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Research Article

VNTR Mutation in Monoamine Oxidase A in Depression and Anxiety: A Quantitative Survey

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ABSTRACT

In this study, the relationship between Monoamine Oxidase A variable tandem repeat number genetic polymorphism and cognitive tests used for depression and anxiety were evaluated. The study aimed to determine whether Monoamine Oxidase A (MAOA) variable sequential repetition count could be an indicator that can be useful in diagnosing depression and anxiety. The beck depression inventory and beck anxiety inventory were applied to 102 individuals aged between 20-25 years. Oral epithelial cells and blood samples were collected for genetic analysis. DNA isolation of blood samples was performed, and the variable number of tandem repeat polymorphism was analyzed. The association between the beck depression and anxiety inventory results variable number of tandem repeat polymorphisms were evaluated statistically by the chi-square test or fisher's exact test. While the results obtained proved a statistically significant relationship between Monoamine Oxidase A (MAOA) variable tandem repeat number genetic profiles in male subjects, a statistically significant impact could not be determined in the female group. The results used in the evaluation were obtained from the beck depression and anxiety inventories. The results obtained from the study provide promising outcomes about the utilization of genetic parameters and diagnostic criteria employed in clinical psychology.

Keywords: Monoamine oxidase A; VNTR polymorphism; depression; anxiety

INTRODUCTION

Depression is an emotional condition that involves the feeling of grief and is used in the descriptions of other conditions such as collapse, sadness, loss of functional and vital activity.

Major depressive disorder, with a lifetime prevalence of 1.5% to 19%, causes severe social problems with high treatment costs, increased mortality, and morbidity rates when not appropriately treated. It can be encountered at any age; however, it is more common in the middle-aged and especially between 25-44 years old individuals [1]. Depression prevalence in Turkey was found between 8-20%. Depression is the most common psychiatric disorder worldwide. Anxiety is a biological condition and process that regulates the 'fight or flight' mechanism, which usually protects a person from a potential threat. Psychological approaches, in which this regulation protects the person against possible external and internal threats and undertakes the stimulation lost its functionality, are classified under anxiety disorder [2]. Depression and anxiety often occur together and accompany other medical diseases frequently; both affect the course of the disease and treatment responses. It is known that neurotransmitters are essential guides for diagnosing and treating psychological disorders. Monoamine Oxidase (MAO), a catabolic enzyme responsible for regulating catecholamines present in synaptic vesicles, plays a vital role in the etiology of various neurological diseases. Likewise, the examination of the gene group encoding the MAO enzyme plays an extensive role in neuropsychology and clinical psychology [3].

It is crucial to examine genetic factors for diagnosing and treating psychological disorders, the same as other diseases. The MAO enzyme in the human body has two isoenzymes as Monoamine Oxidase A (MAOA) with the encoding gene and

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Monoamine Oxidase B (MAOB) with the encoding gene are both the chromosome [4]. MAOA gene is responsible for the breaking down of monoaminergic neurotransmitters in the brain. It has been one of the most significant genes in gene-environment interaction studies, particularly concerning central nervous system disorders and psychiatric disturbances such as schizophrenia, bipolar disorder, and major depressive disorder. Many psychoanalytic theorists have suggested different psychological descriptions of depression. According to the diagnosis based on genetics, three known polymorphisms of MAOA could be utilized; first, the repeat polymorphism of the 'A' nucleotide close to the second exon 23 base pairs, VNTR (Variable Number Tandem Repeat) polymorphism close to the first exon, and 30 bp long VNTR polymorphism. The VNTR polymorphism containing 30 bp repeat sequences containing 3, 3.5, 4, and 5 copies in the promoter region of the MAOA gene was detected [5].

Throughout this study, the connection between the MAOA-VNTR genetic polymorphism and Beck Depression and Beck Anxiety scale tests was investigated. This study aims to add the individuals' genetic predisposition to the existing depression and anxiety diagnostic criteria.

MATERIAL AND METHODS

The study which focuses on screening of VNTR mutation in the MAOA gene and its association with depression and anxiety was found ethically appropriate by the presidency of non-invasive research. Following the permission of the committee, a group of 102 subjects consisting of individuals whose ages vary between 20 and 25 years was chosen for the study [6]. In the present study, due to the location of the selected gene region in the chromosome, the population is divided into two groups based on gender as 72 (70.6%) females and 30 (29.4%) males. From these groups, oral epithelial cells were collected to isolate genomic DNA by using commercially available buccal collection swabs. The epithelial cells were collected from the swabs into 1.5 mL microcentrifuge tubes with the help of a phosphate-buffered saline solution by vortexing for 2 minutes [7]. Collection materials were immediately stored at -20 °C overnight when the isolation step was delayed. RTA Genomic DNA Isolation Kit from Blood was used for DNA isolation for both blood and oral epithelial cell samples. Polymerase Chain Reaction (PCR) was used to observe MAOA-VNTR polymorphisms by the primers given in **Table 1**. PCR mix was prepared for each test subject which contains 12.5µL Lucigen EconoTaq PLUS 2X Master Mix (1X), 1µL forward primer (0.4mM), 1µL reverse primer (0.4mM), 8.5µL nuclease-free water, and 2µL DNA, as the total volume of 25µL. The PCR cycling conditions for MAOA gene were: 95°C for 5 minutes as initial denaturation, 95°C for 1 minute as denaturation, 65°C for 1 minute as annealing, and 72°C for 30 seconds as an extension 72°C for 7 minutes as a final extension [8]. PCR products were fragmented on a 3.5% agarose gel using gel electrophoresis in Tris/Borat/EDTA buffer. The gel was dyed with ethidium bromide and observed under ultraviolet light transilluminator. 100 bp DNA Ladder was used as the standard size for gel electrophoresis.

Primer Sequences	Tm (ºC)	Fragment size (bp)	Activity
F: 5' - CAGCGCCCAGGCTGCTCCAGAAAC - 3'	65	Allele 1; 221 bp (3 repeats)	Low
		Allele 2; 233 bp (3.5 repeats)	High
R: 5' - GGTTCGGGACCTGGGCAGTTGTGC - 3'		Allele 3; 251 bp (4 repeats)	High
		Allele 4; 281 bp (5 repeats)	Low

Table 1: Primers used for screening of MAOA-VNTR polymorphism, allelic information, and diagnosis criteria.

In this study version of Beck Depression and Anxiety Inventories were used for psychological evaluation of depression and anxiety [9]. Beck Depression and Anxiety Inventories were developed in 1961 by Beck, Wart, Mendelson, and in 1988 by Beck and his colleagues. The subjects of the group were asked simultaneously to fill in Beck Depression and Anxiety Inventories. Both tests consist of 21 questions and each question is formed for different categories. The maximum score is 63, and scoring ranges from 0 to 3. For each question, individuals choose the most appropriate level sorted in increasing levels [10]. The gradings of the Beck Depression and Anxiety Scales are divided into 4 groups. The intervals of scores obtained from the Beck Depression Scale test are 0-10 score interval for Minimal Depression, 11-17 score interval for Mild Depression, 18-23 score interval for Moderate Depression, 24-63 score interval for Severe Depression. And, the intervals of scores obtained from the Beck Anxiety Scale test are 0-7 score interval for Minimal Anxiety, 8-15 score interval for Mild Anxiety, 16-25 score interval for Moderate Anxiety, 26-63 score interval for Severe Anxiety [11].

STATISTICAL ANALYSIS

Alleles of the MAOA were divided into two groups; low and high activity. One group exhibited low activity as the alleles 1, and 4 with 3, and 5 repeats, respectively. The other group which represented high activity consisted of alleles 2 and 3 with 3.5 and 4 repeat units, respectively, as described in Table 1. Chi-square tests were used to compare associations for categorical data among groups. IBM SPSS Statistics 25.0.0.1, MacOS 10.14 Mojave software was used for statistical analyses [12]. The analyses were performed separately for male and female subjects, in consideration of the MAOA gene location on the X chromosome. The p-value<0.05 was considered to be statistically significant [13].

Females Depression*Anxiety correlation	Depression	Anxiety	Depression	Pearson Correlation	1	0.635**

Sig.(2-talied)	-	0	
n	72	72	
Anxiety	Pearson Correlation	0.635**	1
	Sig.(2-talied)	0	-
	n	72	72

Table 2: Correlation of depression and anxiety scores in females.

RESULTS

The visualized polymorphism analyses for the MAOA gene are represented in Figure 1. The samples 1-4, 7-9, and 13-14 were interpreted as 3-1 genotype; samples 5 and 10-12 were 3-3 genotype; sample 6 was 4-3 genotype, and sample 15 was 1-1 genotype, with the help of allelic information and diagnosis criteria given [14]. According to the statistical analyses, which were performed separately due to the selected gene region's location in the chromosome, a statistically significant association between genotype and psycho-analytic tests was obtained in male individuals. In contrast, no statistically significant results could be obtained in females [15].

Males correlation	Depression*Anxiety	Depression	Anxiety
Depression	Pearson Correlation	1	0.874**
	Sig.(2-talied)	-	0
	n	30	30
Anxiety	Pearson Correlation	0.874**	1
	Sig.(2-talied)	0	-
	n	30	30

Table 3: Correlation of depression and anxiety scores in males.

In females, the Chi-square values between genetic profile results and depression are 0.537; the Chi-square values between the effects of genetic profile and anxiety are 0.297. The exact test result is 0.540 between genetic analysis and depression scores, while 0.302 was found between genetic analysis and anxiety scores. According to the results, there is no significant connection between MAOA-VNTR genotype, depression, and anxiety scores. According to the bilateral correlation performed between depression and anxiety in females, the Pearson correlation of anxiety score was 0.635, shown in Table 2.

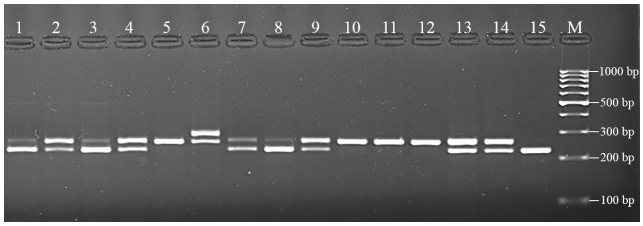

Figure 1: Gel electrophoresis image of VNTR polymorphism in the MAOA gene under UV light; M: 100 bp DNA ladder (bp: base pairs).

Accordingly, a significant association between MAOA-VNTR genotype, depression, and anxiety scores as $p<0.05$ was found in Chi-square analysis. According to a bilateral correlation between depression and anxiety scores in males, the Pearson correlation of anxiety depression score was found 0.874, which is considered high (Table 3).

DISCUSSION

Depression and anxiety often occur together, especially at primary stages. Many of their symptoms are common. These symptoms include fear, disturbance, panic attacks, pain, gastrointestinal complaints, excessive anxiety, agitation, concentration difficulty, sleep disorder, weakness, fatigue, and suicidal thoughts. There is no clear-cut distinction between the symptoms of these two affective disorders. Although the causes are different, the same symptoms can be observed. In some cases, even the causes can be the same. This characteristic of affective disorders makes the distinction between anxiety and depression considerably subtle. Beck depression and anxiety scales give results according to an individual's self-assessment, enabling the test- taker to provide an appropriate answer by evaluating his/her symptoms. In this case, the correlation across scales is expectable. Developments in the field of biotechnology have re-increased the interest in MAO. MAOI prevents the catalytic effect of MAOA protein and contributes to the increase of the Serotonin (HT-5) concentration in the brain. The emergence, development, and progression of psychological and psychiatric diseases due to serotonergic activity are thought to be related to this pathway. MAOA intensity measured by Positron Emission Tomography in the prefrontal cortex, midbrain, and hippocampus regions of patients diagnosed with depression was shown to represent one of the main serotonin-decreasing procedures in patients and to be a marker of major depression. MAOA levels are found to be 34-40% higher in major depression than that of healthy controls in the stated regions of the brain. The 'A' isomer of the MAO gene, together with the other genes involved in neurotransmitter metabolism pathways, has been the primary focus in psychiatric candidate studies.

As a result of research on the MAOB gene, the other isoform of the MAO gene, this gene's activity, and expression have been associated with depression, alcoholism, psychotic disorders, and neurodegenerative diseases. Treatment of neurological diseases is challenging because the treatment method is unknown and hidden within the brain's complex structure; however, this situation can be eliminated by the intersection of different disciplines. Psychological support and continuity of this support

are essential for the treatment of depression to reach an outcome. It is also crucial to examine the genetic factors for diagnosing and treating psychological diseases, the same as other diseases. Research studies aim to discover related genetic factors, especially about the MAOA gene, such as its relationship with bipolar disorder, unipolar disorder, and antisocial personality disorder. Note that even cigarette addiction in the sense of the compounds' response has MAOI properties and is found in cigarettes. The data obtained from empirical studies increase the predictions of candidate studies.

In the MAOA gene meta-analysis study, a significant relationship was established with bipolar disorder. MAO is not only seen in psychological disorders but also in neurological diseases. In another study, with a subject group consisting of temporal lobe epilepsy patients there are significant differences between chronic seizure frequencies and the definitions of MAOA-VNTR polymorphisms. Physical, psychosocial conditions, phylogenetic, ontogenetic developmental phases, and epigenetic differences are essential in acknowledging affective disorders. According to the statistical results, there is a strong association between MAOA-VNTR genotypes and Beck Depression and Anxiety Inventory test scores in male subjects. Additionally, in males, the correlation between depression and anxiety was found high and statistically significant. According to the statistical results, there is no statistically significant connection between MAOA-VNTR genotypes and Beck Depression and Anxiety Inventory test scores in female subjects. Besides, the correlation between depression and anxiety was found lower than that of men. The reason why male individuals exhibit a strong association between their genetic profiles and their psycho-analytic results is thought to be the VNTR polymorphism of MAOA isoenzyme's localization in the X chromosome. Yet the presence of two X chromosomes in females change the diagnosis criteria. It is thought that the evaluation of genetic profiles might pave the way for further research to find better diagnostic options that can be combined with genetic and psycho-analytic tools. In conclusion, the results obtained from the study provide promising outcomes concerning the utilization of biological parameters and diagnostic criteria employed in clinical psychology. The 'A' isoform of MAO as a biomarker should be surveyed in different affective disorders in further studies. Especially, as an addition to the MAOA-VNTR polymorphism profiling, gene expression analysis of the same research group at different time intervals or stages of treatment or for various drugs in therapy would be considered for further enlightening studies.

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