

Association of *BDNF* Gene (rs6265/G196A) Polymorphism with Depression

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ABSTRACT

Depression affects an individual's feelings, thoughts, and behavior. It is known as the most common mental illness worldwide with complex origin. The risk factors for depression include both genetic as well as environmental factors. Depression is affecting more than 300 million individuals globally and is categorized as a major cause of the global burden of disease. Several studies demonstrate the involvement of the *brain-derived neurotrophic factor (BDNF)* gene in the etiology of depressive disorder. This study was designed to assess the association of (rs6265/G196A) polymorphism of the *BDNF* gene in the pathogenesis of depression. The cross-sectional study was conducted consisting of 357 samples from Rawalpindi, Pakistan. Depression was determined through questionnaire, using DSM-IV (Diagnostic and Statistical Manual for Mental Disorders-Version IV). DNA was extracted from the blood samples of study participants. The conventional polymerase chain reaction was performed to amplify the *BDNF* gene and to detect the frequency of rs6265/ G196A SNP in the samples of subjects under study. Statistical analysis was done using Pearson's Chi-Squared test. It was observed that the homozygous GG genotype is more prevalent in study subjects than the homozygous AA or heterozygous AG genotypes. However, depression is likely to be more prevalent in AA genotype i.e., 37.8%, less prevalent in AG genotype i.e., 34.0%, and least prevalent in GG genotype i.e., 28.2%. This data shows the A allele of the *BDNF* gene to be more associated with depression than the G allele, suggesting this polymorphism to be a somewhat potential target for anti-depressants.

Keywords: Depression, *BDNF*, Genetic, Polymorphism, PCR, Pathogenesis

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INTRODUCTION

Depression is one of the most common mental illnesses worldwide, with complex origins, affecting an individual's feelings, thoughts and behavior. It results from an interaction of both genetic and environmental factors (Yang et al.,

2010).) The symptoms are characterized by having a persistent form of sadness and loss of interest in once pleasurable activities and several other physical and cognitive symptoms such as inability to concentrate, changes in sleep and eating habits, physical inactivity and suicidal thoughts. Currently, depression is a

major cause to the global burden of disease affecting more than 300 million individuals globally (Aldogachi et al., 2019). A number of studies demonstrate the involvement of *brain-derived neurotrophic factor (BDNF)* gene in the etiology of depressive disorder.

The *BDNF* is one of the most widely expressed neurotrophins in the mammalian brain that regulates the neural development and plasticity. The *BDNF* gene (MIM: 113505) is located at 11p14.1 and spans 70 kb, containing 11 exons and encoding a small dimeric protein of size 27kD (Sheikh et al, 2010). The highest level of *BDNF* is found in the hippocampus and the cerebral cortex, which are regions of the brain that are involved in many neuropsychiatric diseases. *BDNF* can cross the blood brain barrier and it is critical to the growth, survival, and differentiation of the developing nervous system. According to various researches, this neurotrophin is also involved in hippocampal long-term potentiation, which is related to learning and memory (Tsai et al., 2018) and regulating eating behavior and energy homeostasis (Huiping et al., 2006). The *BDNF* gene contains various single-nucleotide polymorphisms such as, rs6265, rs12273539, rs11030103, rs28722151, rs41282918, rs11030101, rs1048218, rs1048220, rs1048221, rs8192466, rs56164415 and rs139352447 that are associated with certain psychiatric disorders in different populations (Gunstad et al., 2006; Narayanan et al., 2016). The rs6265 is a widely studied functional single-nucleotide polymorphism (rs6265/G196A), which results in a valine to methionine substitution at codon position 66 (val66met) of the 5' pro region of *BDNF* protein (Shen et al., 2018). This nonsynonymous G to A single-nucleotide polymorphism (SNP) exists at position 196 of exon 2 (rs6265), affecting the intracellular packaging of pro-*BDNF*, its axonal transport and, in turn, activity-dependent secretion of *BDNF* at the synapse (Sheikh et al., 2010). The rs6265 doesn't necessarily change the intrinsic biological activity of the mature BDNF protein,

but it can lead to improper protein folding and a reduced binding of the mature *BDNF* to its receptor TrkB, causing impairments in hippocampal function (Narayanan et al., 2016). This polymorphism has been widely implicated in many psychiatric disorders including obsessive-compulsive disorder, schizophrenia, psychosis, major depression and anxiety, as more than 1,100 genetic studies have investigated this polymorphism in the past 15 years.

A number of studies indicate the involvement of the rs6265 polymorphism of *BDNF* gene in the etiology of depressive disorder. It has been found that individuals homozygous for the Val allele have an increased risk of depression in some population (Notaras et. al., 2015). In most populations, the Met allele is associated with less *BDNF* activity and lower serum levels and appears to be associated with major depression, memory impairments, reduced hippocampal activity, and anxiety-related behaviors in animal models (Ribeiro et.al., 2009). Although many reports have demonstrated the possible genetic effects of *BDNF* polymorphism rs6265 on depressive disorder, there is variation of association of disease due to variations in population genetics. The inconsistent findings of *BDNF* Val66Met genetic studies may also result from many other factors such as age, sex, environmental factors, ethnicity, genetic model used for analysis, and gene-gene interaction (Yousset et al., 2018). These findings suggest that this *BDNF* polymorphism has pleiotropic effects on multiple phenotypes; thus, this polymorphism imparts separate advantageous traits and disadvantageous traits in the same organism.

METHODOLOGY

Study Design

The cross-sectional research was conducted in the Rawalpindi, Pakistan region. The reason behind selection of Rawalpindi was that it is one of the regions with most ethnic diversity in

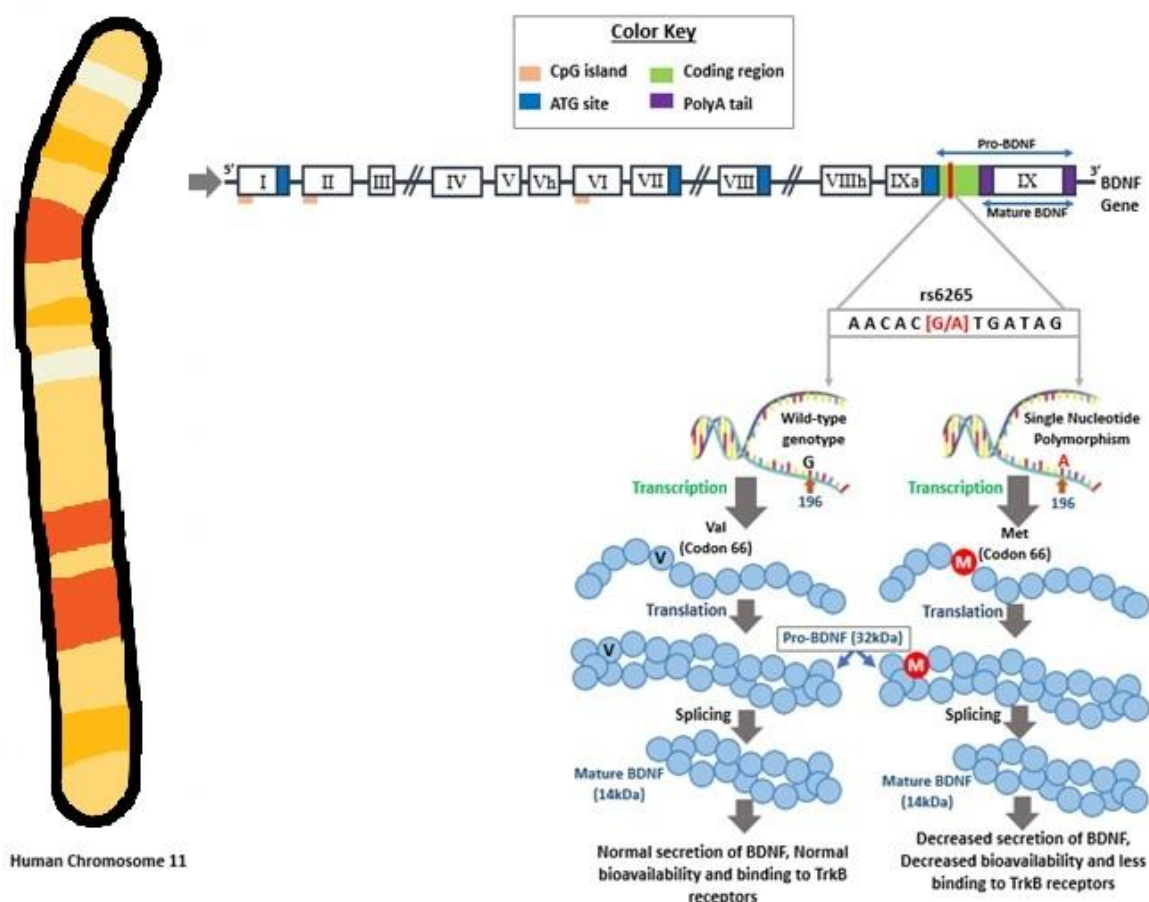


Figure 1: The location of rs6265 on *BDNF* gene and resultant changes in expression of *BDNF* protein

Pakistan as people from all over the Pakistan are resident here.

Sample Size

For the calculation of regional prevalence and correlation, the formula used to calculate sample size was:

$$\text{Sample Size} = \frac{Z^2 * p (1-p) / e^2}{Z^2 * p (1-p) / e^2 N}$$

(Wang et al., 2010) .

In the above formula, N indicates the size of population, e indicates margin error and Z is the score of standard deviations. A total of 318 samples were collected via a blood camp arranged in Kahuta region, Rawalpindi. Margin error was 5% and z- score was 1.96.

Inclusion/exclusion Criteria

Healthy individuals with age ≥ 15 years were included in the research data from Rawalpindi region. The pregnant women and patients with injuries or infections were excluded from the study.

Ethics Statement

This research study was ethically approved from bioethical review committees of Department of Bioinformatics and Biosciences, Capital university of Science and Technology. Questionnaire was designed by reviewing literature and consulting hospital laboratory. All individuals signed informed consent prior to their enrollment in the study stating that their data could be used in future medical research.

Clinical Data

Anthropometric measurements are used to assess the size, shape, composition and other measurements of the human body. The commonly taken anthropometric measurements are blood pressure, weight, height, abdominal circumference, waist circumference and skinfold measurements. BMI is body mass calculation in which weight of a person is divided by square of height. It was calculated by using the formula kg/m^2 and on the basis of BMI individuals were categorized into different groups like underweight, normal weight, overweight, obese class 1, obese class 2, obese class 3 as per WHO classification of obesity for Asian population (Gujral et al., 2014; Nishida et al., 2004).

Biochemical Analysis

Biochemical tests were performed on blood samples to calculate blood glucose and cholesterol levels. Blood glucose level was measured by Lancing device. Cholesterol level was measured in order to check the total lipid profile of individuals. Lipid tests were then performed on blood samples to identify the low-density lipoproteins, high-density lipoproteins and triglycerides profile in the body. Biochemical and physical parameters were finalized in consultation with expert

physicians from Tehsil Headquarter Hospital Kahuta, Rawalpindi.

Determination of Depression

The determination of depression was done through questionnaires, using DSM-IV (Diagnostic and Statistical Manual for Mental Disorders-Version IV). Subjects who were not depressed according to DSM-IV criteria were taken as control.

DNA Extraction and SNP Detection

DNA extraction protocol optimized for extracting DNA from blood samples was a salting out method. Gel electrophoresis was performed to visualize the quality of extracted DNA. DNA bands were then visualized under UV illuminator. Conventional PCR (Polymerase Chain Reaction) was performed followed by RFLP (Restriction Fragment Length Polymorphism) to amplify *BDNF* gene in order to detect the frequency of rs6265 SNP in the samples of subjects under study. A pair of forward and reverse primers was designed using Primer 3 software (version 0.4.0) for amplification of *BDNF* gene region. The amplified fragment of 243 bp was digested with restriction enzyme Afl III (restriction site ACRYGT) which cleaves A variant into two fragments of length 168 bp and 75 bp

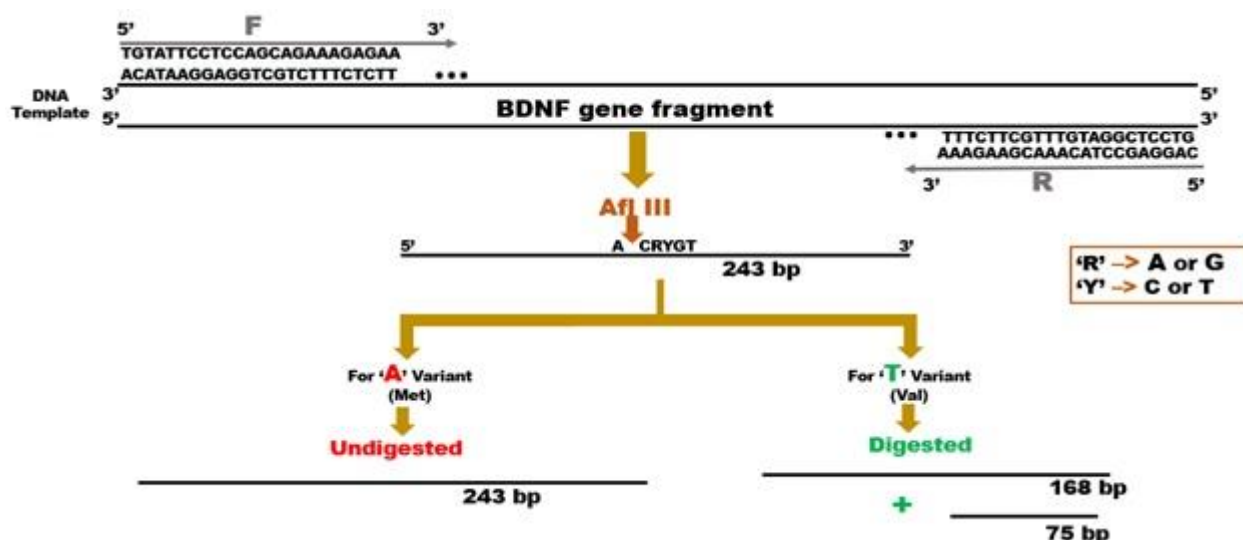


Figure 2: The mechanism of action of PCR-RFLP Assay of *BDNF* gene with Afl III enzyme.

Statistical Analysis

Statistical analysis was done using Pearson’s Chi Squared test. Pearson’s chi-square test is performed to check the independence of distributed traits in a certain population. Each trait is treated like a variable and then the degree of relatability of two or more variables is analyzed. This statistical test checks whether null hypothesis applies to a certain population or not where null hypothesis suggests that two variables are independent in some population. The asymptotic significance or probability value (p-value) is determined for each test. The significance of p-value is more if it is <0.05 and can be used to reject the null hypothesis.

RESULTS AND DISCUSSION

The data was collected via blood camp in Kahuta region which is significant being a

multi-ethnic region. A total of 357 subjects (age range from 18 to 88 years) were recruited of which 176 were males and 181 were females with an overall mean age and standard deviation of 41.63±17.404 years. Age groups were divided into four categories according to standard classification of age and the range of age of samples (Williams et al., 2014). The collected data was used for statistical analysis of anthropometric measurements, biochemical characteristics, disease frequencies and genotype association with depression. The statistical analysis was performed through IBM Statistics SPSS 25.0 software. The anthropometric and biochemical characteristics of samples have been shown below in Table 1. The probability value or p value and odds ratio has also been shown by Pearson’s chi-square test with individual variables to describe the asymptotic significance of variable data used in this research.

Table 1: Analysis of anthropometric and biochemical characteristics of study participants.

Parameters		Control	Depression	Chi-square value (p-value)	Odds Ratio (OR)
Gender	Males (49.3%)	28.8%	71.2%	<0.001	0.323
	Females (50.7%)	44.5%	55.5%	<0.001	3.097
Age	Young Adults (15-24 years) 19.6%	61.3%	38.7%	0.496	0.821
	Middle Adults (25-44 years) 32.7%	59.2%	40.8%	0.659	0.898
	Older Adults (45-64 years) 35.5%	53.6%	46.4%	0.300	1.279
	Retirement (65+ years) 12.2%	57.9%	42.1%	0.954	0.980
Obesity	Non-Obese (21.9%)	59.4%	40.6%	0.709	0.902
	Obese Class-1 (18.7%)	45.8 %	54.2%	0.006	1.175
	Obese Class-2 (17.6%)	53.5%	46.5%	0.057	1.205
	Obese Class-3 (41.8%)	40.9%	59.1%	0.015	0.888
Diabetes		28.8%	71.2%	<0.001	4.983
High BP (mm Hg)		37.8%	62.2%	<0.001	4.084
Cardiovascular Disorders		40.9%	59.1%	0.015	2.214
Eating Disorder		31.1%	68.9%	<0.001	4.655

The overall mean age of study participants was 41.63 as the individuals in young and middle-adult age i.e., 18-35 and 36-55 years were encouraged more for the study. The overall mean and standard deviation of BMI were 30.6±6.9. The BMI was measured using the heights and weights of individuals as it is a crucially important measure for determination of obesity along with waist circumference and lipid profile of study participants. The recommended waist circumference for females is <35 inches while for males is <40 inches. The normal range of total cholesterol (TC) in healthy individuals is <200 mg/dL. The mean of total cholesterol in study individuals were recorded as 194.77 mg/dL with the standard deviation of 40.84 mg/dL.

The blood sugar levels of individuals were checked via random blood sugar test with an overall mean and standard deviation of 115.67±68.812 mmol/L whereas, the normal range of blood sugar lies between 70-125 mmol/L. The mean values and standard deviation of data was calculated using IBM Statistics SPSS 25.0 software. The data for possible causes and outcomes of induced depression was also collected through questionnaires. The questions about lifestyle choices, physical activities, food preferences and complaints regarding eating disorders, cardiovascular disorders (CVD), obesity, diabetes and high blood pressure were also recorded as shown in table 1. This data was

used to analyze possible causative agents for genetic predisposition of depression and its associated outcomes.

Out of 357 totals studied samples 141 individuals' full field DSM IV criteria and were classified to have depression phenotype. Homozygous AA genotype was found in 59 individuals with no depression symptoms making 16% of whole sampled population and 27% amongst individuals with no depression symptom. Homozygous GG genotype was found in 41 individuals with no depression symptoms making 11% of whole sampled population and 19% amongst individuals with no depression symptom. 113 individuals i.e. 31% of total sampled population and 53% of individuals with no depression symptoms were heterozygous at this locus and possess AG genotype. In case of individuals with depression 45 individuals were homozygous AA i.e. 12.7% of total sampled population and 31% of the population with depression. Homozygous GG was found in 36 individuals making 10% of total population and 25.5% of samples with depression symptoms. Among cases with depression 42% i.e. 60 individuals making 16% of total population were heterozygous AG. The frequency of wild-type and mutant genotype was calculated in correlation to depression. This data was used to analyze the degree of independence of both variables as shown in table 2..

Table 2: Association of depression with genotypes of study participants.

		Genotype			Total
		AA	AG	GG	
Depression	Control	Count	45	37	201
		Percentage	22.4%	18.4%	
	Depressed	Count	59	53	156
		Percentage	37.8%	34.0%	
Total		104	90	163	357

The p-value of Pearson's Chi-Square Test was 0.052 that is > 0.05 and cannot be considered significant enough to reject null hypothesis. The count of G allele is high in the sample analysis, showing it as a wild-type allele. The A allele is, however, more likely to be a potential factor to lead to depression as it is more prevalent in patients with depression than G allele, but the count of A allele in control is lower than the one in depression patients, but the difference is not great enough for it to be strongly associated with depression.

CONCLUSION

The homozygous GG genotype is more prevalent in study subjects than the homozygous AA or heterozygous AG genotypes. However, depression is likely to be more prevalent in AA genotype i.e. 37.8%, less prevalent in AG genotype i.e. 34.0% and least prevalent in GG genotype i.e. 28.2%. This data

shows A allele of *BDNF* gene to be more associated with depression than the G allele, suggesting this polymorphism to be a somewhat potential target for anti-depressants. However, depression is a multifactorial disorder and many environmental triggers as well as other relevant genes can be possible

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causative agents for its pathogenesis so, this SNP cannot be relied on as a strong target to fight depression. Moreover, the association of A allele with depression is not strong enough in the population under study.

Large scale studies with various study designs are required in several regions of Pakistan to check "the prevalence of depression and its associated genetic and environmental risk factors. The various statistical method and study designs can be used to assess association. Studies are required to trace out the mechanisms by which genetic risk factors are causing depression. Majority of population is unaware of depression and its causative factors. Healthy lifestyle ultimately leads to relax mind which can help to maintain mental health along with physical health. Education and health departments can play a major role in spreading this awareness among people of different sectors.

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