

The Survey of DBH Gene Polymorphism Rs5320 in Children with Attention Deficit Hyperactivity Disorder (ADHD)

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Abstract

Background

Attention deficit hyperactivity disorder (ADHD) is a common behavioral disorder that affects 8-12% of school-age children. Several environmental and genetic factors play a role in the etiology of this disease. One of the genetic factors involved is dopamine beta-hydroxylase (DBH) gene, which plays an essential role in catecholamine synthesis by converting dopamine into norepinephrine. Here we investigated DBH polymorphisms associated with ADHD in North West of Iran.

Materials and Methods: This descriptive comparative study was performed on 130 children aged 5-14 years who were diagnosed with ADHD by child and Adolescent psychiatrist following a detailed psychiatric assessment and 130 matching healthy children were also selected from local children's Hospital in Tabriz city, Iran. Also, 2ml Peripheral blood sample was obtained from all the participants and RFLP-PCR technique was then used to study the polymorphism position rs5320 and allele and genotype frequency of DBH gene.

Results: The results showed that the frequency of allele A (as the allele causing the disorder) was 15% in ADHD subjects and 6% in healthy subjects ($p < 0.05$). The genotype frequency in ADHD subjects was 4% AA, 26% AG, and 70% GG, and 0%, 12% and 88% for healthy children, respectively ($p=0.017$, $df=2$, $\chi^2=3.14$).

Conclusion: The results suggest that DBH polymorphism, position rs5320, plays a role in the pathogenicity of ADHD in the studied population and therefore can be considered as a candidate gene for future diagnosis.

Key Words: ADHD, DBH gene, Polymorphism, RFLP-PCR.

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1- INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is a behavioral neurological disorder that begins in childhood and has negative effects on various functional aspects (1). Disruption in the performance and acquired social skills associated with ADHD may have a significant impact on the work, life, and academic education of affected people. The global prevalence of ADHD is reported to be 8-12% in children and 4% in adults (2). Also, the prevalence of this disorder in Tabriz was 9.7% among children and 3.8% in adults (3, 4). This is a heterogeneous behavioral neurological disorder with several possible causes, including genetic and environmental factors and, in fact, multiple factors, which can lead to change in neural pathways (5). It has been shown that ADHD has a strong genetic background and various factors contribute to its etiology (6).

Today, the issue of gene polymorphism in different societies, due to the diversity of the gene pool in each community, is an important study of the genes involved in diseases and disorders; because, by studying the gene polymorphism, in addition to finding the genotypic frequency of individuals in a population, its allele frequency is also calculated. As a result, by comparing the allelic abundance in healthy and patient subjects of the society, the link between the gene and the disease or disorder is discovered. Several genetic studies have been performed in this field and in many cases there is a significant relationship between gene polymorphism and ADHD disorder. Some of the investigated genes are: dopamine D4 receptor (DRD4), dopamine D2 receptor (DRD2), dopamine D5 receptor gene, Synaptosomal-associated protein 25 genes, serotonin transporter gene, and dopamine beta-hydroxylase gene (5). The basic neurobiology of ADHD in pharmacological studies, animal models and brain images indicate that the

catecholamine neuronal pathways are involved in this process. Among the important neurotransmitters, dopamine and norepinephrine are involved in neurological functions, as well as concentration and consciousness (7). Furthermore, one of the major genes possibly involved in this disorder is dopamine beta-hydroxylase gene (DBH). The product of this gene, the enzyme dopamine beta-hydroxylase, is responsible for converting dopamine to norepinephrine, which in turn; it inhibits tyrosine hydroxylase, and reduces the amount of dopamine production. DBH is found in the brain tissue and in the catecholamine vesicles of gray matter neurons at the nerve endings (8). As mentioned, dopamine is converted to norepinephrine through a DBH, which is produced by the sympathetic nervous system and easily measured in the plasma and cerebrospinal fluid. The level of this enzyme is significantly decreased in the plasma and urine of children with ADHD (9). The DBH encoding gene has an extension of 23 kb and has 12 exons and is located on chromosome 9 and exactly at position 9q34 (9).

The frequent association between dopamine-norepinephrine system disorders and psychiatric disorders has made DBH an important candidate gene for studying mental neurological diseases such as ADHD. In the DBH gene, the G444A, G910T, C1603T, C1912T, C-1021T, 5'-Ins/Del and TaqI polymorphisms appear frequently (9), and may influence the function of gene products or probably modify gene expression and therefore affect the progression of this disorder. In a study (2007), Nyman et al. investigated the relationship between this gene and ADHD, and acknowledged that the dopaminergic pathway is involved in ADHD etiology, and thus requires more attention in future studies on DBH and DRD2 genes (10). Furthermore, Mehdizadeh et al. (2016)

also recognized the importance of this pathway and suggested further studies on larger population samples and other ethnic groups to explain the linkage between DRD2 polymorphism and risk of ADHD (11). Several studies have shown dopamine beta-hydroxylase enzyme is not responsible for maintaining the balance between dopamine and norepinephrine concentrations in ADHD children. Gharaibeh et al. (2010) have investigated the association between the (GT) repeat in the DBH gene and ADHD in children. Their study showed significant differences between the ADHD group and controls with regard to the plasma levels of dopamine- β -hydroxylase enzyme activity and furthermore they concluded that DBH gene polymorphisms were also significantly linked with ADHD development (12).

Furthermore, Bhaduri et al. have studied the association of exon 2 DBH444g/a gene on 41 children with ADHD in India (2005). They reported no significant relationship between intron5 polymorphism (Taq 1), and this disorder (13). A study by Fonesca et al. on several genes involved in dopaminergic and serotonergic pathways in children with ADHD in Columbia (2015) showed no significant correlation between these genes, especially the DBH gene, and ADHD disorder (14).

One of the proprietary positions in the DBH gene is the rs1611115 shear position which is actually a functional SNP, in which a mutation occurs in the individual allele of the T (15). Previous research, including the work of Bhaduri et al. (2012), on ADHD subjects in India revealed a link between T allele and low levels of plasma DBH activity and cognitive problems (16). A study by Tong et al. (2015) on 794 individuals with ADHD by examining the UTR region of the DBH gene at the rs129882 shear position again revealed that this gene is

associated with the disorder (17). Perhaps due to the multi-factorial nature of this disorder, numerous genetic studies have shown that there is a significant relationship between the polymorphism of the candidate genes and ADHD, but despite all these cases, it is still not well known whether the negative results reported are due to differences in sampling, genetic or heterogenic groups or are indeed represent a real difference between different populations. The aim of this study was to investigate the association of common polymorphism in the DBH gene with ADHD disorder in the Northwest of Iran, in order to clarify these doubts.

2- MATERIALS AND METHODS

2-1. Selection of Samples

The statistical population of the study was all children and adolescents with ADHD, aged 5 to 14 years of age, who referred to specialized children and adolescent psychiatric in clinics of Tabriz University of Medical Sciences. Among them, 130 children were diagnosed and introduced by a child psychiatrist with the aid of Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR), as the case group. Additionally, for the control group, 130 psychologically healthy volunteered children with a similar average age 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. This study population, using convenience sampling method, was selected from the patients referred to the Children's Hospital of Tabriz University of Medical Sciences through the semi-structured clinical interview of K-SADS based on the following inclusion and exclusion criteria: Inclusion criteria were: ADHD impairment based on the criteria specified in the DSM-IV-TR (18) by clinical interview performed by a child and

adolescent Psychiatrist for the age group of 5 to 14 years, from the North West of Iran. Exclusion criteria included: History of head trauma, psychiatric co-morbidity, and epileptic seizure, history of serious medical illness, concomitant medical or psychological treatments, mental retardation, and non-consent of the child's family for continued cooperation.

2-2. Molecular techniques

After describing the study to the parents of the participants and obtaining a written consent approved by the Medical Ethics Committee of Tabriz University of Medical Sciences, 5ml of blood was drawn and stored in tubes containing EDTA at -20°C. DNA extraction was performed using the method used in Tabatabaei et al. (19), using gene amplification or PCR-RFLP technique. The steps of the temperature cycle described in **Table.1**, and by using the specific primers shown in

Table.2, the desired gene was amplified. After ensuring that the desired part is amplified, the products were divided into specific target components using specific limiting enzymes PmlI on the target gene. Subsequently, these digestive cells parts underwent electrophoresis on a 2% agarose gel and at 110 volts and photographed using a UV Transilluminator.

2-3. Statistical analysis

Following the observation and studying the gel, the allele and genotypic frequency of each case and control groups were calculated by the Hardy-Weinberg principle (online HWE calculator, <http://www.oege.org/software/hardy-weinberg.html>). The association between DBH gene polymorphism and ADHD was measured using RFLP-PCR and odds ratio and a 95% confidence interval.

Table-1: PCR Temperature Program for DBH gene amplification

Cycle number	Stage	Temperature	Duration
1	First denaturation	95°C	5 min
30	Denaturation	94°C	20 sec
	Annealing	54°C	20 sec
	Extension	72°C	30 sec
1	Final extension	72°C	7 min

Minute= min, Sec= Second.

Table-2: Specifications and sequences of the pair of primers F and R used for gene amplification

Gene	Primers	SNP
DBH	F: 5`CACTGTCCACTTGGTCTACG3` R: 5`GCTCCTTAATGTAGCACCAG3`	rs5320

SNP= Single Nucleotide Polymorphism; DBH= Dopamine Beta-Hydroxylase.

3- RESULTS

Amongst all 260 children selected for this study, 130 children belonged to the case group and 130 to the control group. The average age of case and control subjects was 2.35 ± 7.64 and 2.02 ± 7.52

years old, respectively ($p = 0.66$). The allele and genotypic frequency for the case and control groups are presented in **Table.3**. Pearson Chi-square test was used to compare observed genotype and allele frequencies with those that are expected in a population with Hardy-Weinberg

equilibrium. As it can be observed, there is a significant difference in the allele frequency of A and G in the ADHD and control groups ($p < 0.05$). Also, there was a significant difference in terms of the three genotypes in the ADHD and control

groups with degree of freedom of 2 and Chi square 3.14 ($p < 0.05$). In other words, the involvement of the allele A in ADHD patients is evident in allele frequency and in genotypic abundance.

Table-3: The frequency of allele and genotype in the case and control groups

Characters		Groups		P- value
		Case, Number (%)	Control, Number (%)	
Allelic frequency (%)	G	110 (85)	122 (94)	0.01
	A	20 (15)	8 (6)	0.001
Genotype frequency (%)	GG	91 (70)	114 (88)	0.005
	GA	34 (26)	16 (12)	0.001
	AA	5 (4)	0 (0)	0.03
Variables		95%CI	OR	P-value
GG vs. GA + AA		0.733-0.867	1.91	0.02
GA vs GG + AA		0.128-0.256	2.72	0.02

Estimated relative risks with odds ratios (OR) and 95% confidence intervals (95% CI), and P-value for association between rs5320 and ADHD risk, vs.: versus.

4- DISCUSSION

ADHD disorder is associated with weakness in communicative, educational, behavioral and emotional functions in affected children (20). The etiology of this disorder has not been fully recognized, but in recent years, some findings have strengthened the theory that genetic factors play an essential role in the development of ADHD. Some studies have reported the heritability of this disorder at about 70 to 90 percent (21). Research indicates that the dopamine system malfunction is involved in the pathogenesis of ADHD (22, 11). It is widely acknowledged that the genes involved in this process are coded for the synthesis of enzymes, receptors, and neurotransmitter material (23). The presence of disturbances in the neurotransmitter dopamine in the etiology of ADHD disorder has already been identified. Production of epinephrine dopamine is induced by dopamine beta-hydroxylase enzyme. Thus, DBH gene polymorphisms have a direct effect on

enzyme activity. Bhaduri et al., in a study in East India in 2010, investigated the association of four DBH gene polymorphisms and ADHD disorder and found positive outcomes (24). A remarkable point in their work was the study of the plasma activity of this enzyme along with the analysis of the polymorphism of this gene which made their work more confident. In another study, Barkley et al. (2006) in addition to the polymorphisms of DAT1 and DRD4 genes investigated the polymorphism of the DBH gene in ADHD patients and the simultaneity of the polymorphisms of the three genes involved in the development of this disorder. They found a significant relationship between this polymorphism and ADHD disorder, and their results were consistent with the present study (25). In a cohort study on 9,000 cases in Finland by Neihan et al in 2007, involvement of two important genes, DRD2 and DBH, were studied. They concluded that allele variation in these genes has a direct

correlation with ADHD (10). This type of study is of great value since the number of cases is investigated. According to genetic studies on ADHD, one candidate gene for studying the etiology of ADHD disorder is DBH (25). The importance of this gene and other genes involved in the pathway of dopaminergic circuits is such that neurodegenerative scholars refer to DBH, and the HTR1B, HTR2C, TH, DRD4, MAOA, SNAP25, SLC6A2, HTR2A, TPH2, DRD3, and CHRNA4 genes as hot genes or top genes (26, 27).

Despite of numerous studies that confirm the direct relation between the DBH gene and ADHD, some other studies indicate that there is no relation between them. One of these studies is Bhaduri et al. (2008), which explains that there is no significant relationship between the polymorphism position of 1021 of the DBH gene and the ADHD disorders in a population in India. This study was also performed on the haplotype of the parents of ADHD children, and the results again revealed that the gene was not associated with the disorder (28). On the contradiction of this research and the present study, the differences between alleles of a particular gene in different populations, the phylogenetics, preservation of a particular allele of that gene in one population and its transformation in the other can be suggested.

5- CONCLUSION

DBH is an enzyme responsible for the conversion of dopamine into noradrenaline. Alteration of the dopamine/noradrenaline levels can result in hyperactivity. The DBH protein is released in response to stimulation. DBH activity, derived largely from sympathetic nerves, can be measured in human plasma. Patients with ADHD showed decreased activities of DBH in serum and urine. The study of gene polymorphism in the population is of particular importance. In

summary, our data supports the association between DBH gene polymorphisms and ADHD. However, further studies on larger population samples and other ethnic groups will be required for explaining the linkage between DBH polymorphism and risk of ADHD.

6- CONFLICT OF INTEREST: None.

7- ACKNOWLEDGMENT

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