

The Association of DRD2 Gene TaqI Polymorphism with Attention Deficit Hyperactivity Disorder a Population Sample of Iranian Azeri-children

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Abstract

Background

Attention deficit hyperactivity disorder (ADHD) is a multi-factorial disorder that has defined by hyperactivity, impulsivity and attention deficits. Various neurotransmitters such as dopamine can play a role in its pathophysiology. The aim of this study was to examine the association of two common single nucleotide polymorphisms in DRD₂ gene, Taq I A (T/C) and Taq I B (G/A), with ADHA risk among Iranian-Azeri population.

Materials and Methods

A study of case-control association was performed with 89 samples with attention deficit hyperactivity disorder and 96 healthy subjects. Peripheral blood samples were used for Genomic DNA extraction by salting-out method. SNP genotyping was carried out by PCR-RFLP technique. The collected data were analyzed through javastant online statistics software, using Chi-square, with a significance level of 0.05.

Results

There was not a significant difference in the allele and genotype frequencies between ADHD and Taq1B polymorphism in cases and controls ($P>0.05$). In the Taq IA of DRD2 gene, TT homozygous dominants and CC homozygous recessives were more frequent in case group than in control group but significant difference was not observed ($P>0.05$). Also, T/C heterozygotes were more frequent among the control group than the case group, and difference was significant ($P<0.05$).

Conclusion

Our data supports lack of association between Taq1A and Taq1B gene polymorphisms and ADHD.

Key Words: ADHD, DRD₂ gene, Biomarkers, Polymorphism.

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1- INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is one of the most common neurobehavioral disorders that begins during early childhood and negatively affects the functionality at various levels (1). ADHD has a strong genetic background, and many different factors are a crucial role in its etiology and course. About its pathophysiology hypotheses implicate various neurotransmitters such as dopamine (2, 3). D2 dopamine receptor seems to be the predominant type of autoreceptors that is involved in the presynaptic regulation of the firing rate, dopamine synthesis and dopamine release. ADHD is as a polygenetic disorder with various candidate genes. The multifactorial concept is constant with high population spread of ADHD (3–6%), high conformity in monozygotic twins (68–81%), but lowest return risk to first-degree relatives (4). The highest levels of D2 dopamine receptors are found in the striatum, accumbens nucleus, and the olfactory tubercle. Also, these receptors are expressed at significant levels in the substantia nigra, ventral tegmental area, hypothalamus, cortical areas, septum, amygdala, and hippocampus (5-8). This receptor seems to be critical for learning and memory mechanisms, such as working memory, that are primarily mediated by the prefrontal cortex (9, 10).

The dopamine receptor D2 (DRD2) gene is localized on human chromosome 11 at q22–q23, extends over 270 kb, and has eight exons (11). An uncommon Taq IA restriction fragment length polymorphism (RFLP), (rs1800497, T/C), has located in region of the 3' flanking of the DRD2 gene. A second polymorphism, Taq IB RFLP, (rs17294542, G/A), has located to regions of the regulatory and structural coding (5'region) of the gene (12). The aim of current study was the possible involvement of the DRD2 gene

polymorphisms in ADHD in a population sample of children in Iranian-Azeri.

2-MATERIALS AND METHODS

2-1. Selection of samples

All participants were diagnosed with ADHD following a detailed psychiatric assessment. At the end 89 ADHD patients were recruited. 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 number: 6/5/12152). For control group, 96 volunteers were recruited from local children's Hospital in the same age range. They were also examined to rule out any neurological, psychiatric, or learning problems. Control group were from those patients that were admitted to Children's Hospital for other reasons. As a result volunteers went under careful examination to rule out any neurological disorder before taking part in this study. Written consent was also obtained from control participants.

2-2. Molecular techniques

DNA extraction was performed from whole blood using standard techniques and subsequently used as a template for determination of DRD2 genotypes. Genotyping for the Taq IA and Taq IB polymorphisms was performed by PCR using a thermal cycler (Sensoquest, GmbH, Germany).

A total of 5–10 ng of genomic DNA was amplified in a PCR containing 0.2 Mm of forward primer and 0.2 Mm of reverse primer, 10xPCR buffer, 1.5mM MgCl₂, 200 mM dNTPs, and 1 unit of Taq DNA Polymerase (Cinnagen, Iran) in a 25 mL volume. Amplification conditions were 95°C for 4 min, 95°C for 30 s, 58° and 60° for 30 s (for Taq I A and B, respectively), 72°C for 30 s, Steps 2–4 were repeated by 35 cycles followed by 72°C for 3 min.

Genotypes of the target sequence containing Taq IB polymorphic site were obtained using the PCR-RFLP with the

following primers: (forward) 5'-GATACCCACTTCAGGAAGTC-3'

and (reverse) 5'-GATGTGTAGGAATTAGCCAGG-3'.

PCR product was digested with TaqI (Thermo Scientefic). The B1 allele showed a band of 459 bp, whereas the B2 allele showed a band of 267 and 192 bp. Genotypes of Taq IA (the coding region of ANKK1) were obtained using PCR-RFLP method with the forward primer 5'-GCACGTGCCACCATACCC-3' and the reverse primer 5'-TGCAGAGCAGTCAGGCTG-3'.

Digestion of PCR products, were performed with TaqI restriction enzyme (Thermo Scientefic) and produced

fragments were visualized via 2-3 percent agarose gel electrophoresis. The A1 allele showed a band of 310 bp, whereas the A2 allele showed a band of 130 and 180 bp.

2-3. Statistical analysis

Allele and genotype frequencies were analyzed through Java stant online statistics software <http://statpages.org/ctab2x2.html>.

When the number of samples was equal or less than 5, the Fisher's exact test was applied to compare the variables. P- value <0.05 was considered as significant level (**Table.1**). To assess Hardy-Weinberg equilibrium, a online HWE calculator, <http://www.oege.org/software/hardy-weinberg.html>, was applied.

Table 1: Table 1: Genotype and allele frequencies of Taq IA and TaqIB of DRD2 gene

Variables	Case (n=89)	Control (n=96)	OR (95% CI)	P-value
Genotypes Taq IB				
B1/B1	73(82.02)	69(71.88)	1.785(0.867-3.689)	0.089
B1/B2	14(15.73)	24(25)	1.692(0.830-3.449)	0.146
B2/B2	2(2.25)	3(3.125)	0.56(0.261-1.194)	0.104
Alleles				
B1	160(89.89)	162(84.38)	1.646(0.882-3.072)	0.115
B2	18(10.11)	30(15.63)		
Genotypes Taq IA				
A1/A1	62(69.66)	57(59.38)	1.57(0.84-2.94)	0.129
A1/A2	22(24.72)	37(38.54)	0.52(0.27-1.00)	0.036
A2/A2	5(5.62)	2(2.08)	2.80(0.50-20.10)	0.173
Alleles				
A1	146(82.02)	151(78.65)	1.209(0.720-2.028)	0.473
A2	32(17.98)	41(21.35)		

Estimated relative risks with odds ratios (OR) and 95 % confidence intervals (95 % CI) and Pvalue for association between Taq IA and Taq IB with ADHD risk.

3- RESULTS

To assess the association of DRD2 Gene Taq IA and Taq IB polymorphisms with attention deficit hyperactivity disorder, a total of 89 patients with attention deficit hyperactivity and 96

healthy subjects with the mean age agreement with the mean age of the patient group were genotyped. 78.5% of cases were male and 21.5% were female. Among 96 controls, 54.44% were male and 45.55% were female. The frequencies of genotypes and alleles did not show a

deviation from Hardy–Weinberg equilibrium in both patient and control groups. For to compare observed genotype and allele frequencies Pearson Chi-square test was performed. For the Taq IB and Taq IA polymorphisms, the frequencies of genotypes and alleles were given in (Table.1). According to statistics, there was not any significant difference in the frequency of the two variant alleles between cases and controls in Taq IB polymorphism. Consistently, no significant difference was detected in the genotypic frequencies between patients and healthy individuals ($P>0.05$) (Table.1). For Taq IA polymorphism, genotypes of TT homozygous dominant and CC homozygous recessive did not show significant difference between two groups, but TC heterozygote had a significant difference in the control group than in the case group [$P=0.036$, OR (95% CI)= 0.52(0.27-1.00)].

4- DISCUSSION

ADHD is characterized by a universal pattern of attention, hyperactive and impulsive behavior that evident early in life. Over a decade ago imaging studies of brain (13-15) detected anatomical abnormalities in ADHD individuals that several brain regions had smaller than normal size. This, seminal observation have been basically confirmed by two decades of intensive research. In addition to an overall reduction in size was detected in childhood and remains in adolescence (16). Regions which have a high density of Dopamine receptors such as the caudate nucleus and globuspallidus were smaller in the ADHD group than in the control group; posterior regions (occipital lobes) and smaller anterior brain regions (right frontal white matter) were larger in ADHD groups. The initial fMRI findings (17, 18) showed decreased activation of the DA (Dopamine) pathway (the cortical-striatal thalamic brain circuit). Since evidence

from pharmacological (19) and studies of positron-emission tomography (20) confirms the role of the dopamine neurotransmitter system in the etiology of ADHD, studies of molecular genetic have mostly focused on genes in these pathways. Because dopamine is involved in a variety of critical functions, therefore many human disorders have been related to dopaminergic dysfunctions. Therefore, recently a case–control study was carried out to assess the possibility of an association between two single nucleotide polymorphisms of DRD2 gene, and ADHD risk with samples from Iranian-Azeri patients. In the current study, significant difference was not found in the allele and genotype frequencies between ADHD and TaqIB polymorphism in cases and controls ($P>0.05$) (Table.1).

In the Taq IA polymorphism of DRD2 gene, TT homozygous dominants and CC homozygous recessives were more frequent in case group than in control group, but significant difference was not observed. In the other hand, T/C heterozygotes were more frequent in the control group than the case group, and difference was significant [OR (95%CI): 0.52 (0.27-1.00), $P=0.036$]. There was not a significant difference in the allele frequency between case and control groups was detected [OR (95%CI): 1.209 (0.720-2.028), $P=0.473$].

6- CONCLUSION

In summary, our data supports lack of association between Taq1A and Taq1B gene polymorphisms 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.

7-CONFLICT OF INTEREST: None.

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