

Study of Polymorphism of the DRD2 Gene (-141C Ins/Del, rs1799732) with Attention Deficit Hyperactivity Disorder a Population Sample of Children in Iranian-Azeri

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Abstract

Background

Attention deficit hyperactivity disorder (ADHD), is a multifactorial disorder and converging evidence has implicated abnormalities of dopamine neurotransmission. The aim of this study was to examine the association of -141 polymorphisms in DRD₂ gene with ADHA among Iranian-Azeri population.

Materials and Methods

A case-control association study included 153 patients with attention deficit hyper activity disorder (case group), and 133 healthy subjects (control group). Genomic DNA was extracted peripheral blood samples by salting-out method. Single nucleotide polymorphism (SNP) genotyping was performed by Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. The data analysis was performed through Chi-square, with a significance level of 0.05.

Results: There was not significant difference in the allele and genotype frequencies between ADHD and -141C Ins/Del polymorphism in cases and controls ($P>0.05$). Ins/Ins homozygous dominants were more frequent in control group than the case group, but there was not significant difference observed ($P>0.05$). Del/Del homozygous dominants were not observed. No significant difference was detected in the allele and genotype frequencies between ADHD and -141 Insertion/Deletion polymorphism in cases and control groups ($P>0.05$).

Conclusion

Our results do not detected association between the -141C Ins/Del, rs1799732, polymorphism and ADHD disorder in population of Children in Iranian-Azeri.

Key Words: ADHD, DRD₂ gene, Iranian children, Polymorphism.

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1- INTRODUCTION

Attention deficit hyperactivity disorder (ADHD), is a most common mental disorders that begins during early childhood and negatively affect the functionality at various levels (1). The principal characteristics of ADHD are extreme motor activity, increased impulsivity and attention deficits (2). ADHD is a complex genetic disorder, and many different factors are a crucial role in its etiology and course (3).

Dysregulation in catecholamine neurotransmission such as dopamine seems to implicate in pathophysiology of ADHD (4, 5). Dopamine is a monoamine catecholamine neurotransmitter and dopamine receptors are key elements of the dopaminergic system that acts by five distinct but closely related G protein-coupled receptors (GPCRs) (6). The predominant type of auto receptors that is involved in the presynaptic regulation of the firing rate, synthesis of dopamine and release of dopamine seems to be D2 dopamine receptor.

The dopamine receptor D₂ (DRD2) gene is localized on human chromosome 11 at q22–q23, and has eight exons (7). The promoter polymorphism of the DRD2 gene (-141C Ins/Del, rs1799732) involving the insertion (I)/deletion (Del) of a cytosine alters its transcriptional activity and thus regulates the expression of DRD2 receptor (8). The aim of current investigation was the possible involvement of polymorphisms of the DRD2 gene in ADHD children of Iranian-Azeri population.

2- MATERIALS AND METHODS

2-1. Selection of Samples

All participants were diagnosed with ADHD following a detailed psychiatric assessment. At the end, 153 ADHD patients were recruited at Sheikhol Rais Hospital in Tabriz, Iran, during 2013 to

2015. Oral and written informed consents were obtained from at one parent of all participants, and the research protocol was approved by the ethics committee of Tabriz University of Medical sciences (ID No.6/5/12152). For control group, 96 volunteers were recruited from local children's Hospital in Tabriz- Iran, in the same age range (3-8 years old). They were also examined to rule out any neurological, psychiatric, or learning problems.

2-2. Molecular techniques

The genomic DNA was extracted from peripheral blood cell using standard techniques and subsequently used as a template for determination of DRD2 genotypes. Determination of -141delC genotype was performed by Restriction Fragment Length Polymorphism (RFLP) analysis of Polymerase chain reaction (PCR) products. A genomic sequence including 156 base pair (bp) of DRD2 was amplified by PCR using forward primer 5'GACCCAGCCTGCAATCAC3' and reverse primer 5'AGGAGCTGTACCTCCTCGG3'.

Genomic DNA of 5–10 ng was amplified in a PCR master mix containing 0.2 mM of forward primer and 0.2 mM of reverse primer, 10x PCR buffer, 1.5 mM MgCl₂, 200 mM dNTPs, and 1 unit of Taq DNA Polymerase (Cinnagen, Iran), in a 25 mL volume. Amplification conditions were Step- 1: 95°C for 3 min, Step- 2: 95°C for 30 s, Step- 3: 65°C for 30 s, Step- 4: 72°C for 30 s, Steps 2–4 were repeated 40 times followed by a final 72°C step for 2 min. Amplified PCR fragments were digested with Thermo Scientific MvaI (BstNI) restriction enzyme digested fragments were visualized via acrylamide gel electrophoresis.

2-3. Statistical analysis

Statistical analysis was performed by Chi-square test. The Fisher's exact test was applied to compare the variables when the number of samples was equal or less than

5. P-value <0.05 were considered as significant level (**Table.1**). To assess Hardy-Weinberg equilibrium, an online

Hardy Weinberg equilibrium test (HWE) calculator, was applied.

Table-1: Genotypic profiles obtained for -141 DRD2 polymorphism

Single nucleotide polymorphism	Primers	Annealing temperature	Restriction enzyme	Fragment size
-141C Ins/Del	F:5'GACCCAGCCTGCAATCAC3' R:5'AGGAGCTGTACCTCCTCGG3'	57°C/	Bst NI	Ins C = 124, 32 Del C = 156

3- RESULTS

To investigate the association of DRD2 Gene -141 C ins/del polymorphism with attention deficit hyperactivity disorder, a total of 153 patients with attention deficit hyperactivity and 133 healthy subjects with the mean age agreement with the mean age of the patient group were genotyped (mean age= 6.2 ± 0.328 years old). 78.57% of cases were male and 21.43% were female. Among 133 controls, 54.44% were male and 45.55% were female. There was not a deviation from Hardy–Weinberg equilibrium in the genotypes and alleles frequencies between patient and control groups.

Observed genotype and allele frequencies were compared with Pearson Chi-square test. The frequencies of genotypes and alleles for the -141 C ins/del polymorphism were given in **Table-2**.

Figures 1 and 2 confirm the results presented in **Table.2**. Consistently, no statistically significant difference in the frequency of the two variant alleles between case and control groups in -141 C ins/del polymorphism was showed. Consistently, no significant difference was showed in the genotypic frequencies between patients and healthy individuals (P>0.05) (**Table 2**).

Table-2: Genotype and allele frequencies of the DRD2 Gene (-141C Ins/Del, rs1799732)

rs1799732	Case (%) n=153	Control (%) n=133	OR (95% CI)	P-value
Genotypes				
Ins/Ins	111(72.55)	110(82.70)	0.553(0.265-1.148)	0.086
Ins/Del	42(27.45)	23(17.29)	1.810(0.872-3.777)	0.086
Del/Del	0	0		
Alleles				
Ins	264(86.27)	243(91.35)	0.595(0.22-1.581)	0.235
Del	42(13.72)	23(8.64)	1.681(0.632-4.543)	0.235

Ins: Insertion; Del: Deletion, OR: odds ratio, CI: Confidence Interval. Estimated relative risks with odds ratios (OR), and 95 % confidence intervals (95 % CI), and P- values for association between rs1799732 and ADHD disorder risk.

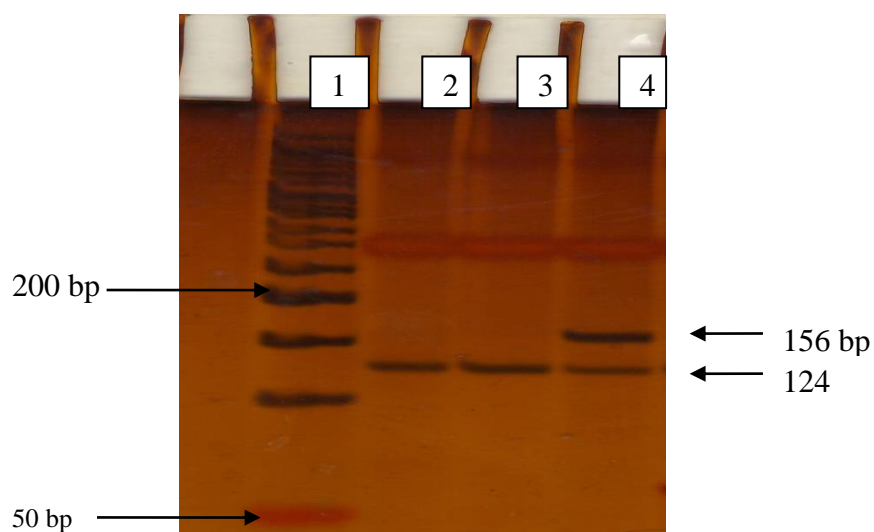


Fig.1: 1; 50 bp DNA ladder; 2,3: Insertion/Insertion genotype; 4; Insertion/Deletion genotype.

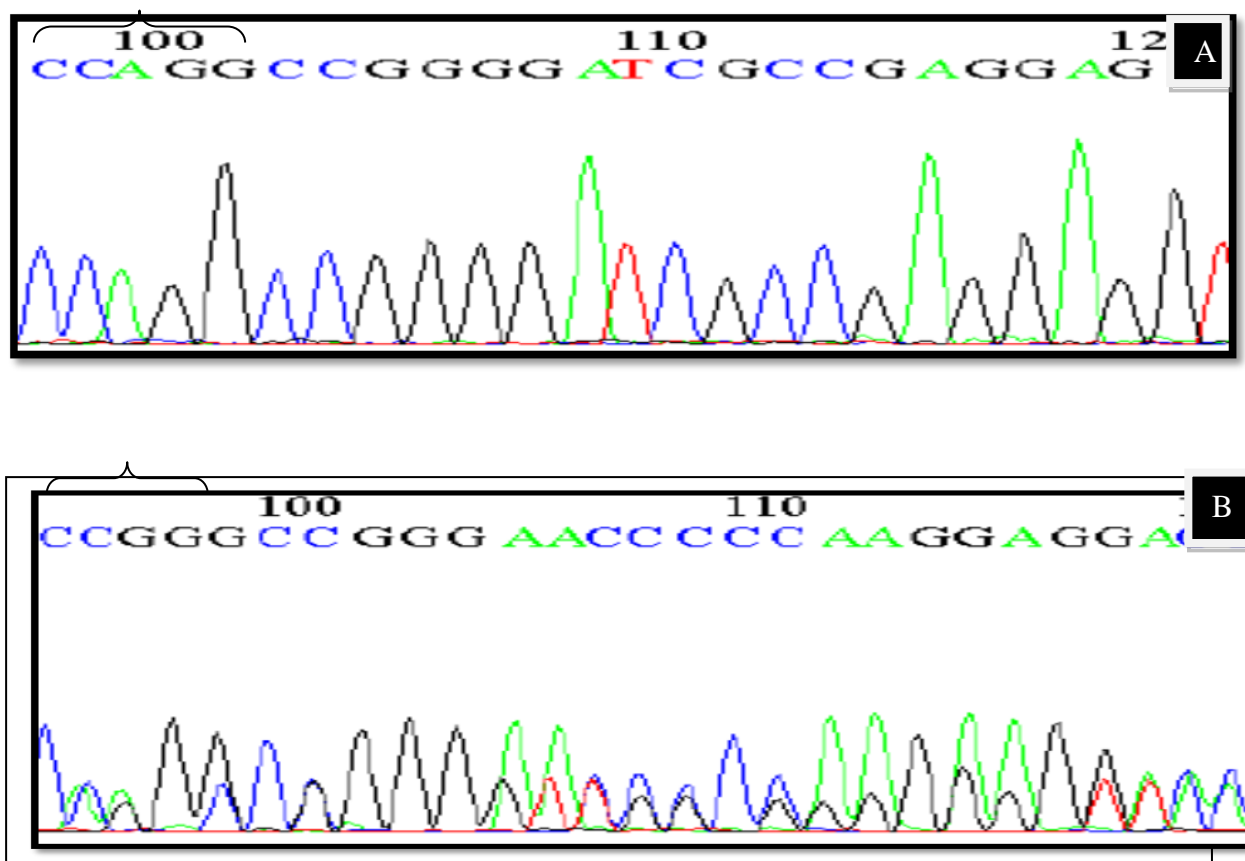


Fig.2: Sequencing, **A:** dominant homozygous, **B:** heterozygous. Mark indicates the position of restriction enzyme recognition sites. In homozygous sample, restriction enzyme has not deleted recognition site but in heterozygous sample, one of strands of DNA is normal and other strand has both of deletion and mutation.

4- DISCUSSION

A number of theories have demanded the involvement of brain dopamine pathways in the attention and executive functions that are believed to be altered in ADHD (9). Magnetic resonance imaging (MRI) and other imaging techniques have detected abnormalities in areas of prefrontal cortex, cingulate gyrus and anterior basal ganglia in children with ADHD, adding some experimental basis to this theoretical framework implicating dopamine in attention control (9-11). However, these data, which support the implicating brain dopamine circuitry in ADHD, and direct evidence of its involvement, is still unclear. Nevertheless, this dilemma is starting to be resolved by genetic research.

Since a number of confirmations from pharmacological (12), and positron-emission tomography studies (13), confirm the role of the dopamine neurotransmitter system in the etiology of ADHD, molecular genetic studies, have mostly concentrated on genes in these pathways. Because dopamine is engaged in a variety of critical functions, it is not shocking that multiple human disorders have been related to dopaminergic dysfunctions. Therefore, recently we performed a case-control study to assess the possibility of an association between a single nucleotide polymorphism of DRD2 gene, and ADHD risk with children samples from Iranian-Azeri. In the current study, significant variation was not showed in the allele and genotype frequencies between ADHD and polymorphism of -141C Ins/Del, promoter (rs1799732), DRD2 gene in cases and controls ($P>0.05$) (Table.2).

In this polymorphism of DRD2 gene, Ins/Ins homozygous dominants were more frequent in control group than the case group, but significant difference was not significant, adding, Ins/Del heterozygotes were more frequent in the case group than the control group, but difference was not

significant. No significant difference in the allele frequency between case and control groups was observed.

4-1. Limitations of the study

One of the restrictions of this research was the low number of participants. We hope this study can be repeated by other researchers in different parts of the country with higher number of subjects so that one can evaluate and scrutinize the data more prudently.

5- CONCLUSION

In summary, our data supports lack of association between -141C Ins/Del, promoter (rs1799732) gene polymorphism and ADHD. Further studies on larger population samples and other ethnic groups will be required for explaining the linkage between DRD2 polymorphism and risk of ADHD.

6- CONFLICT OF INTEREST: None.

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