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MEASUREMENT OF AGGRESSIVE BEHAVIOUR AND MAOA GENE POLYMORPHISM IN ADOLESCENT POPULATION OF TEHSIL CHARBAGH AT DISTRICT SWAT: A CROSS-SECTIONAL STUDY

Original Article

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ABSTRACT

Background: Aggression represents a complex human behavioral trait arising from the interaction between genetic susceptibility and environmental influences. Dysregulation of monoaminergic neurotransmitters, including serotonin, dopamine, and noradrenaline, has been strongly implicated in aggressive and impulsive behaviors. The monoamine oxidase A (MAOA) gene plays a central role in this process by encoding an enzyme responsible for the metabolic breakdown of these neurotransmitters. Variations within the MAOA gene, particularly variable number tandem repeat (VNTR) polymorphisms in its promoter region, have been associated with altered enzyme activity and behavioral differences, especially among males due to X-linked inheritance.

Objective: The study aimed to assess aggression levels among adolescent males residing in Tehsil Charbagh, District Swat, and to examine their association with MAOA VNTR polymorphisms.

Methods: A cross-sectional study was conducted involving 70 male participants recruited from different localities of Tehsil Charbagh. Aggressive behavior was evaluated using a 23-item structured questionnaire administered over a 30-day period. Aggression scores were categorized into normal, less aggressive, and highly aggressive groups. Buccal swab samples were collected from all participants, followed by genomic DNA extraction. MAOA VNTR polymorphisms were identified using polymerase chain reaction amplification, and allele sizes were determined through agarose gel electrophoresis. Statistical analysis was performed using the Chi-square test to assess associations between genetic variants and aggression categories.

Results: Of the 70 participants, 56 (80.0%) were classified as less aggressive, 8 (11.42%) as highly aggressive, and 6 (8.57%) as normal. Molecular analysis identified three MAOA VNTR alleles: 321 bp in 15 individuals (21.4%), 351 bp in 33 individuals (47.1%), and 381 bp in 22 individuals (31.4%). The distribution of MAOA VNTR variants differed significantly across aggression categories. Chi-square analysis demonstrated a statistically significant association between MAOA VNTR polymorphisms and aggression levels ($\chi^2 = 12.764$, df = 4, $p = 0.012$).

Conclusion: The findings suggest that MAOA VNTR polymorphisms may contribute to inter-individual variation in aggressive behavior among adolescent males. Although aggression appears to be influenced by multiple factors, genetic variation within the MAOA gene may represent an important biological component underlying behavioral differences in this population.

Keywords: Aggression, Behavior, Gene Polymorphism, Male Adolescents, Monoamine Oxidase A, Pakistan, VNTR.

INTRODUCTION

Violence has been recognized by the World Health Organization as a major global public health concern because of its profound physical, psychological, and social consequences for individuals and communities worldwide (1). Among the core behavioral traits contributing to violence, aggression occupies a central position, as it encompasses intentional acts aimed at causing harm, injury, or damage to others. Such behaviors not only undermine social cohesion but also impose long-term mental and physical health burdens on both victims and perpetrators, making aggression an important focus of biomedical and behavioral research (2) Aggression is not a fixed trait; rather, it shows substantial inter-individual variability influenced by a complex interplay between genetic predisposition and environmental exposures. Evolutionary perspectives suggest that stabilizing selection maintains aggression within a certain range, as both excessively low and excessively high levels may be maladaptive in social contexts (3). Contemporary research increasingly emphasizes that genetic susceptibility may manifest as aggressive or antisocial behavior particularly under adverse environmental conditions, such as unstable family settings, social deprivation, or psychosocial stress. Among the genetic factors implicated in aggression, the monoamine oxidase A (MAOA) gene has received considerable attention. Located on the short arm of the X chromosome, MAOA encodes an enzyme responsible for the degradation of key neurotransmitters, including serotonin, dopamine, and norepinephrine, which are crucial regulators of mood, impulse control, and emotional processing (4). Evidence from pharmacological and clinical studies further underscores the relevance of MAOA, as its inhibitors are widely used in the treatment of psychiatric and cardiovascular disorders, highlighting the enzyme's central role in neurobehavioral regulation (5).

The link between MAOA and aggressive behavior was first highlighted through the identification of Brunner syndrome in a Dutch family, where affected male members exhibited impulsive aggression, antisocial behavior, and emotional dysregulation due to a functional deficiency of MAOA (6). Subsequent studies have expanded this observation, demonstrating that genetic variations, or polymorphisms, within the MAOA gene can influence enzyme activity and behavioral outcomes. These polymorphisms include single nucleotide polymorphisms and variable number tandem repeats (VNTRs), although only a subset has been shown to exert functional effects on gene expression or enzymatic activity (7,8). Of particular importance is a 30-base-pair VNTR located in the upstream regulatory region of the MAOA gene (MAOA-uVNTR). This polymorphism results in alleles associated with either low or high transcriptional activity of the enzyme. Commonly reported alleles include 2R, 3R, 3.5R, 4R, and 5R, with substantial evidence indicating that the 2R and 3R variants confer lower MAOA activity, whereas the 3.5R and 4R variants are linked to higher activity levels (9). Neuroimaging and behavioral studies suggest that individuals carrying low-activity MAOA variants may exhibit impaired emotional regulation and increased antisocial tendencies, particularly when exposed to adverse environmental conditions (10). Importantly, several molecular genetic studies have demonstrated that MAOA-uVNTR interacts with psychosocial stressors to predict aggressive and criminal behaviors, especially in males, due to the gene's X-linked inheritance pattern (11). Despite the growing international literature on MAOA polymorphisms and aggression, there remains a notable lack of population-specific data from many regions, including northern Pakistan. Sociocultural, environmental, and developmental factors unique to specific communities may modify gene–behavior relationships, underscoring the need for localized research. To date, no systematic investigation has examined the association between MAOA gene polymorphisms and aggressive behavior among adolescent males in Tehsil Charbagh, District Swat. Therefore, the objective of the present study is to investigate the correlation between MAOA gene polymorphisms, particularly MAOA-uVNTR variants, and aggressive behavior in adolescent males of Tehsil Charbagh, District Swat, with the rationale of addressing a critical regional knowledge gap and contributing to a more context-specific understanding of the biological underpinnings of aggression.

METHODS

A cross-sectional analytical study was conducted in Tehsil Charbagh, District Swat, to examine the association between MAOA gene polymorphisms and aggression levels in male participants. A total of 70 males aged 16–28 years were recruited from different localities of the tehsil using a non-probability convenience sampling approach. Participants were included if they were permanent residents of the area, biologically male, and willing to provide informed consent and biological samples. Individuals with a self-reported history of diagnosed psychiatric illness, neurological disorders, substance dependence, or current use of psychotropic medication were excluded to minimize potential confounding effects on behavioral assessment. Aggression was assessed using a 23-item structured questionnaire specifically developed by subject experts to evaluate behavioral tendencies relevant to aggression. The instrument was translated into Urdu to ensure linguistic clarity and cultural appropriateness, and it was administered over a 30-day data collection period. All participants completed the questionnaire independently after receiving standardized instructions. Total aggression scores were calculated

and transformed into a categorical variable, whereby scores of <30% were classified as "Normal," scores between 30% and 79% as "Less aggressive," and scores ≥80% as "Highly aggressive," enabling comparative statistical analysis across behavioral groups. For genetic analysis, buccal swab samples were collected from each participant using sterile techniques. Genomic DNA was extracted following standard laboratory protocols suitable for downstream molecular analysis. Amplification of the MAOA gene was performed using polymerase chain reaction (PCR) with a forward primer sequence of 5′-ACA GCC TGA CCG TGG AGA AG-3′ and a reverse primer sequence of 5′-GAACGTGACGCTCCATTCGGA-3′. Each 15 µl PCR reaction mixture comprised 7.5 µl of Green Master Mix, 0.5 µl of each primer, 2 µl of template DNA, and 4.5 µl of PCR-grade water (12). Thermal cycling 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, with a final extension step at 72 °C for 5 minutes.

PCR products were resolved on a 2% agarose gel prepared in 1× TAE buffer, and electrophoresis was performed alongside a 100 bp DNA ladder to estimate fragment sizes. Band sizes were visually compared with reference standards to identify MAOA-uVNTR polymorphisms corresponding to approximately 321 bp, 351 bp, and 381 bp fragments. These fragment sizes were used to categorize VNTR alleles for subsequent association analysis. Statistical analysis was carried out using IBM SPSS version 2021. Descriptive statistics were computed for demographic variables, aggression categories, and genotype frequencies. The association between MAOA-uVNTR polymorphisms and categorized aggression levels was evaluated using the Chi-square test, with statistical significance assessed at an appropriate alpha level (12). All analyses were performed under the assumption of independence of observations. Ethical approval for the study was obtained from the relevant institutional ethical review committee prior to data collection; written informed consent was secured from all participants, and for those below 18 years of age, consent was additionally obtained from parents or legal guardians. Confidentiality of personal and genetic information was strictly maintained, and samples were used solely for the purposes outlined in the study protocol.

RESULTS

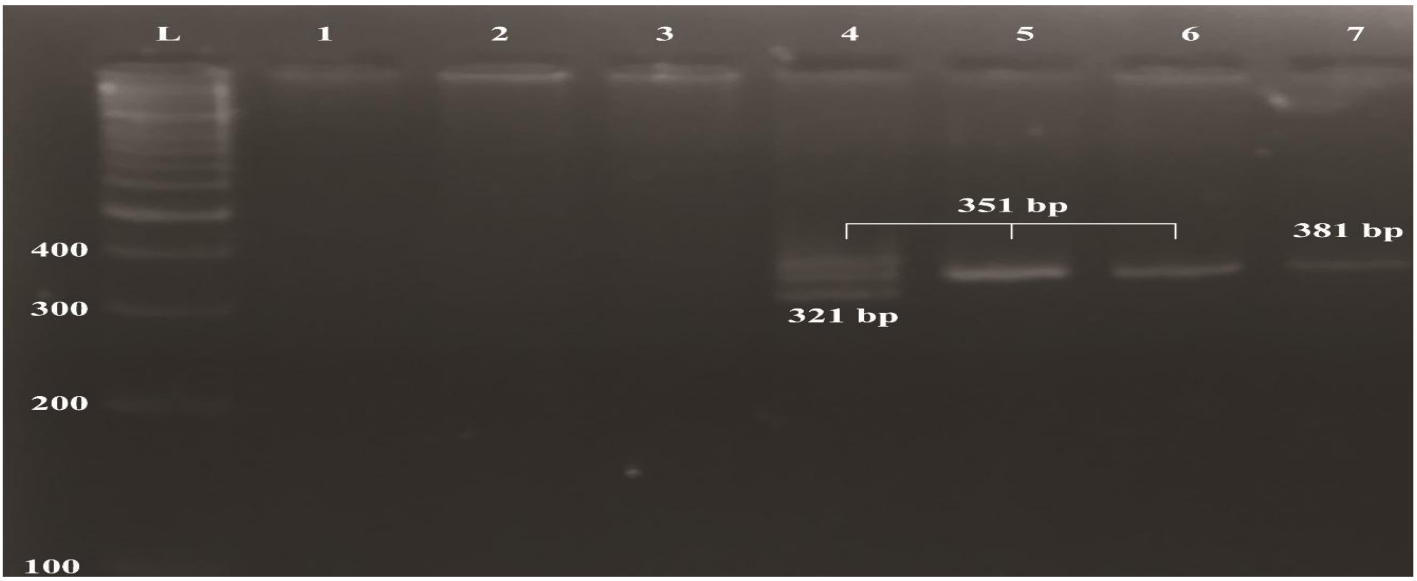
A total of 70 male participants from Tehsil Charbagh, District Swat, were successfully evaluated for behavioral aggressiveness and MAOA VNTR polymorphisms. Based on aggression scores derived from the standardized questionnaire, participants were categorized into three behavioral groups. The majority of individuals were classified as less aggressive, accounting for 80.0% (56/70) of the sample, while 11.42% (8/70) were categorized as highly aggressive and 8.57% (6/70) fell within the normal range. This distribution indicated a predominance of mild-to-moderate aggressive tendencies within the study population. Genetic analysis was successfully completed for all collected buccal swab samples. Three distinct MAOA-uVNTR allele sizes were identified following PCR amplification and agarose gel electrophoresis. The 351 bp allele was the most frequently observed variant, present in 47.1% (33/70) of participants, followed by the 381 bp allele in 31.4% (22/70) and the 321 bp allele in 21.4% (15/70). This distribution demonstrated a clear predominance of the intermediate-sized VNTR allele in the study cohort. When aggression categories were examined in relation to MAOA VNTR polymorphisms, notable differences in allele distribution across behavioral groups were observed. Among highly aggressive individuals, the 351 bp allele was the most common, followed by the 321 bp and 381 bp variants. In the less aggressive group, all three alleles were represented, with the 351 bp and 381 bp variants showing higher frequencies compared to the 321 bp allele. In the normal aggression category, comparatively fewer individuals were observed overall, with the 351 bp allele again being more frequent than the other variants. Across all behavioral categories, the 321 bp allele remained the least prevalent. Statistical analysis using the Chi-square test demonstrated a significant association between MAOA-uVNTR polymorphisms and categorized aggression levels ($\chi^2 = 12.764$, df = 4, $p = 0.012$). The likelihood ratio test further supported this association (value = 13.553, df = 4, $p = 0.009$), indicating that variability in MAOA VNTR alleles was significantly related to differences in aggressive behavior within the study population. No significant linear-by-linear trend was observed, suggesting that the relationship was not strictly ordinal across aggression categories.

Table 1: Aggression × Genotype association

Aggression Category	321 bp	351 bp	381 bp	Total
High Aggressive	2	5	1	8
Less Aggressive	11	25	20	56
Normal	2	3	1	6
Total	15	33	22	70

Table 2: Chi-Square test results

Test	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	12.764	4	0.012
Likelihood Ratio	13.553	4	0.009
Linear-by-Linear Association	0.684	1	0.408
N of Valid Cases	70	—	—



Well 1:100bp Ladder (Thermo Scientific gene ruler ref# SM0323), **Well 4:** 321bp MAOA Gene (Homozygote), **Well 5-6:** 351bp MAOA Gene (Homozygote), **Well 7:** 381bp MAOA Gene (Homozygote)

Figure 1. PCR Amplification Results

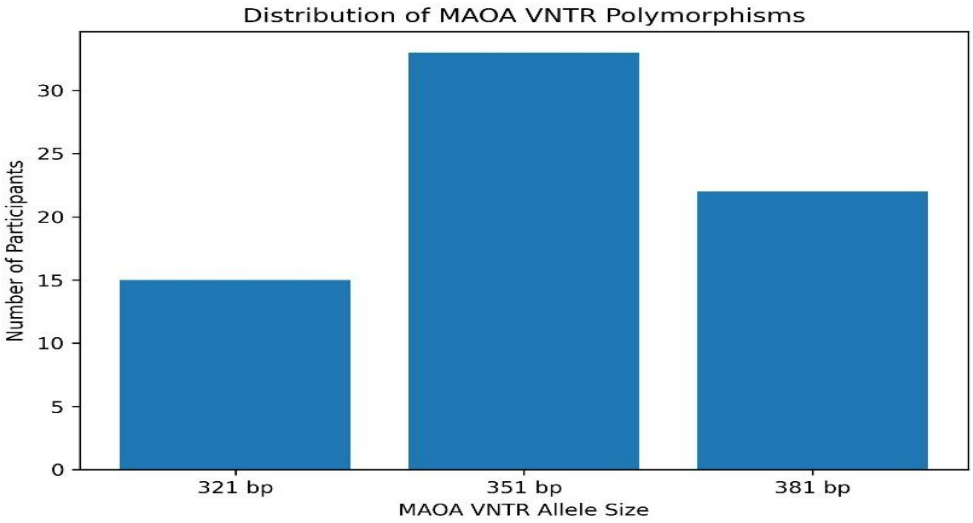


Figure 2 Distribution of MAOA VNTR Polymorphisms

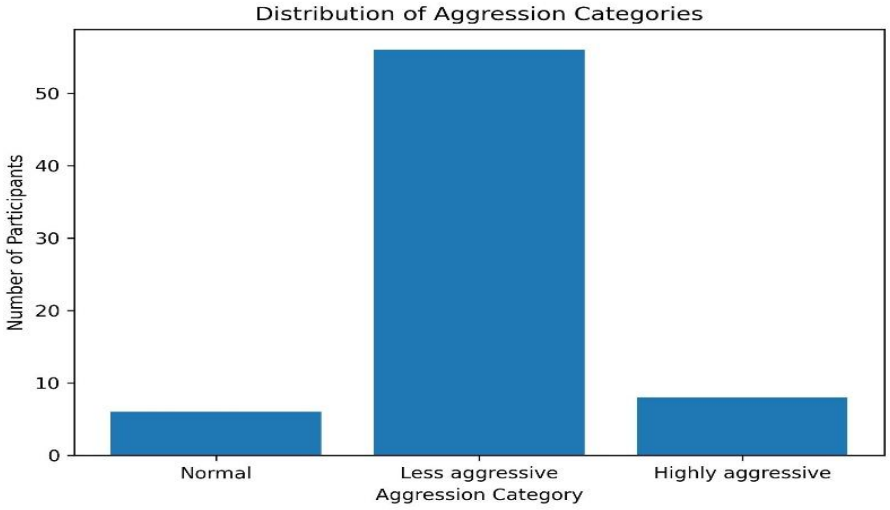


Figure 3 Distribution of Aggression Categories

DISCUSSION

The present study explored the relationship between MAOA VNTR polymorphisms and aggressive behavior among male adolescents from Tehsil Charbagh, District Swat, and provides population-specific insights into a gene–behavior association that has been widely investigated but remains context dependent. Most participants demonstrated low to moderate levels of aggression, with a relatively small proportion classified as highly aggressive. This distribution is consistent with behavioral research suggesting that extreme aggression is less prevalent in community-based adolescent samples and that subclinical or moderate aggression represents the dominant behavioral phenotype in most populations (10,12). Molecular analysis identified three MAOA VNTR alleles—321 bp, 351 bp, and 381 bp—with a predominance of the longer alleles and relative rarity of the 321 bp variant. This allelic pattern contrasts with findings reported in other regional populations, even within the same broader geographic district, where different VNTR distributions have been observed (13). Such variation supports the concept that MAOA VNTR allele frequencies are not uniform across populations and may reflect underlying genetic stratification influenced by ethnicity, migration patterns, and local demographic history. These findings emphasize the importance of conducting region-specific genetic studies rather than extrapolating results from genetically distinct populations. The observed association between MAOA VNTR polymorphisms and aggression aligns with earlier evidence indicating that functional

variation within the MAOA promoter region influences transcriptional efficiency and enzyme activity, thereby modulating monoaminergic neurotransmission involved in impulse control and emotional regulation (14,15). Lower-activity variants have been repeatedly associated with increased vulnerability to antisocial and aggressive behaviors, particularly in males due to the hemizygous expression of X-linked genes. In the present study, longer VNTR alleles were more frequently observed among individuals with higher aggression scores, suggesting that MAOA-related behavioral effects may not be solely attributable to a simple low- versus high-activity dichotomy and may instead reflect more complex regulatory mechanisms or population-specific functional consequences.

Importantly, the findings also support the broader gene–environment interaction framework, wherein MAOA polymorphisms exert their behavioral influence in conjunction with environmental exposures rather than acting as isolated determinants. Aggressive behavior is well established as a multifactorial outcome shaped by early life stress, socioeconomic adversity, family instability, and trauma, which may interact with MAOA genotype to amplify or buffer behavioral risk (16,17). Given the socioculturally diverse and potentially stress-prone environment of the study area, it is plausible that unmeasured environmental factors interacted with genetic susceptibility to influence aggression levels in this cohort. This reinforces the view that genetic predisposition confers risk rather than inevitability and that environmental modulation remains a critical determinant of behavioral outcomes (18-20). Several strengths of the study merit consideration. It represents one of the first attempts to investigate MAOA VNTR polymorphisms in relation to aggression within this specific population, thereby addressing a clear regional research gap. The integration of behavioral assessment with molecular genetic analysis provides a biologically informed approach to understanding aggression, and the use of standardized laboratory techniques ensured reliable genotyping outcomes. However, important limitations must also be acknowledged. The modest sample size limited statistical power and may have constrained the detection of subtler genotype–phenotype associations. The exclusive inclusion of male participants, while methodologically justified given the X-linked nature of MAOA, precluded evaluation of sex-specific effects and limits the generalizability of findings to females. Additionally, environmental risk factors were not quantitatively assessed, restricting the ability to formally test gene–environment interactions. The cross-sectional design further limited causal inference, as temporal relationships between genetic variation, environmental exposure, and aggressive behavior could not be established.

Future research would benefit from larger, ethnically diverse samples and the inclusion of both sexes to enable more comprehensive modeling of MAOA-related behavioral effects. Longitudinal designs incorporating validated measures of childhood adversity, psychosocial stress, and socioeconomic status would be particularly valuable in clarifying developmental pathways linking MAOA polymorphisms to aggression. Functional analyses distinguishing low- and high-activity alleles and their neurobiological correlates may also enhance interpretability of behavioral associations (21). In summary, the study provided evidence that MAOA VNTR polymorphisms were associated with variation in aggressive behavior among adolescent males from Tehsil Charbagh, while also highlighting notable regional differences in allele distribution. The findings were consistent with the broader literature supporting a gene–environment interaction model of aggression and underscore the need for early preventive and psychosocial interventions during adolescence. Although constrained by sample size and design limitations, the results contribute meaningful population-specific data and lay the groundwork for future multidisciplinary research integrating genetics, environment, and behavioral health.

CONCLUSION

This study addressed the objective of exploring the relationship between MAOA VNTR polymorphisms and aggressive behavior among adolescent males from Tehsil Charbagh, District Swat, and demonstrated that genetic variation within the MAOA gene may play a contributory role in shaping aggression patterns in this population. The predominance of specific MAOA alleles alongside generally mild aggression levels suggests that genetic influences on behavior are subtle and likely modulated by broader biological and environmental contexts rather than acting in isolation. Although a definitive association could not be established, the findings add valuable population-specific insight and highlight the importance of integrating genetic perspectives into behavioral research. These results underscore the need for larger, well-designed studies to better clarify the role of MAOA variation in aggression and to inform future prevention and early intervention strategies aimed at reducing aggressive and antisocial behaviors during adolescence.

AUTHOR CONTRIBUTIONS

Author	Contribution
Hasnain Ali Khan	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Syed Ihteshamullah*	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Attia Rehman	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Isra khan	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Sidra Bano	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Hafsa	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published
Inam Ullah	Contributed to study concept and Data collection Has given Final Approval of the version to be published
Zeeshan Khan	Writing - Review & Editing, Assistance with Data Curation
Muhammad Israr*	Writing - Review & Editing, Assistance with Data Curation

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