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Association of MAOA Gene Polymorphisms with aggressive behaviors of some prisoners in Erbil city / Kurdistan region, Iraq

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ABSTRACT

Molecular genetic studies of personality traits have made use of continuous genomic information, but the molecular mechanism for developmental change and stability of personality has not been fully investigated. The human monoamine oxidase A (MAOA) gene has been suggested as a possible contributor to aggressive behaviors. The purpose of this study was to discover a single nucleotide polymorphism (SNP) in the MAOA gene that is linked to aggressive behavior and beyond with a history of criminal behavior and antisocial attitudes. A total of 35 samples were obtained from people who have history of crimes participated in this study and the blood DNA was extracted according to the manufacturer's instructions. The software (https://eurofinsgenomics.eu/en/dna-rna-oligonucleotides/oligo-tools/primer-design-tools/) was used for designing primers to amplify exon 7 and exon 8. The SNP was analyzed by performing a Gradient PCR. The PCR products are sequenced then using and Mega 6 for multiple sequence alignment and analysis. The serum samples were

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analyzed using an ELISA kit to check the level of dopamine. Of the total of 35 criminal participants, there were only two MAOA gene variants identified, the first one was in exon 7 of the MAOA gene (rs1181634890) and the second was in exon 8 containing (rs6323) variants, %96.6 of participants had (rs6323) variants. Raised dopamine serum levels, in both variants, were reported. Our data confirmed a possible role for MAOA gene variants in the progression from aggressiveness to criminal behavior.

1. Introduction

Polymorphism, as related to genomics, refers to the presence of two or more variant forms of a specific DNA sequence that can occur among different individuals or populations. The most common type of polymorphism involves variation at a single nucleotide (also called a single-nucleotide polymorphism, or SNP). Other polymorphisms can be much larger, involving longer stretches of DNA (Collins, Brooks, & Chakravarti, 1998).

The genetic polymorphism present in the human genome has been linked to a variety of diseases (Akbari, Badrlou, et al., 2022; Ghafouri-Fard et al., 2022; Hussen et al., 2021; Qader et al., 2021; Taheri et al., 2022) such as psychiatric disorders (Akbari, Eghtedarian, et al., 2022). Recent developments in neurocriminology have revealed new aspects, including the possibility of genetic influences on violent criminal conduct (Coppola, 2018). Moreover, analysis of data from study showed that MAOA, a gene encoding an enzyme involved in catabolizing amine neurotransmitters like dopamine, serotonin, and noradrenaline, has the strongest association between genetic variation and aggression (Coppola, 2018).

MAO-A preferentially oxidizes the biogenic amines serotonin and norepinephrine and is involved in controlling levels of monoamine neurotransmitters. In MAOA-L carriers, a distributed network of node links with a significantly increased connectivity was determined in brain, compared to MAOA-H carriers (Fan et al., 2010).

Additionally, findings have provided information on the link between an allelic mutation of the MAOA gene and criminal behavior (Sohrabi, 2015). Since 1993, there has been a resurgence of interest in aggression and monoamine oxidases (MAOs) due to the reported aberrant behavioral indications, including overtly aggressive and

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violent conduct, in a Dutch family with an X-linked nonsense mutation in the MAOA genes (Sohrabi, 2015). Interestingly, it has been found that those who inherit a variant of the MAOA gene that causes expression levels to be low (MAOA-L) are more likely to display aggressive personality traits. As a result, this demonstrates that MAOA-L has a unique neurobiological vulnerability associated with characteristic aggression (Klasen et al., 2018). A functional variation in the MAOA gene can reduce the impact of early-life trauma on the development of aggressive tendencies in humans. High rates of antisocial behavior in adulthood were seen among children with the low activity form of MAOA who had also been maltreated (Caspi et al., 2002). Aggression is an individual or collective social behavior carrying adaptive benefits as well as maladaptive antisocial consequences when enacted excessively or pathologically (Gazzillo et al., 2020). Aggressive behavior is common in antisocial people and can be divided into two major kinds; Reactive aggression, also known as impulsive aggression, is characterized by emotionally motivated and frequently irrational acts with the sole purpose of causing harm to others. These incidents frequently take place in response to perceived environmental or social threats, though they can also be used to start or fuel aggressive interpersonal relationships (Mentis, Dardiotis, Katsouni, & Chrousos, 2021). The other type of aggression is called instrumental or indirect aggression. It includes the behavior of physical or mental harm that is planned and mainly aimed to succeed, which often includes hurting social relationships (Harman, Kruk, & Hines, 2018).

The MAOA gene's regulatory variable number tandem repeat (VNTR) in the promoter region has received the most attention since it appears to be linked to variation in impulsivity and aggression in healthy male participants (Manuck, Flory, Ferrell, Mann, & Muldoon, 2000). Gene-environment interactions implicating this polymorphism have also been reported In particular, it was discovered that maltreated kids were more likely to exhibit violent and antisocial conduct as adults than healthy kids (Martin & Njoroge, 2005), and this finding has been further replicated (Foley et al., 2004). These results seem to be consistent in males, but not in females (Sjöberg et al., 2007). Similar to this, results for female adolescents were less significant, while male adolescents with the low MAOA-VNTR had a twice-increased likelihood of

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violent delinquency compared to the other MAOA-VNTR variations (Guo, Ou, Roettger, & Shih, 2008).

There is another gene related to aggressive behavior (CDH13 Gene). This gene encodes a member of the cadherin superfamily. The encoded protein is localized to the surface of the cell membrane and is anchored by a GPI moiety, rather than by a transmembrane domain (Rivero et al., 2015).

The aim of this study was to study the effect of MAOA gene polymorphisms on personal aggressiveness in criminal participants. In addition to that it was also to find the mentioned impact would change over time or not.

2. Materials and Methods

In this study 5 mL blood samples were obtained from 35 subjects of criminal Participants, in both Ethylene Diamine Tetra Acetic Acid (EDTA) tubes for gene detection and gel tubes for determining serum dopamine levels, from prisons in the Kurdistan region. The total genomic Deoxyribonucleic Acid (DNA) was extracted from peripheral blood samples using the genomic DNA extraction kit (ROCHE, Germany) according to the manufacturer's protocols and the quality and quantity of DNA samples was assessed using NanoDrop^{TM} One (Thermo Scientific) and stored at -20°C for later downstream applications. The control samples for the MAOA gene mutation were screened for mutations in exons 7 and 8 of the MAOA gene. Primer for MAOA gene forward 5'GTGGCCTGTGACTTTCTGGA3' and MAOA reverse 5'-GTGTGGCCAAGGATATGAGG3'. 40-80 ng of participants' DNA were used for Polymerase Chain Reaction (PCR) amplification (780 bps). Using AddStart Taq DNA Polymerase kit (ROCH) according to the manufacturer's protocol, 3 µl buffer, 2 µl Magnesium Chloride (MgCl2), 2 µl deoxynucleotide Triphosphate (dNTP), 0.4 l of Taq DNA polymerase and 1 ml of each primer, 13.6 µl PCR grade water and 2 µl template DNA were mixed in PCR tube and amplified in the following PCR conditions for MAOA gene. PCR amplification products were separated using 1.5% agarose gel electrophoresis at 90 V, 100 mA, 9 W for 1 h after initial denaturation for 5 min at 94c°, followed by 35 cycles at 94c° for 30 s, 61c° for 30 s, and 72c° for 30 s, followed by a final extension at 72c° for 7 min. Then, the PCR products were sequenced by the Sanger sequencing method using the 3130 Genetic Analyzer (Applied Biosystems,

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Hitachi High- Technologies, Tokyo, Japan). The PCR products were sent to (Immunogene Center-Erbil) for sequencing then using (BioEdit.exe) and Mega 6 (http://www.megasoftware.net/) for multiple sequence analysis and alignment.

The serum samples were analyzed using an ELISA kit (Human Dopamine, DA ELISA Kit, EA0041Hu China) to check the level of dopamine. Statistical differences in the dopamine level data between MAOA mutants and non-mutant participants were determined using a U-Mann Whitney test. Normality tests, namely D'Agostino and Pearson omnibus, Shapiro-Wilk, and KS normality tests.

3. Results

Standard protocol for detecting and finding mutations was used. The SNP was analyzed by performing a Gradient PCR (AB Applied Biosystems, USA). The PCR products were sequenced then using (BioEdit.exe) and Mega 6 (http://www.megasoftware.net/) for multiple sequence analysis and alignment. After receiving genetic counseling, among participants, 30 participants (%85.7) had a point missense mutation in the exon 7 and exon 8 of the MAOA gene (Table 1). A heterozygous missense mutation was identified using Sanger sequencing of the gene's coding region, in which %3.4 in the exon 7 MAOA gene (rs1181634890) and %96.6 in the exon 8 MAOA gene (rs6323) (Table 2).

The Alanine to Threonine amino acid switch is caused by the A>G mutation in the MAOA gene (rs1181634890), which is located in exon number 7 and belongs to chromosome ChrX: 43,731,269 cytogenetic p arm (Figure 1), and the valine to phenylalanine amino acid switch is caused by the G>T mutation in the MAOA gene (rs6323), which is located in exon 8 and belongs to chromosome X:43731789 cytogenetic p arm (Figure 2).

Table 1: Percentage of mutant and non-mutant MAOA gene within criminal group

Participants	Frequency	%
Mutant MAOA	30	85.7
Non-mutant MAOA	5	14.3
Total	35	100.00

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Table 2: The percentage of a variant of mutations.

Mutation	Frequency	Percent
Rs6323	29	96.6
Rs1181634890	1	3.4
Total	30	100.00

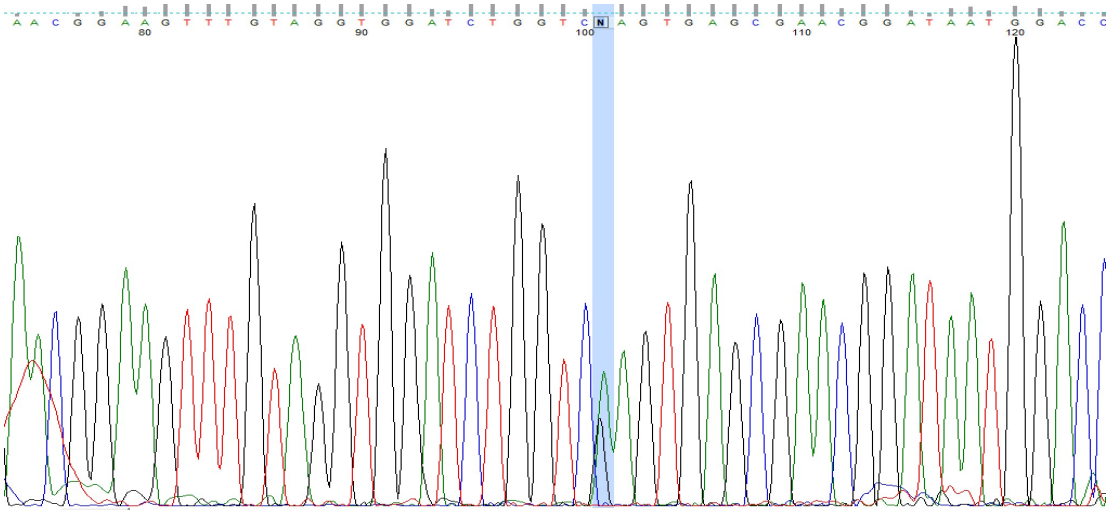

Figure 1: Sequencing of mutated MAOA gene (exon 7) (rs1181634890, heterozygous). which is located in exon number 7 and belongs to chromosome ChrX: 43,731,269 cytogenetic p arm.

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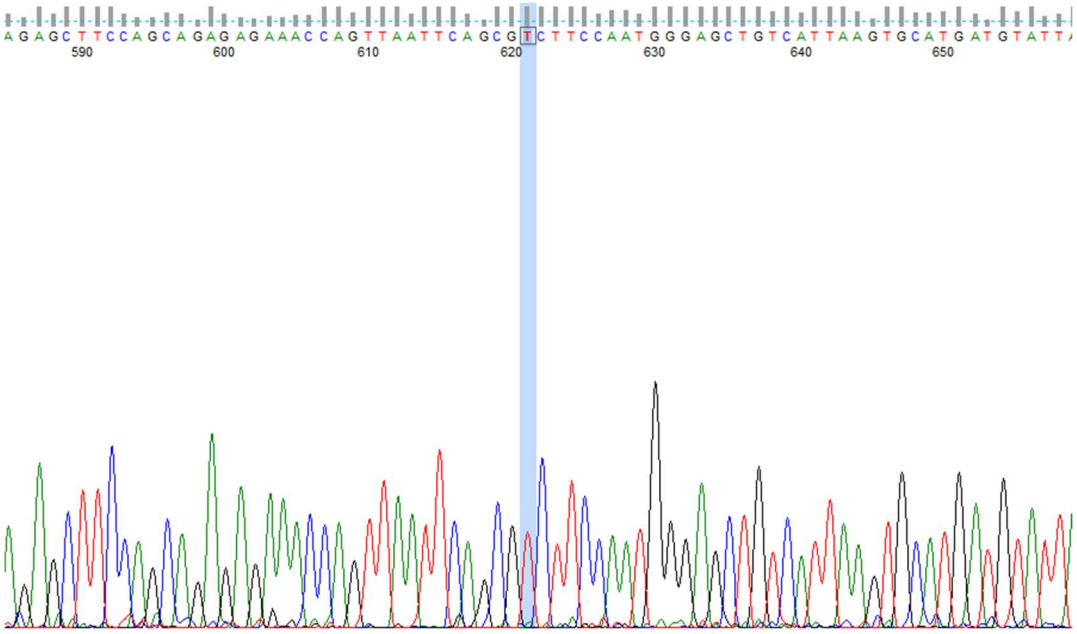

Figure 2: Sequencing of mutated MAOA gene (exon 8) (rs6323, heterozygous). which is located in exon 8 and belongs to chromosome X:43731789 cytogenetic p arm.

A substantial elevation of serum dopamine levels was found in both variants and found a significant difference in the level of serum dopamine(ng/ml) (p-value P<0.01) between MAOA gene mutated participants and MAOA no mutated participants (Figure 3).

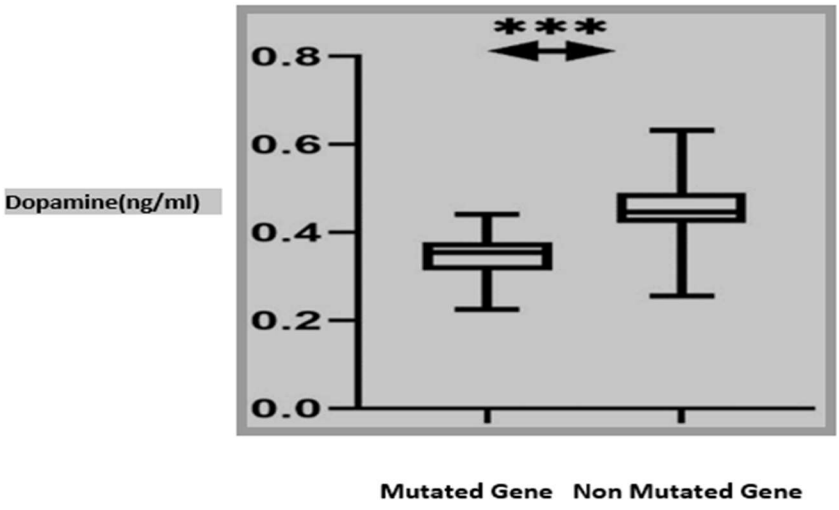

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Figure 3: Dopamine level between mutated and non-mutated genes.***=P-value < 0.001

4. Discussion

This research identified an MAOA gene substitution (Missense). A potential mutation in MAOA was detected in a group of participants utilizing targeted Sangar sequencing as first research on the MAOA gene mutation in Kurdish participants. The MAOA gene is the first gene related to aggression (Odintsova et al., 2019). A heterozygous substitution mutation (rs1181634890 A>G) in a single participant is situated on the chromosome ChrX: 43,731,269 cytogenetic P arm which is located in exon number 7 causes variation in amino acid from Alanine to Threonine, this missense mutation changes the characteristics of proteins and their function by decreasing the activity of the MAOA enzyme, our result is supported by (Carvalho et al., 2022). While (rs6323 G>T) variants among other participants in which situated on the chromosome X: 43,654,907-43,746,817 of the MAOA gene cytogenetic P arm which is located in exon number 8 cause variation in amino acid from valine to phenylalanine. Our results were similar to the study of (Sarwar & Hasnain, 2021).

Both variants cause the low activity of the MAOA enzyme, the low activity form of the MAOA enzyme (MAOA-L) has been linked to increased levels of aggression (Imran, Al-Thuwaini, Al-Shuhaib, & Lepretre, 2021) which is in agreement with violence our results.

An elevation of serum dopamine level in the mutated MAOA gene was determined in our study, and supported by Van (Rhodes et al., 2022) which revealed that the monoamine content in the brain is controlled by MAOs, which catabolize monoaminergic neurotransmitters. The discovery that changes in dopaminergic neuronal activity directly influence impulsive and violent behavior is consistent with the abundant expression of the MAOA gene in dopaminergic (DA) neurons (Pinto et al., 2014).

Conclusion:

The results of our research highlight the significance of bringing a variation in the MAOA gene effect into the field of behavioral genetics and mutation in this gene considering increasing personal violence. The significance of the MAOA gene in

personality development is unclear, and future research using a longitudinal design to investigate the gene-environment interplay, with large sample size, may provide insight into the issue

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الملخص
استفادت الدراسات الجينية الجزيئية لسمات الشخصية من المعلومات الجينومية المستمرة ، ولكن لم يتم بعد التحقيق في الآلية الجزيئية للتغير النمائي واستقرار الشخصية.
تم اقتراح جين MAOA البشري (monoamine oxidase A) كمساهم محتمل في السلوكيات العدوانية. كان الغرض من هذه الدراسة هو اكتشاف تعدد أشكال واحد للنيوكليوتيدات (SNP) في جين MAOA المرتبط بالسلوك العدواني وما بعده مع تاريخ من السلوك الإجرامي والمواقف المعادية للمجتمع. شارك 35 رجلاً لديهم تاريخ من الجريمة في هذه الدراسة وتم استخراج الحمض النووي للدم وفقًا لتعليمات الشركة المصنعة. تم استخدام البرنامج التمهيدي المحدد لتصميم بادئات لتضخيم exon 7 و exon 8. تم تحليل SNP عن طريق إجراء. يتم تسلسل منتجات PCR ثم باستخدام و Mega 6 لمحاذاة وتحليل تسلسل متعدد. تم تحليل عينة المصل باستخدام مجموعة ELISA ، DA ELISA Kit، للتحقق من مستوى الدوبامين. من إجمالي 35 مشاركًا إجراميًا ، كان هناك نوعان فقط من المتغيرات الجينية MAOA تم تحديدها ، وكان أحد المتغيرات في exon 7 من جين (rs1181634890) MAOA ومتغيرات أخرى في exon 8 تحتوي على (rs6323) متغيرات (rs6323) المتغيرات. تم الإبلاغ عن ارتفاع مستويات مصل الدوبامين في 96.6 ٪، من المشاركين لديهم كلا المتغيرين. تشير هذه النتائج إلى دور محتمل لمتغيرات الجين MAOA في التطور من السلوك العدواني إلى السلوك الإجرامي.

پوخته
توێژینەوە جینایەتیە گەردیلەییەکان لەسەر تایبەتمەندییەکانی کەسایەتی سوودیان لە زانیارییە جینۆمییە بەردەوامەکان وەرگرتووە, بەڵام هێشتا لێکۆڵینەوە لە میکانیزمی گەردیلەیی بۆ گۆڕانکاری و سەقامگیری کەسایەتی نەکراوە. جینی MAOA (مۆنۆئەمین ئۆکسیدایز A) ی مرۆڤ وەك بەشداریکی ئەگەری لە رەفتارە شەرەنگێزەکاندا پێشنیارکراوە. ئامانج لەم توێژینەوەیە دۆزینەوەی پۆلی مۆرفیزمی تاك نیوکلۆتایتد (SNP) بوو لە جینی MAOA کە پەیوەندی بە رەفتاری شەرانگێزەوە هەیە و لەوەش زیاتر بە مێژووی ڕەفتاری تاوانکاری و هەڵوێستی دژە کۆمەلایەتی. 35 پیاو کە پێشینەی تاوانیان هەبووە لەم توێژینەوەدا بەشداریان کردووە و DNA خوێن بەپێی ڕێنمییەکانی بەرهەمهێنەر دەرهێنراوە (nomic DNA Extraction پG Kit,ROCHE,Grmanyپ). پرایمەری نەرمەکاڵای تایبەت بۆ دیزاینکردنی پرایمەرەکان بۆ گەورەکردنی ئێگزۆن 7 و ئێگزۆن 8 بەکارهات. SNP بە ئەنجامدانی Gradi پnt PCR (AB Appliپd Biosyst (ms, USA شیکرایەوە. بەرهەمەکانی PCR ڕێك دەخرێن پاشان بە بەکارهێنانی (BioEdit.پxپ) و پM ga 6 (/tپn.پgasoftwarپhttp://www.m) بۆ ڕێکخستن و شیکاری چەندین زنجیرە. نمونەی سیرۆمەکە بە بەکارهێنانی کیتی ELISA (Human

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(DA ELISA Kit,EA0041Hu China,پDopamin) شـیکرایـەوە بۆ پشـکنینی ئـاسـتی دۆپامین.لە کۆی گشـتی 35 بەشـداربووی تاوانبار,تەنها دوو جۆری جینی MAOA دەسـتنیشـان کرابوون, جۆرێـك لـە ئێگزۆنی 7ی جینی MAOA بوو (rs1181634890) و جۆرەکـانی تر لـە ئێگزۆنی 8 کە جۆرەکانی (rs6323) تێدابوو, 96.6% لە بەشـداربووان (rs6323) یان هەبوو. جۆرەکـانی بـەرزبوونـەوەی ئـاسـتی سـیرۆمی دۆپـامین لـە هـەردوو جۆرەکـەدا راپۆرت کرا. ئـەم ئەنجامەئاماژە بە رۆلێکی ئەگەری بۆ جۆرەکانی جینی MAOA لە پێشـکەوتن لە شـەرانگێزییەوە بۆ رەفتاری تاوانکاری دەکەن.