

The Effect of BDNF Val66met Polymorphism in Mood Disorders

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Abstract

Background: Depression is one of the most common mood disorder that affects the quality of life, individual performance, psychological activities and interpersonal communications; and in severe forms, it causes disability and suicide. Using advanced biology techniques, can be identified susceptible individuals with a disorder or disease, and the predisposing factors are avoided or reduced, or be neutral. The aim of this study was to determine the role of biological factors (gene candidate BDNF val66met) affecting the prevalence of mood disorders, including depression. **Materials and Methods:** This was a cross-sectional study and in total, 73 patients with mood disorders, and 55 non-depressed subjects were evaluated. After collecting blood samples and DNA extracted from lymphocytes, a polymorphism of the gene were analyzed by ARMS-PCR technique. **Results:** The results showed that depression is no significant relationship with BDNF val66met polymorphism ($p = 0.005$). **Conclusion:** Although genetic predisposition, a person can be prone to mood disorders, but environmental factors can play a major role in modulating or strengthen their risk. Therefore, the study of etiology and risk factors

of depression, helps to mental health system staff, to apply the appropriate treatment and care measures based on the needs of the community.

Keywords: mood disorders, depression, BDNF val66met polymorphism

Introduction

Mood disorders are the most common of mental disorders, including major depressive disorder and bipolar disorder. Suicide is a major medical problem. There are the suicidal thoughts in mood disorders that reported 79% with bipolar depression, its rate for bipolar disorder was 15 times of the general population [1]. Depression affects the quality of life, individual performance, psychological activities and interpersonal communications; and in severe forms, it causes disability and suicide. The etiology of depression is multifactorial, related to biological, psychological, and social mechanisms [2]. In the present study to identify genetic factors associated with mood disorders, we examined Brain-derived neurotrophic factor gene.

BDNF is a candidate gene, a biomarker and a genetic precipitating factor [3] for depressive disorders. BDNF is an important member of the neurotrophins family [2, 4], promotes the survival and differentiation of serotonergic neurons, and maintenance of neurons in the peripheral and central nervous systems, and has beneficial effects on promoting synaptic plasticity [5-8]. The BDNF val66met polymorphism (rs6265), which consists of a single-nucleotide polymorphism G/A substitution at codon 66(val66met), was analyzed as described previously, val/val versus val/met or met/met. The met allele is less active than the val allele [9]. Some studies reported the met allele results in a reduction of activity-dependent BDNF secretion and a loss of BDNF protein at the synapse due to abnormal trafficking pattern [10, 11].

Gene-environment interactions studies indicated that genetic influences affecting individual differences in response to the environment [12]; and also demonstrated differential susceptibility, and promoted the understanding of the influence genetic variation. Social factors such as, severe social deprivation are associated with a range of behavioral problems. Hence, gene-environment interactions contribute to the etiology of mood disorders [13]. Duruy et al. indicated the cumulative genetic plasticity might be associated with the greatest sensitivity to the caregiving environment [7], and exposed to early severe social deprivation supporting a differential susceptibility model in developmental psychopathology. Some studies reported that the BDNF val66met polymorphism are no significantly associated with hippocampal volume [14] or function in a geriatric population [15]. Earlier studies indicated the significant relationship between mood disorders and the stressful life events [13]. Some studies demonstrated the positive association results; Kim et al. were found significant interactions of stressful life events with BDNF and SLC6A4 genotypes [16]. In a meta-analytic study, Verhangen et al. reported the negative association results that BDNF val66met was not significantly associated with depression, though a significant effect was found in men [17].

We examined functional polymorphisms of BDNF as predictors of mood disorders. We hypothesized met allele of BDNF may be associated with more mood disorders.

Methods and Materials

The tetra-primer amplification refractory mutation system–polymerase chain (ARMS–PCR) reaction is a simple and economical method to genotype single-nucleotide polymorphisms (SNPs). It uses four primers in a single PCR and is followed just by gel electrophoresis [18].

Samples. The initial sample (N=73) were recruited from patients with mood disorders in chronic diseases treatment Centers under the State Welfare Organization were hospitalized; and healthy volunteers (N=55) were recruited as control group. In total, 128 participants entered the study with consent. Mean age was 37.8±12.41years, 66% were female and 37% were male. Of 73 patients, 48 (66%) were BD and 25 (34%) were MDD.

Genotyping. The BDNF Val66Met polymorphism, including a single-nucleotide polymorphism (SNP) G/A substitution at codon 66 (the rs6265 SNP), were analyzed by using DNA isolated from the blood. After collecting blood samples, DNA was extracted lymphocytes and the BDNF gene polymorphisms amplified by ARMS-PCR technique. The polymerase chain reaction (PCR) was performed with a final volume of 25 µl, containing 1.5 µl of forward inner primer & reverse inner primer, 1.0 µl of each forward outer primer & reverse outer primer; 1.0 µl DNA; YTA Mastermix 10 µl (Cat No:YT1591; YTA Taq polymerase (5U/µl) in separated vial). The polymerase chain reaction temperature (PCR) for gene BDNF 30 times cycle include: 5 minutes at 95°C, denaturation, at 95 ° C for 30 seconds, annealing at 61 ° C for 30 seconds, elongation at 72 ° C for 30 seconds, the final amplification at this temperature (72 ° C) for 5 minutes. The amplified product from each sample was run on the 1.5% agarose gel, and the pictures was taken by using Gel Doc system. The resulting bands can be included 389 bp or 239 bp or 200 bp, were analyzed. The ARMS-PCR result detected on the agarose gel picture (wild-type and mutant).

Table 1. Tetra primers used in BDNF *val66met* (rs6265) polymorphism assay

Forward inner primer (A allele): 298 TGGCTGACACTTTTCGAACCCA 318	Melting temperature
Reverse inner primer (G allele): 346 TGGTCCTCATCCAACAGCTCTTCTATAAC 318	66
Forward outer primer (5' - 3'): 108 CAGGTGAGAAGAGTGATGACCATCCT 133	66
Reverse outer primer (5' - 3'): 496 CTCATGGACATGTTTGCAGCATCTAG 471	66
Product size for A allele: 200	
Product size for G allele: 239	
Product size of two outer primers:389	

Statistical analysis. Data collected were analyzed by SPSS statistical software, version 22 windows 10, using Chi-square test and Fisher's exact test and odd's ratio.

Results

In this study, we used the ARMS-PCR to detect the wild-type and mutant in samples, and were compared three types of genotype frequency of gene BDNF. The genotyping included val/val, val/met, and met/met were correctly detected as shown in the gel picture presented in Fig.1.

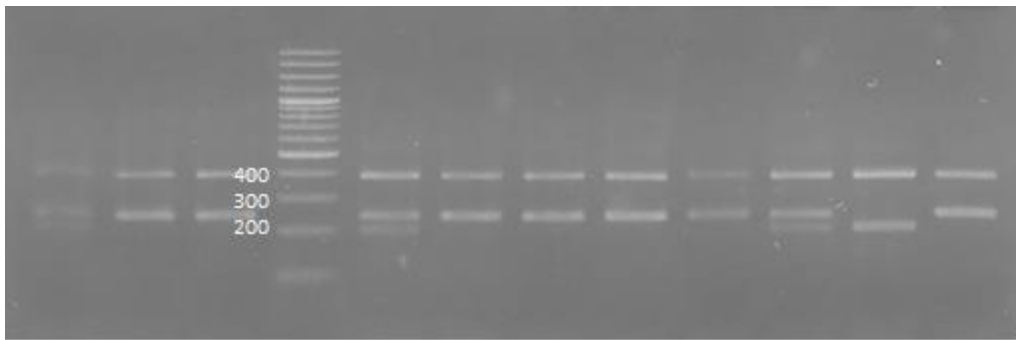


Figure 1. Schematic of the brain derived neurotrophic factor (BDNF) gene

Only one band was expected for non-depressed participants the wild-type variant, while two bands of the same molecular size were expected for patients with mood disorders the wild-type and mutant. The results showed that 87.5% of non-depressed participants and patients were homozygous alleles; and 11.7% were heterozygous genotype in both groups. (Table 2)

Table 2. Allelic distributions of polymorphism in BDNF gene in non-depressed and patients.

BDNF gene genotype frequency	Frequency	Percent
Val/Val	112	87.5
Val/Met	15	11.7
Met/Met	1	0.8
Total	128	100

Chi-Square test results showed that there was significant differences between genotype val/val, and other genotypes in all groups ($P=0.001$); and by comparing two groups of patients and non-depressed participations, there was no significant relationship between the BDNF gene polymorphism and risk of disease ($p=0.44$). Odds Ratio between the disease and the non-depressed participants and patients genotyping showed in Table 3.

Table 3. Odd's ratio of BDNF gene genotype

Depression status	val/val genotype	Valine allele	val/met genotype	met/met genotype	Methionine allele
OR patient / Non-depressed	0.56	1.04	1.58	1.01	1.04

Results calculated from the odd's ratio showed that there was no risk of the disease with genotype val/val and val/met and met/met. Thus, polymorphisms of this gene had no effect on the disease. In total, 85% of men and 88% of women entering the study, regardless of disease status had a version homozygous val/val of the BDNF gene. The analyzed results found no significant correlation between the incidence of this genotype and gender ($p=0.53$). Also 14.9% of men and 9.9% of women with heterozygous genotype (val/met), regardless of disease status, had a genotype val/met of the BDNF gene, that this was not a significant relationship between gender and the incidence of this genotype ($p = 0.39$). (Table 4)

Table 4. Relationship gender and frequency of BDNF gene polymorphism regardless of the disease

BDNF gene genotype	Male	frequency percentage	Woman	frequency percentage	Chi-square test	Fisher's exact test
val/val	40	85%	72	89%	0.53	0.58
No val/val	7	15%	9	11%		
val/met	7	14.9%	8	9.9%	0.39	0.4
No val/met	40	85%	73	90%		
met/met	0	0%	1	0.1%	0.44	1.0
No met/met	47	100%	80	99%		

Discussion

An important category of mental disorders are mood disorders that have a high prevalence and variety, these disorders are life-threatening (due to suicide), and a leading cause of death are in the world [19]. By the frequent recurrence can be considered very high burden to their patients [20], and annually, medical and psychological costs imposed on society and their families [21].

The main findings of this research did not show a significant correlation between the different genotypes of BDNF, gender and the type of depression ($p > 0.05$). However, gene-gene interactions, such as the direct influence of gene may be observed only in the presence of environmental stressors. Wang et al (2014) found no significant relationship between depression and rs6265 polymorphism in hemodialysis patients to be pathogenic. Brain-derived neurotrophic factor (BDNF) is an important member of the neurotrophin family and is able to pass from the blood. BDNF promotes survival, differentiation and protection of neurons in the central nervous system [2].

A number of studies support an important role for BDNF, Kim et al. (2012) indicated that BDNF val66met polymorphism was a factor risk of depression in patients with medical illness, such as patients who had suffered a heart attack and breast cancer patients after mastectomy. While the results of this study showed that there was no significant relationship between depression and the rs6265 polymorphism. Etiology of depression is multifactorial and it seems as a mechanism of social, biological and physiological. So, with regard to biological mechanisms, elements of inflammation and neurotransmitters, nutritional factors, as well as genetic predisposition, all of whom are potentially pathogenic [2].

The reasons can be cited for this paradoxical results:

One of the main reasons for inconsistent results that can be obtained from biological etiology, that is, the social environment factors, as research Kaufman et al (2004) reported a positive interventions such as social protection of children who are genetically prone to maladaptive behavior, the risk of depression makes more mild [22].

One of the other reasons that can be explained that the control group of this study were affluent society, but social class are not included in the research. Often the environmental factors that trigger or worsen the disorder are less observed in affluent society. In other words, the environment is often more favorable. And if the person with the disease or disorder, further benefited medical facilities, welfare and family-social support.

The effect of individual's environment is a dose-response effect, and this could lead to different results in different populations [23]. So it is very important that the population be studied. If a traumatized sample is investigated, certainly would have different results from a sample of less traumatized. As a result, environmental conditions and the type of population studied, lead to different results.

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