research objects. The peripheral venous whole blood of the subjects was drawn, genomic DNA was extracted, modified with bisulfite, and directly sequenced to quantitatively detect the methylation status of the CpG island in the promoter region of brain-derived neurotrophic factor (BDNF). **Results:** The results showed that the methylation levels of CPGl, CPG2, CPG3, CPG4, CPG5, CPG6, CPG7, CPG9, CPGl2, CPGl3, CPGl4, CPGl5, CPGl6, CPGl7, and CPGl8 in THE BDNF promoter were significantly different between the HAN PTSD group and the Li PTSD group ($P < 0.001$). **Conclusion:** It is suggested that CPG methylation in the promoter region of BDNF gene is closely related to patients with PTSD. There is a statistical difference in the level of CpG methylation in the promoter region of BDNF gene in PTSD between Li and Han ethnic groups in Hainan Province. CpG methylation in the promoter region of BDNF gene may be used as a biomarker for the diagnosis of PTSD.

Keywords

Ethnicity, Brain-Derived Neurotrophic Factor, DNA Methylation, Post-Traumatic Stress Disorder, Epigenetics

1. Introduction

Posttraumatic Stress Disorder (PTSD) is the most typical type of stress mental illness [1]. It is mainly manifested as traumatic experience repeatedly intruding into consciousness or dreams, continuous increased alertness and avoidance of anything that can cause this trauma. In the scene of sex memory, the patient's psychological and social functions are seriously damaged. Epidemiological studies have shown that more than 1/2 of PTSD patients are often accompanied by comorbidities such as depression, other anxiety disorders, and drug abuse [2]. The suicide rate of PTSD patients is 6 times that of the general population, and it is one of the mental diseases that seriously impairs the ability to work. Among the many candidate genes for PTSD genetic susceptibility, BDNF is the second neurotrophic factor discovered after nerve growth factor (NGF) [3]. The precursor has 247 amino acid residues, and 119 are produced by post-translational processing [4]. A mature basic protein composed of amino acid residues has 3 pairs of disulfide bonds in the chain, which exist as dimers in the body. BDNF is the most abundant neurotrophic factor in the body. In the central nervous system, BDNF is mainly synthesized in neurons, transported from anterograde axoplasm to axon terminals, and after release; it mainly acts on target cells through the high-affinity receptor tyrosine protein kinase B (TrkB) [5]. In addition, BDNF can also be secreted by target cells acted on by neurons, and inversely nourish neurons. Immunohistochemistry confirmed that in the central nervous system, BDNF immunopositive neurons are widely distributed in the rat brain, especially in the hippocampus, thalamic striatum, and cortex. In the hippocampus, BDNF mRNA content is 20 - 30 times higher than NGF mRNA content [6]. In the peripheral nervous system, more BDNF was found in the distal part of the nerve stump after nerve injury. BDNF is also expressed in small amounts in ovaries, heart, lungs, and skeletal muscles other than nerve tissues.

The content of BDNF is high in human serum, and a large amount of BDNF in whole blood is located in platelets, but the source of BDNF in platelets is still unclear. Due to the high content of BDNF in the blood, research on the repair and regeneration effects of BDNF on the peripheral nervous system has been focused on. Based on the weak areas of the blood-brain barrier, such as the hypothalamus, BDNF may pass through the blood-brain barrier. The mutual inductance between peripheral blood and BDNF in the nerve center cannot be ruled out [7]. It is necessary to explore whether changes in peripheral blood BDNF content can be used as a direct indicator of PTSD. So how does BDNF gene polymorphism affect PTSD? At present, there is no relevant research on the

association with post-traumatic stress disorder, and there is no report on the association of BDNF gene polymorphism with gene frequency distribution between Li and Han ethnic groups and PTSD. Therefore, we focused on the relationship between BDNF and the gene frequency distribution and PTSD between the Li and Han ethnic groups in Hainan Province [8]. At the same time, we tested the BDNF gene methylation level in the Li and Han people diagnosed with PTSD in Hainan Province and the normal Li and Han controls. Analyze the relationship between BDNF gene methylation level and the incidence of PTSD, and whether there are differences in BDNF gene methylation level between the Li and Han ethnic groups in Hainan Province, and conduct more in-depth research on the susceptibility genes of Li ethnic group.

2. Materials and Methods

2.1. Research Objects

From August 2018 to March 2019, 300 patients with sporadic post-traumatic stress disorder of the Han nationality in Hainan province were selected from an epidemiological survey in Hainan Province. The control group included 150 patients with post-traumatic stress disorder of Li nationality in Hainan province. All patients underwent a clinical examination, were tested with the DIAGNOSTIC Scale for PTSD, met the diagnostic criteria for the Diagnostic Statistical Manual of Mental Disorders (DSM-V), and were diagnosed by two psychiatrists (at least two of whom were senior psychiatrists). Exclusion criteria 1) Physical diseases that may cause impairment of cognitive function; 2) Had taken drugs affecting cognitive function in the past three months; 3) Obvious mental retardation. This study was approved by the Ethics Committee of Hainan Provincial People's Hospital, and all subjects signed informed consent.

2.2. Research Methods

2.2.1. Specimen Collection and Preservation

After completing the epidemiological investigation and test scale evaluation, all the research subjects will collect 5 - 10 ml blood from the anterior cubital vein, EDTA anticoagulation, and send it to the Central Laboratory of Hainan Provincial People's Hospital at −20˚C Frozen.

2.2.2. DNA Extraction

Use the whole blood genomic DNA extraction kit (OMEGA, USA) to extract genomic DNA with a spin column method. The concentration and purity detection meet the PCR amplification requirements, and the DNA methylation research is carried out.

2.2.3. Experimental Method

The first IV BDNF gene launched the determination of DNA methylation:

1) CpGIsl and prediction: CpGplot is applied online.

2) Genomic DNA was treated with bisulfite and BDNF IV promoter fragment

acquisition: PCR amplified the DNA fragment containing BDNF 1V promoter CpG island with a length of 297 bp (upstream primer CCCTGGAACGGAACTCTTCT; Downstream primers (ATTGCATGGCGGAGGTAATA).

3) MS-snuPE elongation reaction was carried out on 19 CpG sites in the CpG island by SNaPshot Multiplex Kit (Applied Biosystems Company) (the primers used were as follows:

CCTGGAACGGAACTCTTCTAATAAAAGATGTATCATTTTAAATGCGC TGAATTTTGATTCTGTAATTTCGGCACTAGAGTGTCTATTTCGAGGCAG CGGAGGTATCATATGACAGCGCACGCACGTCAGGCACCGTGGAGCTCC CACCCACTTTCCCATTCACCGCGGAGAGGGCTGCCTCGCTGCCGCTCC CCCCGGCGAACTAGCATGAAATCTCCCTGCCTCTGCCGAGATCAAAT GAGCTTCTCGCTGATGGGGTGCGAGATTACCTCCGCCATGCAAT. Note: The underlined parts are CpG dinucleotide sites. The reaction conditions were as follows: template DNA 4 L, upstream and downstream primers 0.8 L each, Mixl0ul, and sterilized deionized water were added to the total volume of 20 L.

4) Thermal cycling steps: 950C pre-denaturation for 3 min, 950C denaturation for 30 s, 58.50C annealing for 1 min, 720C extension for 45 s, cycling for 45 times, 720C final extension for 5 min, and termination at 40C. 15 L PCR product was taken and sent to sequencing company for sequencing (Shanghai Genomic).

2.2.4. Data Processing

Epidata3.1 was used for data entry, and SPSS19.0 was used for statistical analysis. The measurement data is expressed by and the counting data is expressed by frequency and percentage. For comparison of inter-group differences, if the measurement data of small samples meet the requirements of normality and homogeneity of variance, t-test is used for comparison of inter-group differences, and analysis of variance is used for comparison of multi-group differences. If normality or homogeneity of variance is not satisfied, use Rank sum test; t/z/F test for large sample measurement data. The chi-square test was used to compare the differences between the enumeration data groups. The test level a is 0.05, $P \leq 0.05$, the difference is considered statistically significant.

3. Results

1) Demographic Characteristics Analysis

The average age of PTSD group was 39.20 ± 6.30 years, 182 males, 118 females, 257 married and 43 unmarried. In the Li people group, there were 150 participants, aged 26 to 64, with an average age of 38.75 + 9.14 years, 79 males, 71 females, 132 married and 18 unmarried. There were no significant differences in age (t = −0.611, P = −0.5416), sex composition (2 = 0.3721, P = 0.5412), marital status (2 = 1.623, P = 0.203) and education (year) (12.65 + 5.72 vsl 3.14 + 4.77, t = 0.4848, P = −0.6288).

2) Comparison of BDNF promoter CPG methylation between PTSD group and normal control group. The t test was used to compare the methylation of

BDNF promoter CPG in THE TSD group and the control group. The results showed that the methylation levels of CPGl, CPG2, CPG3, CPG4, CPG5, CPG6, CPG7, CPG9, CPGl2, CPGl3, CPGl5, CPGl6, CPGl7, CPGl8 in the BDNF promoter were significantly different between the HAN PTSD group and the Li PTSD group ($P < 0.001$). It suggested that CPG methylation in BDNF gene promoter region was closely related to PTSD in Li and Han nationality patients in Hainan province. CpG methylation in the BDNF gene promoter region may be a biomarker for the diagnosis of PTSD (**Table 1**).

4. Discussion

With PTSD often suffer from depression, brain derived neurotrophic factor (BDNF) BDNF regulate pain and fear, a lack of will lead to posttraumatic stress disorder, its survival, differentiation, growth and development of neurons play an important role, and to prevent neuronal damage death, and improve the pathological state of neurons, and promote the regeneration of damaged neurons and the differentiation of biological effects [9]. As previously described, a gene-gene interaction between the DRD2 TaqlA locus (RSL800497) and the BDNF Val66 allele Val66Met (RS6265) predicts PTSD severity [10]. Although

Table 1. Comparison of methylation degree between groups ($\bar{x} \pm s$).

PromoterHan CPG	PTSD group (n = 300)	Li PTSD group (n = 150)	*t*	*P*
CPG1	3.61 ± 0.29	8.39 ± 0.99	77.329	<0.001
CPG2	3.34 ± 1.14	14.49 ± 2.59	63.344	<0.001
CPG3	1.51 ± 0.41	4.59 ± 0.89	50.254	<0.001
CPG4	1.74 ± 0.57	3.89 ± 0.89	31.023	<0.001
CPG5	0.79 ± 0.57	5.69 ± 2.09	37.922	<0.001
CPG6	6.04 ± 1.02	14.49 ± 1.69	65.897	<0.001
CPG7	10.56 ± 1.69	14.71 ± 1.71	24.460	<0.001
CPG8	16.34 ± 3.89	15.79 ± 1.09	1.698	0.090
CPG9	4.19 ± 4.79	28.49 ± 19.29	20.606	<0.001
CPG10	10.24 ± 2.17	9.89 ± 1.09	1.861	0.063
CPG11	62.01 ± 5.05	61.89 ± 4.59	0.244	0.807
CPG12	5.60 ± 1.05	12.31 ± 1.79	49.993	<0.001
CPG13	4.14 ± 1.09	59.69 ± 4.89	187.838	<0.001
CPG14	5.44 ± 0.77	6.29 ± 0.59	11.885	<0.001
CPG15	3.69 ± 0.69	62.40 ± 6.50	154.878	<0.001
CPG16	8.04 ± 1.74	74.29 ± 50.89	22.547	<0.001
CPG17	3.19 ± 0.77	32.40 ± 13.19	38.270	<0.001
CPG18	1.89 ± 0.47	2.29 ± 0.29	9.551	<0.001

animal experiments, neurobiochemical, neurophysiological and endocrine studies have suggested that BDNF may be associated with the development of PTSD, no association has been found between three SNPS of BDNF (G-712A, C270T, Val66Met) and PTSD [11].

DNA methyltransferase blockers can improve depression-like behaviors while causing hypomethylation [12]. These findings suggest that abnormal methylation in the BDNF promoter region may be involved in the onset of depression, and methylation status may become a biological marker for the diagnosis of depression and the prediction of drug treatment effect, providing a new direction for the development of antidepressant drugs. PTSD is a multigene complex disorder. Epigenetic mechanisms have been proposed in recent years as a new explanation for the pathogenesis of various human diseases, including mental disorders, especially PTSD, and have made some progress. With the rapid development of epigenetics and DNA methylation detection technology, it is possible for humans to study the pathogenesis of PTSD from the perspective of DNA methylation and search for disease-causing genes [13]. The study of CpG methylation levels in BDNF gene promoter region suggests that epigenetic mechanisms, particularly DNA methylation, play an important role in the pathogenesis and treatment of PTSD. A comprehensive study of epigenetic changes in the pathogenesis of PTSD would improve our understanding of the pathogenesis of PTSD and could serve as a biomarker for the diagnosis of PTSD, providing scientific basis for the prevention of the disease and the development of new anti-PTSD drugs. Although studies have suggested a possible link between DNA methylation and THE pathogenesis of PTSD, the findings are controversial, possibly due to inadequate techniques, limitations or the complexity of metabolic processes. With the increasing social pressure in modern life, PTSD has received more and more social attention. With the application of biochemistry, genetics and human genome engineering in the etiology of PTSD, it is believed that new breakthroughs will be made in the study of its pathogenesis and new research directions will be provided [14]. In recent years, a large number of studies on the regulation of BDNF gene expression have found that epigenetic modification has a significant impact on the expression of BDNF gene. Fuchikami [15] to 18 of 20 patients with major depression and healthy controls for DNA methylation research shows that the depression of BDNF gene methylation and 29 normal methylation exist obvious exceptions, found in the BDNF gene exon promoter CpG island area has 2 CpG loci associated with depression, the results show that the classification, based on the DNA methylation of the CpG of BDNF gene may be a valuable diagnostic biomarker of depression. At present, studies have found that BDNF levels in the hippocampus and prefrontal cortex are significantly reduced in patients with depression [16]. Studies have found that although the levels of BDNF in brain tissue and blood are orders of magnitude different, there is a positive correlation between BDNF in serum and cerebral cortex, so serum BDNF can indirectly reflect the level of BDNF in the brain. DNA methylation

also regulates the expression of BDNF gene in hippocampus in the process of cognitive memory in rats. Many studies have shown that the expression of BDNF changes under stress. For example, rats with post-traumatic stress disorder (PTSD) show down-regulation of BDNF gene expression in the hippocampus. There is an interaction between BDNF and serotonin neurons, and they can regulate each other to maintain a certain dynamic balance. It is now clear that the relationship between the serotonin nervous system and depression. Many studies have shown that BDNF can be linked to depression through the serotonin nervous system. The levels of BDNF in the peripheral serum and plasma of patients with depression are reduced. For example, Karege *et al*. [17] found that the levels of BDNF in the serum and plasma of depression patients are lower than those of healthy people. Research on DNA methylation of brain-derived neurotrophic factor in MDD (Major Depressive Disorder): Studies have found that there is a decrease in brain-derived neurotrophic factor (BDNF) levels in MDD patients. The methylation level of BDNF exon 1 region was significantly increased, and MDD patients with elevated methylation were found to be significantly correlated with antidepressant treatment. The methylation ratio of MDD patients receiving antidepressant treatment was (4.1370, 28%; n = 140) was significantly higher than the MDD patient group (1.7270, 28%; n = 25; P = 0.0019) and the healthy control group (2.0170, 13%; n = 278; P = 0.0001) without antidepressant treatment); But it is not related to clinical symptoms, such as the severity of depression [18]. MDD patients with suicidal ideation and suicidal behavior also found elevated levels of methylation in the BDNF promoter region in the brain, similarly to MDD patients with suicidal ideation who were ineffective in antidepressant treatment [19]. DNA methyltransferase blockers can improve depression-like behaviors while causing hypomethylation [20]. These findings suggest that abnormal methylation in the promoter region of BDNF may be involved in the pathogenesis of depression. The methylation status may become a biological marker for the diagnosis of depression and the prediction of the effect of drug treatment, providing a new direction for the development of antidepressant drugs. PTSD is a multi-gene complex disease. Epigenetic mechanism is a new explanation for various human diseases, including mental diseases, especially the pathogenesis of PTSD, and has made certain progress. With the rapid development of epigenetics and DNA methylation detection technology, it has become possible for humans to study the pathogenesis of PTSD from the perspective of DNA methylation and to find therapeutic genes [21]. To study the level of CpG methylation in the promoter region of BDNF gene, it can be speculated that epigenetic mechanisms, especially DNA methylation, play an important role in the pathogenesis and treatment of PTSD. A comprehensive study of the epigenetic changes in the entire process of the onset of PTSD is more conducive to improving our knowledge and understanding of the onset of PTSD. It can be used as a biomarker for the diagnosis of PTSD, providing for the prevention of disease and the development of new anti-PTSD drugs. Although

studies have shown that DNA methylation may be related to the pathogenesis of PTSD, there is still controversy about its conclusions, which may be related to the insufficiency of related technologies, limitations or the complexity of the metabolic process. In the modern life with increasing social pressure, PTSD has received more and more attention from society. With the application of biochemistry, genetics, human genome engineering, etc. in the etiology of PTSD, it is believed that there will be new research on its pathogenesis and provide new research directions for it [22]. BDNF is PTSD candidate genes, but may be the object of study of age, illness, confounding factors such as nationality, course, and the influence of the number of cases less, not a detailed research and analysis of the [23], tend to make the correlation research conclusion persuasive, sometimes the lack of comparability between different results, even the conclusion on the contrary, its influence factors is worth careful consideration in PTSD correlation studies. Therefore, we further investigated the correlation between BDNF gene methylation and post-traumatic stress disorder, aiming to explore the role of BDNF in the occurrence, development and prognosis of PTSD among ethnic groups, and to provide evidence for the inconsistent results of the current association. The results show that the methylation levels of CPGl, CPG2, CPG3, CPG4, CPG5, CPG6, CPG7, CPG9, CPGl2, CPGl3, CPGl4, CPGl5, CPGl6, CPGl7, and CPGl8 in BDNF promoter were significantly different between the HAN PTSD group and the Li PTSD group ($P < 0.001$). It suggested that CPG methylation in BDNF gene promoter region was closely related to PTSD in Li and Han nationality patients in Hainan province. CpG methylation in BDNF gene promoter region may be a biomarker for the diagnosis of PTSD. In summary, based on the PTSD epidemiological investigation of the Li and Han ethnic groups in Hainan Province, we adopted the method of case-control association analysis, using polymerase chain reaction (PCR) and polyacrylamide gel electrophoresis (PAGE) technology to detect the methylation level of BDNF gene in Li and Han patients with PTSD diagnosed in Hainan Province and normal Li and Han controls. Analyze the relationship between the level of BDNF gene methylation and the incidence of PTSD, and whether there are differences in the level of BDNF gene methylation between the Li and Han ethnic groups in Hainan Province, explore the susceptibility factors of PTSD in the Li ethnic group in Hainan Province, and the incidence of this disease There will be a deeper understanding of the mechanism, which will provide scientific basis for the clinical diagnosis of PTSD patients, provide basic data for the study of the pathogenesis of PTSD and its prevention and treatment, and provide genetic data for anthropology, forensic identification and PTSD association studies.

5. Conclusion

This study is more meaningful for larger samples. In addition, population differences, especially the degree of linkage disequilibrium among other potential variants in the tested genes, may have contributed to this inconsistent discovery.

However, these findings have opened up a new avenue for research and may help to understand the relationship between genetic polymorphism and post-traumatic stress disorder.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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