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The rs6323 and uVNTR Polymorphisms in the MAOA Gene are Associated with Attention Deficit Hyperactivity Disorder in Iranian Azeri Children

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Abstract

Background: ADHD is the most prevalent psychiatric health issue in youth, which may also affect adults. Environmental and genetic factors both contribute significantly to the development and progression of this condition. Monoamine oxidases, which catalyze the metabolism of dopaminergic neurotransmitters, are involved in the pathogenesis of ADHD. The purpose of this study was to determine the connection between polymorphic variations rs6323 and uVNTR in the (Un translate variable nucleotide tandem repeat) MAO-A gene and the risk for ADHD in Iranian-Azeri children.

Methods: Clinical evaluation was used to recruit 137 ADHD patients (female 22, male 115) and 100 controls (female 48, male 52) from the East Azerbaijan region in northern Iran. Genomic DNA was taken from their peripheral blood samples and genotyping was performed using PCR-based amplification of target sites. SPSS (Version 16) and the javastat online statistics program (http://statpages.org/ctab2x2.html) were used for statistical analysis.

Results: The rs6323TT genotype was shown to be a significant risk factor for ADHD (OR 3.619, 95 percent CI 0.878-17.213, p = 0.044). In comparison, no significant differences in allele frequencies were observed between ADHD patients and the control group (p > 0.05). The 5R allele of uVNTR was shown to have a substantial protective impact against the development of ADHD (OR0.349, 95 percent confidence interval 0.151-0.797, p = 0.006).

Conclusion: Our findings indicate that MAOA gene polymorphisms may play a role in the start and development of ADHD in Iranian-Azeri youngsters. However, more research with larger sample sizes is necessary to corroborate these results.

Key Words: Attention Deficit Hyperactivity Disorder (ADHD), Genotyping, Iranian-azeri children, Molecular genetic investigation, Monoamine Oxidase-A (MAOA) gene, PCR-based amplification, Polymorphism, TT genotype.

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1- INTRODUCTION

Attention-deficit/hyperactivity disorder (ADHD) is the most prevalent mental health condition. It is characterized by a number of chronic difficulties, including trouble paying attention, hyperactivity, and impulsive conduct (1). The global prevalence of ADHD has been assessed to be approximately 5%, while the prevalence of all ADHD kinds has been calculated to be 12.12% in Tabriz, Iran (2, 3). ADHD develops in genetically predisposed people who are exposed to particular variables that have a significant role in its genesis (4, 5).

The genetic basis of ADHD is still unknown. According to researchers, genetic polymorphisms in the dopaminergic, serotonergic, and noradrenergic pathways all contribute to the development of ADHD (4). Consequently, the role of the dopamine D4 receptor (DRD4), the dopamine D5 receptor (DRD5), the dopamine D2 receptor (DRD2), the dopamine D5 receptor gene, the synaptosomal-associated protein 25 (SNAP-25) gene, the serotonin transporter gene (SLC6A4), the dopamine beta-hydroxylase (DBH), and the norepinephrine transporter protein (NET) gene are reported in the onset and development of ADHD (6, 7). Due to the MAOA gene's significance in a variety of psychopathologies in adults and children, including antisocial behavior, autism spectrum disorder, and impulsivity, the present molecular genetic investigation examined two functional polymorphisms in MAOA genes (4, 8).

The MAOA gene is located on the X chromosome (Xp11.23) and encodes the enzyme monoamine oxidase A, which is required for the catabolism of neurotransmitters such as dopamine, norepinephrine, epinephrine, melatonin, and serotonin (9, 10).

Numerous studies have shown an association between ADHD and the upstream variable number tandem repeats (uVNTR) in the promoter and the MAOA gene variant rs6323 (4).

The polymorphism rs6323 (R297R / Arg297Arg) is a functional single nucleotide polymorphism situated in exon 8 that is related to altered enzyme activity (11). The substitution of T for G at this position results in an amino acid shift from valine to phenylalanine, which enhances MAOA activity (12).

uVNTR is a polymorphic variation situated about 1.2 kb upstream of the MAOA gene transcriptional regions. It consists of 30 bp repetitions of "the -ACCGGCACCGGCACCAGTACCCGCACCAGT-5' " sequence. Alleles varied in the number of copies (2, 3, 3.5, 4, 5, or 6) (13, 14). According to previous studies, some uVNTR polymorphisms operate as a mediator in the connection between certain environmental risk factors and child behavioral issues (15, 16).

2- MATERIALS AND METHODS

2-1. Participants

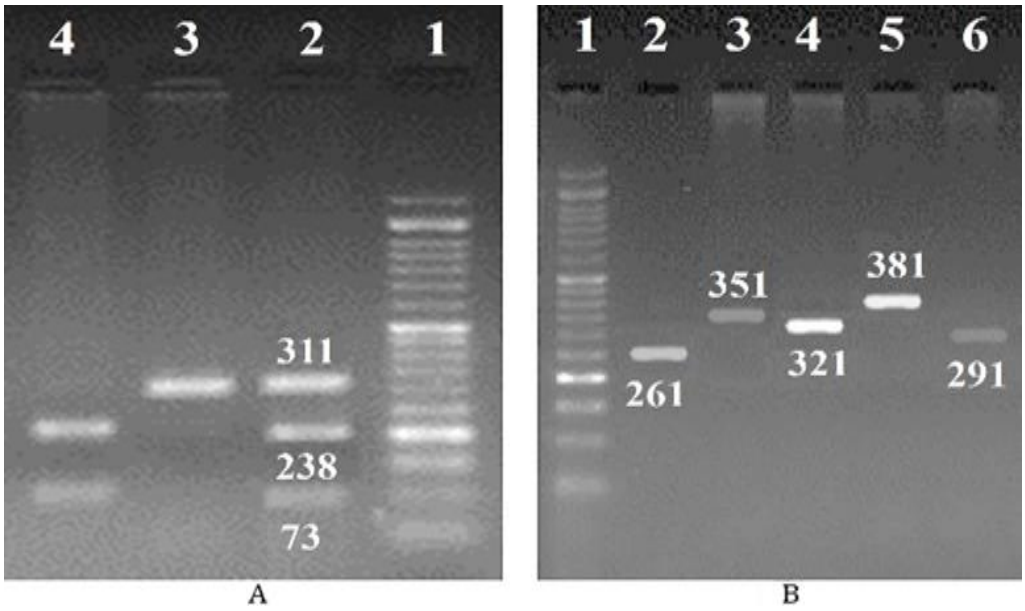
This case-control research included 100 healthy participants and 130 ADHD sufferers ranging in age from 5 to 14 years. Between 2013 and 2015, all patients were recruited from the Sheikhol Rais Hospital in Tabriz, Iran. Physical examinations of the control participants were performed to rule out any neurological, psychological, or learning abnormalities.

2-2. Procedural procedures:

The salting-out approach was used to isolate genomic DNA from peripheral blood samples. PCR (Polymerase Chain Reaction) and PCR RFLP (Restriction Fragment Length Polymorphism) analyses were used to identify the uVNTR and rs6323 polymorphisms, respectively.

The forward primer 5'-TGTTGCCTCACAGTTGCC-3' and the reverse primer 5'-AGCCTACCCTTCTTCC-3' were used to amplify a 312 bp DNA fragment, including the rs6323 polymorphism, and the genotype analysis of uVNTR was performed using the forward and reverse primers 5'-AGCCTGACCGTGGAGAAGG-3' and 5'-AGGTGTCGTCCAAGCTGG-3', Each PCR experiment included 5–10 ng of genomic DNA, 0.2 mM forward and reverse primers, 10xPCR buffer, 1.5 mM MgCl2, 200 mM dNTPs (DeoxyNucleotide Three Phosphates), and 1 unit of Taq DNA Polymerase (Cinnagen, Iran).

Fnu4H I (New England BioLabs, Beverly, Mass) was used as the restriction enzyme for rs6323 RFLP, which cleaves the G allele to create fragments of 238 and 73 bp in size (**Fig. 1/A**). uVNTR 2R (261 bp), 3R (291 bp), 4R (321 bp), 5R (351 bp), and 6R (381 bp) PCR products were found (**Fig. 1/B**).



A: lane1, 50 bp DNA ladder; lane 2 heterozygous samples; lane 4 homozygous C; and lane 3 homozygous T; all from ADHD patients. B: lanes 1 and 2, 50 bp DNA ladder Lane 2 has two repetitions of uVNTR, Lane 3 contains five repetitions of uVNTR, Lane 4 contains four repetitions of uVNTR, Lane 5 contains six repetitions of uVNTR, and Lane 6 contains three repetitions of uVNTR.

Fig 1: Gel image showing two MAOA polymorphisms

2-3. Data Analysis:

The data were analyzed statistically using the statistical program for social sciences, version 16.0. (SPSS, Chicago). To determine the departure from Hardy-Weinberg Equilibrium (HWE) (http://www.oege.org/software/hardy-weinberg.html) of SNPs and to compare MAOA allele and genotype frequencies in patients and controls, the Java astat online statistics program (http://statpages.org/ctab2x2.html) was used. The connection between the genotypes or alleles of the MAOA rs6323 and uVNTR polymorphisms and the risk of ADHD was assessed using logistic regression to calculate odds ratios (OR) with 95 percent confidence intervals (CI). The P-value was judged significant at a level of 0.05.

3- RESULTS

3-1. ADHD's clinical features include the following

All patients in this research were from northwestern Iran; a total of 137 patients (22 females and 115 males) ranging in age from 3 to 8 years (mean age 8.315 1.66 years) were evaluated. There were no statistically significant variations in the mean age of participants (p=0.16), between ADHD and disease-free people (**Table1**). Additionally, 48% of the 100 controls were female, whereas 52% were male.

Table-1: Frequencies of alleles and genotypes of the rs6323 MAOA gene polymorphism in ADHD patients and controls

Genotype/ Allele	Case (n=137)	Control (n=100)	OR (95%CI)	P-Value
GG	81(59.12%)	55(55%)	1(Reference)	
GT	37(27%)	42(42%)	0.598(0.311-1.147)	0.096
TT	16(11.67%)	3 (3%)	3.619(0.878-17.213)	0.044*
G	199 (74.25%)	152 (76%)	1(Reference)	-
T	69 (25.74%)	48 (24%)	1.098(0.551-2.190)	0.776

(CI, confidence interval; N, number; OR, odds ratio)

3-2. ADHD with rs6323 and uVNTR of MAOA

In this research, we examined the relationships between rs6323 and the uVNTR of MAOA and the risk of ADHD. In sum, the TT genotype was associated with an increased incidence of ADHD (OR 3.619, 95% CI 0.878-17.213, p=0.044). In comparison, no significant differences in allele frequencies were observed between ADHD patients and the control group (p > 0.05) (**Table 1**). Additionally, the 5R was shown to have a substantial protective impact against the development of attention deficit/hyperactivity disorder (OR0.349, 95 percent confidence interval [CI] 0.151-0.797, p=0.006) (**Table2**).

Table-2: MAOA uVNTR allele and genotype frequencies in ADHD patients and controls

Genotype	Case (n=137)	Control (n=100)	OR (95%CI)	P-Value
2 repeats	3(2.2%)	1(1%)	1.554(0.11-44.083)	0.718
3 repeats	42(30.7%)	34(34%)	0.638(0.310-1.309)	0.184
4 repeats	62(45.3%)	32 (32%)	1(Reference)	-
5 repeats	21(15.3%)	31 (31%)	0.349(0.151-0.797)	0.006*
6 repeats	9 (6.6%)	2(2%)	2.331(0.395-17.652)	0.302

(CI, confidence interval; N, number; OR, odds ratio)

4- DISCUSSION

Attention deficit/hyperactivity disorder is a multifaceted illness that is influenced by genetic predisposition and environmental circumstances. MAOA has, previously, been identified as a potentially susceptible gene for behavioral attributes and psychiatric disorders (17-20). The present study examined the connection of the MAOA rs6323 and uVNTR polymorphisms with ADHD in Iran's northwestern population. We identified departures from the Hardy-Weinberg equilibrium in the ADHD loci rs6323 and uVNTR (p 0.05). Thus, the case group demonstrates a genetic link to illness (21). Our findings suggest that the rs6323

polymorphism has a substantial impact on the development of ADHD in Iranian-Azeri youngsters.

Additionally, there is a correlation between the TT genotype of the MAOA rs6223 variation and ADHD (p 0.05) (**Table 1**).

Wook Hwang et al. (2018), on the other hand, demonstrate that the TT genotype of rs6323 is protective against ADHD in Korean children. In another research, Wei et al. (2011) showed that the MAOA functional polymorphism had no significant connection with schizophrenia in Han Chinese. Nonetheless, rs6323T-rs1799836G was the schizophrenia risk haplotype in female patients (11). Additionally, Karmakar et al. (2017) demonstrate in a pilot investigation that maternal transmission of rs6323'G' to male probands is statistically significant in ADHDs (22).

According to our findings, 5R, a low-expressing allele of uVNTR, was significantly more prevalent in controls than in ADHD patients (p 0.05) (**Table 2**).

In comparison to healthy persons, Ahmed. Salem et al. (2013) found that the low-expressing MAOA uVNTR allele was usually more prevalent in female autistic patients than in healthy individuals (23). According to Ying-HuiWu et al. (2007), Alzheimer disease patients with a long MAOA-VNTR genotype (composed of 3.5-or 4-repeat alleles) exhibited increased MAOA activity and gene expression in the brain, as well as participation in alterations in monoamine metabolism (24). Beverly H. Brummett et al. (2009) suggested that persons with less active MAOA-uVNTR alleles may be more susceptible to depressive symptoms and poor sleep (25).

4-1. Strengths and limitations of the study

The study's small sample size was a significant restriction. In comparison, our research benefited from matching people by sex and age to account for the impact of these characteristics, and using questionnaire survey and clinical examination for sample selection enabled us to separate the pure case and control groups.

5- CONCLUSION

In conclusion, this research gives insight into the association between rs6323TT SNPs in eight exons, ADHD, and the protective impact of the 5R uVNTR polymorphism on the MAO-A promoter area on the development of ADHD in Iranian-Azeri children. Thus, our findings suggest that polymorphisms in the monoamine oxidase-A gene are likely to have a significant influence on the onset and progression of ADHD. However, more research using a larger sample size is needed to corroborate these results.

6- ETHICAL CONSIDERATIONS

The study protocol was approved by the ethical committee of Tabriz University of Medical Sciences (ID No. 6/5/12152), and all participants provided us with verbal and written permissions.

7- ACKNOWLEDGMENTS

We would like to thank all the participants and their parents for their contribution to this study.

8- COMPETING INTERESTS

None.

9- AUTHORS' CONTRIBUTIONS

LM designed and planned this study. NV designed the primers, analyzed, and supervised the laboratory examination, and LM and SA were major contributors to writing the manuscript. ST and SA performed laboratory examinations. MH supplied the laboratory and machines. All authors read and approved the final manuscript.

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