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Polymorphism of dopamine transporter gene 3'-UTR VNTR in Iranian Azeri Turkish patients with ADHD

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Abstract

Background: Attention Deficit Hyperactivity Disorder (ADHD) is the most common neurobehavioral disorder among children, which several studies have indicated the role of genetic factors in its development. Dopamine transporter gene (*DAT1*) is one of the candidate genes and a 40-bp variable number of tandem repeat (VNTR) in 3'-UTR of the gene is reported to have a role in ADHD. Here we examined the association between *DAT1* VNTR and ADHD susceptibility among Iranian Azeri Turkish children.

Methods: The study included 202 patients and 143 controls aged 6-12 years. ADHD children were diagnosed according to DSM-IV and "conners scale" and *DAT1* 3'-UTR VNTR was genotyped by PCR technique.

Results: The genotypes and allelic distribution of the *DAT1* 3'-UTR VNTR were not significantly different between the case and control groups ($p>0.05$) but a significant difference was found in allelic frequencies when the analysis was confined to females ($p=0.029$).

Conclusion: Our results do not support the role of 10 or 9-repeat alleles as risk alleles in the studied subjects but an association was found between 11-repeat allele and the susceptibility to ADHD in females.

Key Words: Attention deficit hyperactivity disorder, ADHD, *DAT1*, SLC6A3, 3'-UTR VNTR.

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1- INTRODUCTION

Attention Deficit Hyperactivity Disorder (ADHD) is among the most common psychiatric disorders that affects 3-10% of children all around the world (1). Its incidence is four times higher among boys than girls and usually lasts until adolescence/adulthood (2). ADHD is characterized by symptoms like extreme and inappropriate levels of motor activity, impulsivity and inattentiveness and the patients may mainly reveal the inattentiveness or hyperactivity or more commonly the combination of the symptoms (ADHD combined type) (3). A study on Iranian population has reported the prevalence of the combined type of ADHD from less than 5% to 20% in different cities (4).

Etiological studies suggest that genetic factors influence the susceptibility of ADHD development (5). Dopaminergic system, is one of the main targets in ADHD genetic studies (6) and since methylphenidate, a psychostimulant that inhibits dopamine transporter, is often used for ADHD treatment, the gene *DAT1* (or SLC6A3) codes for the dopamine transporter was considered to be associated with ADHD according to its function (7, 8).

The *DAT1* gene has a variable number of tandem repeat (VNTR) in its 3'-UTR which is a 40-bp variant (rs28363170). Ten different alleles with 3-13 copies have been identified for rs28363170 and the 10-repeat allele, which is the most widespread variant, is considered to be associated with children and adolescent ADHD susceptibility (9). Nine-repeat allele is also common in some populations and recently 9-repeat allele carriers have been introduced as being associated with ADHD in adults (10).

Association of *DAT1* 3'-UTR VNTR alleles is not limited to ADHD, but has been implicated in several disorders, including Depression (11), Posttraumatic Stress Disorder (12), and Obsessive–Compulsive Disorder (13) which are all associated to the 9-repeat allele.

This study aims to investigate the association between *DAT1* 3'-UTR 40-bp VNTR and ADHD susceptibility among Iranian children with Azeri Turkish ethnicity.

2- METHODS AND MATERIAL

2-1. Subject

The sample of the study included 202 ADHD patients, aged 6-12 years from Azeri Turkish ethnic who were diagnosed in Tabriz Children Hospital with ADHD symptoms and the diagnosis was confirmed by the "Conners test". They consisted of 41 girls and 161 boys diagnosed with the ADHD combined type. The control group consisted of 64 girls and 79 boys aged 6-12 years. They were selected from children who went to the hospital for other reasons and had referred to a blood sampling unit for diagnosis. These children did not have any symptoms of ADHD. We used the remaining of their testing blood to extract DNA.

2-2. Ethical considerations

Written informed consents were obtained from the parents of all subjects. Also the research protocol was approved by the Ethics Committee of Tabriz University of Medical Sciences (ID number: 1384.772).

2-3. DNA extraction and Genotyping

Blood samples of the participants in both of the experimental and control groups were used for DNA extraction by salting out method and the PCR technique was performed for genotyping. rs 28363170 polymorphism was proliferated by using two specific primers F 5'-TGCGGTGTAGGGAACGGCCTGAG-3' and R 5'-CTTCCTGGAGGTCACGGCTCAAGG-3' in a thermal cycler (Bio-Rad, USA). The

PCR mixture (20 µl) was made of 10µl Taq DNA polymerase 2X master mix (Ampliqon, Denmark), 10 pM of each forward and reverse primers and 100 ng DNA for each sample. The PCR reaction was consisted of one cycle of 95 ˚C for 5 mins, 35 cycles of 95 ˚C for 30s, 61 ˚C for 30s, 72 ˚C for 30s and one cycle of 72 ˚C for 10 mins. The PCR amplification products were subjected to electrophoresis on 2.5% agarose gel.

2-4. Statistical analysis

Statistical analysis was conducted by Microsoft Excel 2010 and SPSS version 20. Chi-square test was used to investigate possible associations of ADHD with genotypes and alleles. The distribution of demographic characteristics (gender) and genotype were analyzed by χ^2 test, odds ratio (OR) with a 95% confidence interval (CI) analysis. $p \leq 0.05$ was considered statistically significant.

3- RESULTS

3-1. Population study

The demographic data of the study is shown in **Table 1**. Total number of the children was 345 (202 cases and 143 controls). The case group consisted of 161 boys (79.70%) and 41 girls (20.30%) and the control group consisted of 79 boys (55.24%) and 64 girls (44.76%). The mean age of the participants in the case and the control group was the same. A significant difference between the case and the control groups was observed in terms of gender distribution, confirming that ADHD is more common in boys than girls, in our studied population (**Table 1**).

Table-1: The frequency of ADHD among males and females.

Gender	ADHD (%)	Controls (%)	Chi-square	p value
Female	41 (20.30)	64 (44.76)	23.66	0.00001>
Male	161 (79.70)	79 (55.24)		
Total	202	143		

3-2. The association between *DAT1* 3'-UTR 40-bp VNTR and ADHD susceptibility

Investigating different alleles of *DAT1* 3'-UTR 40-bp VNTR, we observed various bands with different sizes. Different genotypes of the studied VNTR are shown in **Fig. 1**. The genotypes and allelic distribution of the *DAT1* 3'-UTR VNTR were not significantly different in the case and control groups (**Tables 2** and **3**). The association was also non-significant when the analysis was confined to males (**Tables 4** and **5**) but we found a significant association between 11-Repeat allele frequency and ADHD in females (p=0.011). The OR of female ADHD children having the 11-Repeat allele compared with their control counterparts was 2.61 (95% CI 1.2282-5.5606, p=0.0126) (**Table 5**).

4- DISCUSSION

The association between *DAT1* 40-bp VNTR and ADHD was first mentioned in a study by Cook et al., suggesting that the 10-Repeat allele is the risk allele (p=0.006) (14); However, our results were different. Here, the 10 and 9-repeat alleles were the most common alleles in both of the case and control groups, respectively. Nevertheless, there was not a significant association between 10 or 9-repeat alleles and the disorder. A recent study by Banoei et al., also reported a similar finding. They analyzed 100 ADHD and 130 Iranian control children for the 10-repeat allele

and they described this allele as the most common allele but their findings did not support the contribution of 10 repeat allele to ADHD susceptibility (p<0.9) (15).

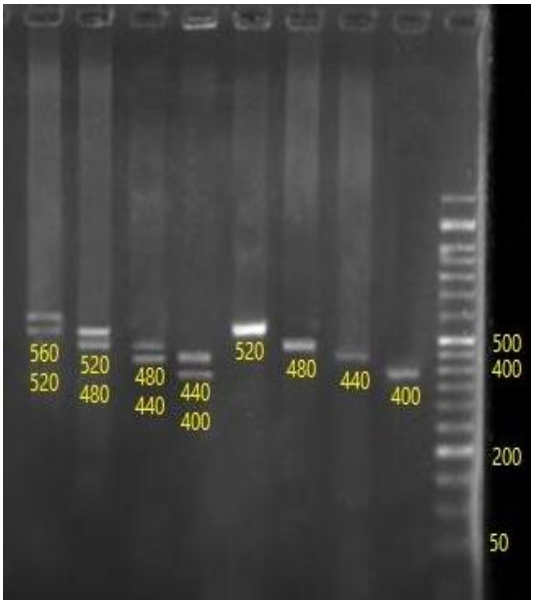

Fig. 1: Detection of various genotypes in the *DAT1* 3'-UTR 40-bp VNTR. PCR products electrophoresed on 2.5% agarose gel. Various alleles have been observed: 400bp (8 repeat allele), 440bp (9 repeat allele), 480bp (10 repeat allele), 520bp (11 repeat allele) and 560bp (12 repeat allele).

Table-2: Genotype distribution of *DAT1* 3'-UTR 40-bp VNTR in ADHD children and the control group

Genotype	ADHD	ADHD-freq	Controls	Co-freq	Individual p value	p value
8R/8R	2	1	1	0.70	0.759	
8R/9R	3	1.48	7	4.90	0.088	
9R/9R	25	12.37	15	10.48	0.626	
9R/10R	45	22.27	35	24.48	0.613	
9R/11R	2	1	0	0.00	0.398	0.44
10R/10R	78	38.61	54	37.76	0.728	
10R/11R	21	10.39	15	10.48	0.815	
11R/11R	24	11.88	16	11.20	0.751	
11R/12R	2	1	0	0.00	0.398	

Table-3: Allelic frequencies of *DAT1* 3'-UTR 40-bp VNTR in ADHD children and the control group

Allele	ADHD	Freq	Controls	Co-freq	Individual p value	p value
8R	7	1.73	9	3.15	0.258	
9R	100	24.75	72	25.18	0.97	
10R	222	54.95	158	55.24	0.998	0.425
11R	73	18.07	47	16.43	0.392	
12R	2	0.5	0	0.00	0.137	

Table-4: Genotype frequencies of *DAT1* 3'-UTR 40-bp VNTR in the female and male ADHD children and the controls

Gender	Genotype	ADHD	ADHD-freq	Controls	Co-freq	Individual p value	p value
Female	8R/8R	0	0.00	0	0.00	0.826	
	8R/9R	0	0.00	4	6.25	0.226	
	9R/9R	2	4.88	3	4.69	0.964	
	9R/10R	11	26.83	15	23.44	0.695	
	9R/11R	0	0.00	0	0.00	0.826	0.38
	10R/10R	14	34.15	31	48.44	0.151	
	10R/11R	6	14.63	7	10.93	0.576	
	11R/11R	7	17.07	4	6.25	0.088	
	11R/12R	1	2.44	0	0.00	0.342	
Male	8R/8R	2	1.24	1	1.27	0.988	
	8R/9R	3	1.86	3	3.80	0.377	
	9R/9R	23	14.29	12	15.19	0.852	
	9R/10R	34	21.12	20	25.32	0.465	
	9R/11R	2	1.24	0	0.00	0.557	0.72
	10R/10R	64	39.75	23	29.11	0.109	
	10R/11R	15	9.32	8	10.12	0.841	
	11R/11R	17	10.56	12	15.19	0.303	
	11R/12R	1	0.62	0	0.00	0.809	

Table-5: Allelic frequencies of *DAT1* 3'-UTR 40-bp VNTR in the female and male ADHD children and the control group

Gender	Allele	ADHD	Freq	Controls	Freq	Individual p value	p value
Female	8R	0	0.00	4	3.13	0.233	
	9R	15	18.29	25	19.53	0.824	
	10R	45	54.89	84	65.62	0.119	0.029
	11R	21	25.60	15	11.72	0.011	
	12R	1	1.22	0	0.00	0.343	
Male	8R	7	2.17	5	3.16	0.521	
	9R	85	26.40	47	29.75	0.440	
	10R	177	54.97	74	46.84	0.094	0.448
	11R	52	16.15	32	20.25	0.276	
	12R	1	0.31	0	0.00	0.811	

Here, we also reported the 8, 11 and 12-repeat alleles in the studied population and the OR of ADHD female children having the 11-repeat allele compared with controls was 2.61, which shows a significant association in allelic frequencies between *DAT1* 3'-UTR 11-repeat VNTR and ADHD risk in female children. Our findings are compatible with El-Tarras' and Qian's results (16, 17). El-Tarras by studying 120 ADHD children and 160 healthy age matched controls identified two alleles (7 and 11 repeats) ($p<0.01$, OR=2.5, 3.3) as risk alleles and two genotypes (11/11 and 11/7) ($p<0.01$, OR= 4, 3) as risk genotypes in Saudi-Arabian children (16). In the same line, Qian recruited 548 Han Chinese children (332 ADHD and 216 controls) in a case-control study that found that 11 and 12-

repeat alleles (p=0.032) were associated with the disorder (17).

Other researchers reported different results. Wohl did not find any significant association between *DAT1* 3'-UTR VNTR alleles and genotypes and ADHD risk among French children (18). Similarly, Kim using a family-based study showed no association between ADHD susceptibility and *DAT1* 3'-UTR VNTR 6-11 repeat alleles and genotypes in Korean children (19). In the last two studies, the 10-repeat allele and 10/10 genotype were the most common alleles and genotypes in the studied subjects. Inconsistently, Wiguna reported the 10-repeat allele and 10/10 genotype as a risk factor for ADHD in a small sample of 47 ADHD and 48 control Indonesian children (20). Asherson, recruiting 383 ADHD children from eight European countries also showed that the 10-repeat allele is associated with ADHD risk (p=0.019) (21). These different results might be due to the variations in sample size, ethnicity, diagnostic criteria, recruitment strategies, and the techniques used for genotyping.

Finally, Grunblatt in a meta-analysis consisting of 14821 cases and 25860 controls concluded that there is a weak association between the 10-repeat allele and child ADHD which came to significance only in the European population (22).

Explorations regarding the functional outcome of the *DAT1* 3'-UTR 40-bp VNTR variants which mainly focused on long alleles, appear to have some mixed results (23, 24). In vitro studies have shown either 9 or 10-repeat alleles to increase *DAT1* gene expression, while ex vivo studies have shown incompatible findings. Regardless of these results, Grunblatt has recently introduced the long allele as the risk allele in ADHD, particularly in children and adolescents with Caucasian, European and Asian ethnicity (22). As they have mentioned "this could indeed be due to the possible functional effect of the variant's length in controlling the expression of the *DAT1* gene" which should be approved by more precise functional studies.

5- CONCLUSION

In conclusion, our results do not support the role of 10 or 9-repeat alleles as the risk alleles in the studied subjects. However, we found an association between 11-repeat allele and the susceptibility to ADHD in females; though due to the small number of our participants, this should be confirmed with a larger sample size. Moreover, association of the dopamine transporter gene in ADHD susceptibility calls for investigation into other functional polymorphisms within the *DAT1* gene in the Iranian Azeri Turkish ADHD subjects.

6- CONFLICT OF INTEREST

None.

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