

MEASUREMENT OF AGGRESSIVE BEHAVIOR AND ITS ASSOCIATION WITH MAO-A GENE POLYMORPHISM IN POPULATION OF SWAT, KHYBER PAKHTUNKHWA

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Abstract

Aggressive behavior holds adverse responses toward conditions. Aggression is a human and animal behavioral phenotype that is deeply concerned with genetics and environmental factors. Literature states the MAO-A (monoamine oxidase A) gene is responsible for aggression and violence. Monoamine Oxidase-A gene translates the MAOA enzyme present in mitochondria, which has a role in degrading neurotransmitters such as dopamine, serotonin, and nor-adrenaline. A high level of MAOA leads to several abnormalities of depression, anxiety, etc. while a low level of MAOA can lead to criminal and aggression disorders. The VNTR (Variable number tandem Repeat) polymorphism was detected in MAOA gene. This Polymorphism are highly found in the Asian and African populations. This study has big importance for the defense agencies of the area and has broad forensic value.

Objectives. The main focus of this study is to investigate aggressive individuals and find their association with MAOA gene polymorphism.

Methods. Aggressive patients were determined using a questionnaire. Total of 128 buccal swab samples was extracted from the patients. For identifying polymorphism conventional PCR was perform.

Results. All the individuals' behavior was assessed and found 48 individuals with high aggression, 48 with very low aggression and 32 as normal. We genotyped 128 individuals and successfully find out 3 different types of polymorphism 321bp, 351bp, and 381bp. In comparison with other populations, we have 321bp size as more repetitive. Chi Square test show the linkage (P value 0.00) of MAOA gene polymorphism and behavior variation.

Conclusion. Statistical analysis show that aggressive behavior has a moderate association with MAOA gene polymorphism. The disturbance of the MAOA gene leads to etiological disorders.

INTRODUCTION

Violence is considered a big issue for society by the world health organization (WHO)^[1,2]. Violence is a complex trait that is triggered by aggression, a behavior that responds to a stimulus abnormally. Aggressive behavior holds adverse responses toward conditions. Aggression is a human and animal behavioral phenotype that is deeply concerned with genetics and environmental factors.^[3,4] Literature states the MAO-A (monoamine oxidase A) gene is responsible for aggression and violence^[5,6].

The Monoamine Oxidase-A gene also called the "Warrior gene"^[7] translates the MAOA enzyme present in mitochondria, which has a role in degrading neurotransmitters such as dopamine, serotonin, and noradrenaline^[8]. After any nerve impulse to the brain MAOA enzymes have a role in degrading the used neurotransmitters in the synaptic clefts^[9]. The functional VNTR (Variable tandem repeat) polymorphism is determined in the MAOA gene on chromosome XP 11.23^[10,11]. This contains 31 base pairs polymorphism which categorizes the activity of MAOA gene as low (MAOA-L) and high (MAOA-H). This polymorphism can affect the function of the MAOA enzyme, which lead to over-degrading or low degrading of neurotransmitters. This abnormality reaches aggressive and violent behavior^[12,13].

High levels of MAOA lead to several abnormalities of depression, anxiety, etc^[14]. While low levels of MAOA can lead to criminal and aggression disorders^[15]. Variability in the MAOA sequence is 2,3,3.5,4 and 5 repetitions and hence termed polymorphism^[16]. The low level of MAOA refers to 2 and 3 copies of the sequence of MAOA, while the high level of MAOA contains 3.5 and 4 copies. These repetitions are highly found in Asian and African populations^[17]. This repetition means the repeated polymorphic sequences to the MAOA gene. Environmental aspects have a sufficient role in the genetics of MAOA. People who have been maltreated in their childhood have more chances to

become violent in their post age. Children who have been treated badly such through physical punishment, verbal abuse, disgracing activities, and many more are the inducing factor of MAOA polymorphism, A positive polymorphism has seemed in childhood maltreatment individuals^[18,19].

This study gain the novelty in the study area for such observations. A dense patient of aggression in the study area was observed which aimed to this study. This study has big importance for the defense agencies of the area and has broad forensic value^[20,21].

MATERIAL & METHODS:

A total of 128 male patients from the Baidara Valley of Swat, aged 20-30, were examined using a 23-item questionnaire to evaluate their levels of aggressiveness [22,23]. Buccal swab samples were collected from all participants for genomic DNA extraction, which was performed using the manual PCIA method^[22]. Amplification of the MAOA gene was conducted using the forward primer 5'-ACA GCC TGA CCG TGG AGA AG-3' and the reverse primer 5'-GAACGTGACGCTCCATTCGGA-3'^[23]. A 15 µl PCR mixture (7.5 µl Green Master Mix, 0.5 µl of each primer, 2 µl template DNA, 4.5 µl PCR water) was used for amplification. The PCR conditions included an initial denaturation at 95 °C for 5 minutes, followed by 35 cycles of denaturation at 94 °C for 1 minute, annealing at 55.5 °C for 1 minute, and extension at 72 °C for 1 minute. A final extension was performed at 72 °C for 5 minutes. The PCR products were visualized on a 2% agarose gel (1X TAE buffer), and a 100 bp DNA ladder was used to estimate the size of the MAOA gene bands. The size of all bands was compared to known standards for measurement.

All the questionnaires were scored and categorized into three behavioral groups: individuals with a 0-40% aggressiveness score were labeled as "Low aggressive," those with 41-70% as "Moderately

aggressive," and those with 71-100% as "Highly aggressive," making this a categorical variable^[24]. The second variable was different gene sizes. A Chi-square test was conducted using IBM-SPSS-2021 to compare the mean values across all groups.

RESULTS AND DISCUSSION:

The activation and expression of specific genetic features are greatly influenced by environmental variables. Stress, anxiety, despair, mistreatment, and unethical behavior all play a big part in disrupting

the normal transcription of the MAOA gene, which results in antisocial behavior and excessive aggressiveness.^[4]

In this investigation, the study aimed to look the association between violent behavior and MAOA gene polymorphism in 128 male participants from the Baidara Valley who were between the ages of 20 and 30. A 23-item questionnaire was used to classify the participants' violent behavior, and buccal swab DNA samples were used to examine the MAOA gene sizes of the individuals.

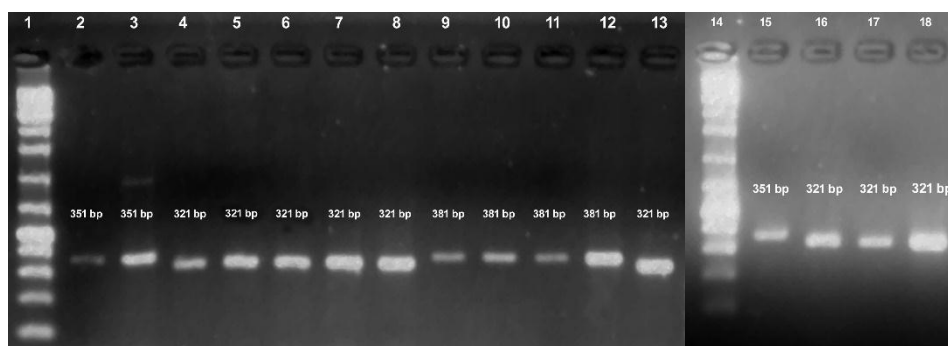


Figure 1. PCR products of MAOA gene on 2% Agarose gel, Line 1=100 bp DNA Ladder, Line 2-7 = High Aggressive individuals, Line 8-13= Low Aggressive individuals, Line 14=100 bp DNA Ladder, Line 15- 18= Moderate Aggressive individuals.

Aggressiveness Categories and Gene Sizes:

Based on questionnaire 3 different categories was obtained from the data. This is High, Low and moderate level of aggression^[24]. Their results are listed below

- High Aggressive: Out of 48 individuals, 16 (33.3%) had a 351 bp MAOA gene, and 32 (66.7%) had a 321 bp MAOA gene.

- Low Aggressive: Out of 48 individuals, 32 (66.7%) had a 381 bp MAOA gene, while 16 (33.3%) had a 321 bp MAOA gene.

- Moderate Aggressive: Out of 32 individuals, 8 (25%) had a 351 bp MAOA gene, and 16 (50%) had a 321 bp MAOA gene.

The 321 bp MAOA gene was found to be the most frequent gene size, particularly in the high-aggressive group. This pattern is further illustrated in the table below:

Table 1: Aggressive Behavior x MAOA Gene_Size Cross tabulation

		MAOA_Size			Total
		321bp	351bp	381	
Behavior	High	32	16	0	48
	Low	16	0	32	48
	Moderate	24	8	0	32
Total		72	24	32	128

Statistical Analysis

The relationship between aggressive behavior and the MAOA gene polymorphism was investigated using a Chi-square test. A statistically significant association was found by the test, with a significant p-value of 0.00 showing in table 2. The null hypothesis, according to which there is not an association

between aggression and MAOA gene polymorphism, was refuted by this findings. The distribution of gene sizes among the aggressiveness categories implies that there could have an association between increased levels of aggression and specific gene polymorphisms, specifically the 321 bp size.

Table 2: Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	75.852 ^a	4	.000
Likelihood Ratio	93.726	4	.000
Linear-by-Linear Association	.160	1	.689
N of Valid Cases	128		

Discussion

Scientific evidence shows the linkage between aggressive behavior and MAOA gene polymorphism in male individuals from the Baidara Valley, aged 20-30, is presented in this study. Particular, this is observed that the 381 bp gene was more common in the low-aggressive group and that people with the 321 bp MAOA gene size were primarily found in the high-aggression category.

Previous research has connected the MAOA gene, also known as the "warrior gene," to behavioral traits like aggression and impulsivity. Our findings lend credence to the theory that variations in this gene could impact aggressive conduct. Interestingly, people who carry the 321 bp polymorphism seem to be more aggressive than people who carry the 381 bp polymorphism, which seems to be linked to lower levels of aggression.

There are different polymorphisms observed previously in different populations which are 291bp (1.5 repeats), 321bp (3.5), 351bp (4.5), 381bp (5.5)^[25]

Among the populations studied, In the White/non-Hispanic group, 32.7% had 3 repetitions (3R), a small percentage of 0.5% had 3.5 repetitions (3.5R), 64.9% had 4 repetitions (4R), and 1.8% had 5 repetitions (5R). The Asian/Pacific Islander group had 61% with 3 repetitions (3R), 1.2% with 3.5 repetitions (3.5R), and 37.8% with 4 repetitions (4R). Hispanic/Latino population exhibited 29.3% with 3 repetitions (3R) and 70.7% with 4 repetitions (4R)^[6].

German European populations demonstrated 35.9% with 3 repetitions (3R), 0.8% with 3.5 repetitions (3.5R), 61.1% with 4 repetitions (4R), and 2.2% with 5 repetitions (5R)^[26]. Another study on German Europeans displayed 0.8% with 2 repetitions (2R), 35.9% with 3 repetitions (3R), 0.8% with 3.5 repetitions (3.5R), 61% with 4 repetitions (4R), and 1.5% with 5 repetitions (5R)^[27]. The New Zealand European population had 0.3% with 2 repetitions (2R), 33.8% with 3 repetitions (3R), 0.9% with 3.5 repetitions (3.5R), 63.8% with 4 repetitions (4R), and 1.2% with 5 repetitions (5R)^[19]. The Italian European population had 1.7% with 2 repetitions (2R), 40% with 3 repetitions (3R), 56.6% with 4 repetitions (4R), and 1.7% with 5 repetitions (5R)^[26]. The Afrikaner population showed 28.1% with 3 repetitions (3R), 68.4% with 4 repetitions (4R), and 3.5% with 5 repetitions (5R)^[28]. The American population showed 36.2% with 3 repetitions (3R), 2.9% with 3.5 repetitions (3.5R), 60.5% with 4 repetitions (4R), and 0.4% with 5 repetitions (5R)^[29]. Iraqi Population have 1.1% of 1.5R, 35.8% of 3R, 52% of 4R and 11.1 % of 5R^[16].

African Americans exhibited 59% with 3 repetitions (3R), 2.3% with 3.5 repetitions (3.5R), 36.4% with 4 repetitions (4R), and 2.3% with 5 repetitions (5R)^[6]. Among the Chinese population, 0.5% had 2 repetitions (2R), 57% had 3 repetitions (3R), 42% had 4 repetitions (4R), and 0.5% had 5 repetitions (5R)^[30].

Comparing our results with the above populations we have 321 bp (3.5R) size 56.3%, 351bp (4.5R)

18.8%, 381bp (5.5R) 25%, 3 repetition 0%, and 4 repetition 0%. Rather than being only the result of genetic makeup, the departure from the anticipated gene size in each aggression category like 351 bp in aggressive group may also be influenced by some other factors. Furthermore, data variability may be introduced by participants' potential to give false or incorrect responses on the aggressiveness questionnaires. Our population has differences from the previous populations. These findings indicate that a complex interaction between hereditary and environmental variables shapes violent behavior and antisocial inclinations within the Pukhtoon population. To clarify the precise processes behind these genetic and behavioral trends, more research is required. Our research adds to the library of knowledge on how genetics and the environment interact.

Conclusion:

This study confirm the moderate association between behavioral variability and MAOA gene polymorphism. This finding highlights the potential role of genetics in the development of aggressive behavior.

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