Clinical Epidemiology and Global Health 8 (2020) 290–294

Contents lists available at ScienceDirect

Clinical Epidemiology and Global Health

journal homepage: www.elsevier.com/locate/cegh

Determination of association between the polymorphism in exon 3 of dopamine receptor gene type 4 with attention deficit-hyperactivity disorder

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ARTICLE INFO

Keywords:
Attention deficit hyperactivity disorder
Polymorphism
Dopamine receptor gene type
Iran

ABSTRACT

Introduction: Evidences suggest that attention deficit-hyperactivity disorder (ADHD) is a hereditary disorder and at least 20 potential genes associated with ADHD have been identified. Dopamine receptor gene type 4 (DRD4) has been more considered due to a stronger relationship with ADHD. However, no study has yet been conducted on the Iranian population to assess the association.

Objective: In this study, the association between polymorphism of DRD4 gene with ADHD has been studied among capital of Iran population.

Materials and methods: This study is a case-control study conducted on children aged 6–12 years with ADHD referred to child and adolescent psychiatric clinic Imam Hussein (AS) and normal subjects in 2011. Diagnosis was done based on the DSM-IV-TR criteria and interviewing by two child and adolescent psychiatrists. If parental were consent, then saliva samples of subjects were prepared and DRD4 gene and related allele were evaluated using PCR method. The K-SADS questionnaire was also used to assess comorbid disorders.

Results: In this study, 114 patients in ADHD group and 109 patients in the control group were studied. The most frequency was obtained for allele 4 allele that has been observed in about 90% of both case and control groups. However, frequency of allele 6 in the case group was 8.8% where the frequency was 5% in the control group ($p = 0.02$). The presence of repeat of allele 6 increased chance of suffering from ADHD to 1.809 (95% equal to 3.871–0.845).

Conclusion: For the first time this study showed that in Iranian population repeat of DRD4 gene allele 6 unlike the other geographic areas is relatively common and it will increase the chances of suffering from ADHD. However, additional studies are required.

1. Introduction

Attention deficit and hyperactivity disorder (ADHD) is a syndrome characterized by inattention, distractibility, hyperactivity and restlessness, impulsivity, and other defects in executive function.[1] The disorder is one of the most common psychiatric disorders with onset in childhood that can infect 5–12% of children worldwide.[2] This disorder can create deficiencies in the performance of the individuals and affect all aspects of life.[1] Frequent job changes, divorce, financial problems in the management of life, increased risk of substance abuse, problems

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https://doi.org/10.1016/j.cegh.2019.08.016
Received 4 February 2019; Received in revised form 19 August 2019; Accepted 24 August 2019
Available online 26 August 2019

related to driving including high-speed motor vehicle collisions, and side effects such as depression, anxiety, increased risk of anti-social behavior are the main problems cases ADHD symptoms.[1,3]

Genetic and environmental factors are known to be effective in the etiology of this disorder. Twin and adoption studies have mentioned the heritability in the range of 76%.[4] Different genes are included in the etiology of this disorder, so that researchers have reported at least 20 types of genes including DAT1, DRD4 and DRD5.[2] Among the expressed genes Dopamine D4 receptor gene (DRD4) is the most important gene.[5] This gene is located on chromosome 11 in the region 11P 15,15 and is also the candidate for etiologic psychiatric disorders such as schizophrenia and mood disorders and behavioral traits and behavioral traits such as drug use and diversity.[1–4,6]

Based on result different genetic and neuropsychological neuroimaging studied, it seems that this gene has a key role in the pathophysiology of ADHD. On the other hand in addition to polymorphisms can make the person prone to this disorder[6] they can also disturb the response to drugs.[3] This gene contains 4 exons and some polymorphisms in the encoding area of gene. Most of these polymorphisms occur in exon III. These polymorphisms are repeated on a number of variables (VNTR) of 48 base pairs (bp) with repeats 2 to 11 (D4/2 to D4/10). Recent studies have shown that polymorphisms in these genes can be of interference in the appearance of the disorder. However, there is no adequate study in this field.

Various studies have reported that the incidence of repeats in exon III of DRD4 gene in Asian populations is different from other regions.[7] So far, no study has been conducted in Iran to investigate the prevalence of allele frequency of this gene. This study aimed to evaluate the association of this gene with ADHD inattention, hyperactivity in a group of Iranian population.

2. Material and methods

2.1. Study and samples

A total number of 223 children aged between 6 and 12 years were chosen for case-control study (one hundred and fourteen cases and 109 controls). Calculation of sample size was performed using a standard error of 5% or less, 95% confidence interval and frequency of DRD4 alleles in ADHD.[8]

This cross-sectional study was conducted to in Tehran city, Iran. Cases were chosen from Imam Hossein Hospital, a large hospital located in the center of Tehran from October 2011 to August 2012. Imam Hossein hospital is a tertiary-care hospital with 500 beds, located in the east of Tehran.[9]

Because of location of hospital, referred patients represent a variety of socioeconomic status. Accordingly, the control group consisted of normal children who were chosen during the same study period from kindergarten and schools from all districts of Tehran city. Group matching was based on age and gender. Inclusion criteria were: children (6–12 years old), recorded ADHD diagnosis, and IQ score above 70.

ADHD diagnosis was made using a structured diagnostic interview by two Pediatricians. Cases were diagnosed as ADHD based on the documentation of symptoms that were associated with functional impairment from multiple environments according to the DSM-IV criterion. Children who may have a history of autism, other pervasive developmental disorders and mental retardation which might interact with ADHD diagnosis were excluded. The IQ of all children was assessed with the Wechsler Intelligence Scale for Children-III Thai version (WISC-III). Children with IQ less than 70 were excluded from the study. Also, children who may have a history of autism, other pervasive developmental disorders and mental retardation which might interact with ADHD diagnosis were excluded.

Parents of cases and controls were asked to sign consent forms participate in this study. This project was approved by research Ethics committee of Shahid Beheshti University of Medical Science. All statistical analyses were carried out by the SPSS 13. The collected data were analyzed through chi-square test (x^2), with a significance level of 0.05. A P-value less than 0.05 was considered statistically significant.

2.2. Laboratory methods

In order to DNA extraction, one saliva sample (no less than 30 min after eating of drinking) was collected from each eligible child. Each sample was collected after mouth washing with water. Then the samples were placed into plastic tubes and were stored at *-20*°C until analysis.

Genomic DNA was extracted from saliva samples for 114 ADHD subjects and 109 normal controls using PUREGENE® (www.gentra.com). The DRD4 polymorphism was amplified, with forward primer 5′-ACGTCGCGCCAAGCTGCA-3′ and reverse primer 5′-TGCGGGTCTGC GGTGGAGTC-3′. Reactions were performed in a 96-well format in a total reaction volume of 25 μl containing 2.6 μl of 10 ηg/μl DNA, 1 μl of each 10 μM primer, 2.5 μl of PCR buffer, 1.5 μl $MgCl_2$, 2 μl of 10 mM dNTP mix, 0.1 μl of Taq polymerase 5U/μl and 14.3 μl of H_2O. PCR was performed using 1 cycle of denaturation for 5 min at 94 °C, 45 cycles of annealing for 30 s at 94 °C, 30 s at 65 °C, and 45 s at 72 °C, followed by 1 cycle of extension for 5 min at 72 °C. PCR products were run out on a 3% agarose gel at 100 V for 100 min.

3. Results

In this study, 238 patients participated in two groups including children with attention deficit hyperactivity disorder (ADHD) and control children group that 15 of them were excluded from the study because of lack of appropriate samples and finally 114 subjects in the ADHD group and 109 patients in the control group were studied. Table 1 shows the age distribution of the participants separated by the two groups. As it can be seen, the average age of children in the ADHD group is 9.1 ± 1.9 years and in control group 10 ± 1.2 years that the observed difference is statistically significant (p = 0.001).

In Table 2, the gender distribution of the participants has been shown separated in two groups. As it was expected, most participants in both ADHD groups were boys (78.9%). According to the matching of the control group with ADHD group, distribution of boys in these groups is also the same.

In Table 3, the distribution of ADHD subtype in children with this disorder has been identified. Accordingly, the maximum frequency of subgroups can be observed in the subgroups composing from 87.7% of patients. Subtypes of attention deficit and hyperactivity-impulsivity disorder were in the second and third ratings (Table 3). Additionally, in Table 4 comorbidity of associated disorders in ADHD group has been presented according to which 48.2% of children have had comorbidity of associated disorders that most frequent case was the confrontational disorders (ODD) that included 43% of all patients.

DRD4 gene polymorphisms distribution has been presented in Table 5 based on the allele distribution. As it can be observed the most frequency of alleles distribution is observed in the allele 4 that about 90% was obtained in both case and control groups. However, allele 6 frequency in case group was 8.8% and the frequency in the control group was 5% (p = 0.02). The presence of 6 allele repeats increased the chance of suffering from ADHD to 1.809 (95% equal to 3.871–0.845). Also, DRD4 gene genotype distribution between the two groups in the

Table 1
The age distribution of the study participants into separated groups.

p-value	Standard deviation	Average age (years)	
0.001	1.9	9.1	case group (ADHD)
	1.2	10	Control group

Table 2
The gender distribution of the participants separated by the study groups.

p-value	Gender Abundance (%)		
	Girl	Boy	
0.846	24 (21.2%)	89 (78.8%)	case group (ADHD)
	22 (20.2%)	87 (79.8%)	Control group

Table 3
Distribution of ADHD subtypes in the case group.

Frequency percentage	Abundance	
87.7%	100	Combined
7%	8	Inattentive
5.3%	6	Hyperactive-inpulasive

Table 4
Distribution of comorbid disorders with ADHD in the case group.

Frequency percentage	Abundance	
43%	49	**(ODD) ppositional-defiant disorder**
5.3%	6	Conduct disorder(CD)
51.8%	59	Without comorbidity

Table 5
Distribution of DRD4 gene polymorphisms based on the allele types between the two groups.

p-value	Control group	group ADHD (case)	Alleles
0.02	201 (92.2%)	205 (89.9%)	4
	1 (0.5%)	–	5
	11 (5%)	20 (8.8%)	6
	3 (1.4%)	–	7
	2 (0.9%)	–	8
	–	1 (0.4%)	10
	–	2 (0.9%)	11

Table 6
DRD4 gene genotype distribution between the two groups in the case and control groups.

p-value	Control group	group ADHD (case)	Type of genotype
0.04	92 (84.4%)	92 (80.7%)	4.4
	1 (0.9%)	–	4.5
	11 (10.1%)	19 (16.7%)	4.6
	3 (2.7%)	–	4.7
	2 (1.8%)	–	4.8
	–	1 (0.9%)	4.10
	–	1 (0.9%)	4.11
	–	1 (0.9%)	6.11

case and control groups showed in Table 6.

4. Discussion

The obtained evidences from the family information, twins, and adoption to indicate that ADHD is a hereditary and family disorder (91-75%).[2,4,10] At least 20 potential susceptibility genes have been identified in relation to ADHD.[4] Pharmacological evidences, brain imaging, and animal studies have emphasized the involvement of neurotransmitter systems especially dopaminergic pathway in ADHD.[11–13] Now half a century has been passed from the beginning of consumption of driving of drugs which have mechanism of action on the dopaminergic system, as the initial treatment in ADHD.[10] Therefore, the

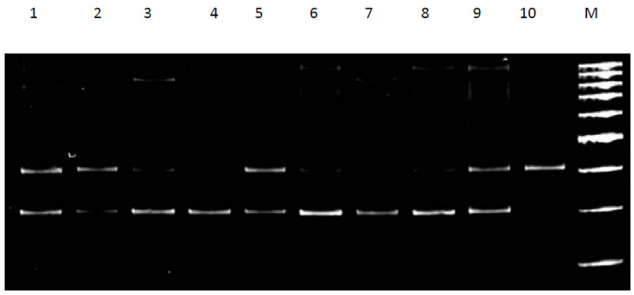

Fig. 1. PCR analysis of the *DRD4* repeats polymorphism. Lane 1, 2, 5 and 9: heterozygote with 4 repeats and 6 repeats. Lane 3, 4, 6, 7 and 8, homozygote with 4 repeats. Lane 10: homozygote with 6 repeats.

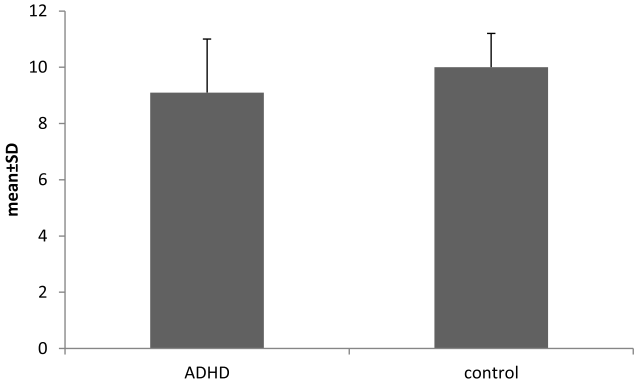

Fig. 2. The age distribution of the study participants separated by the groups.

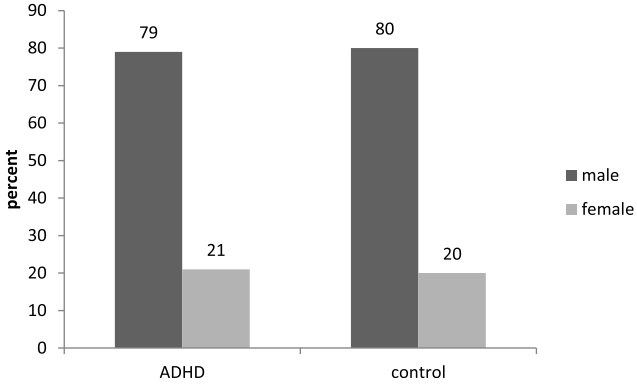

Fig. 3. The gender distribution of the participants to the separate groups studied.

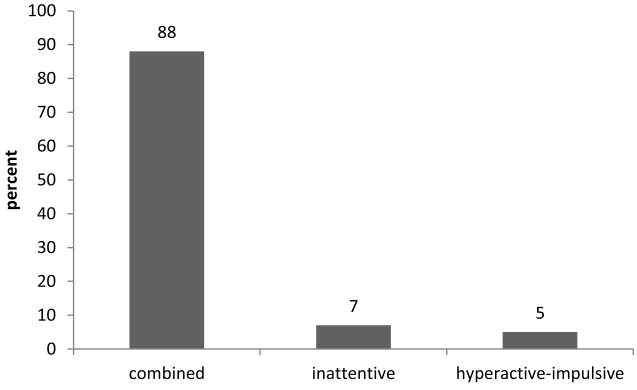

Fig. 4. Distribution of ADHD subtypes in the case group.

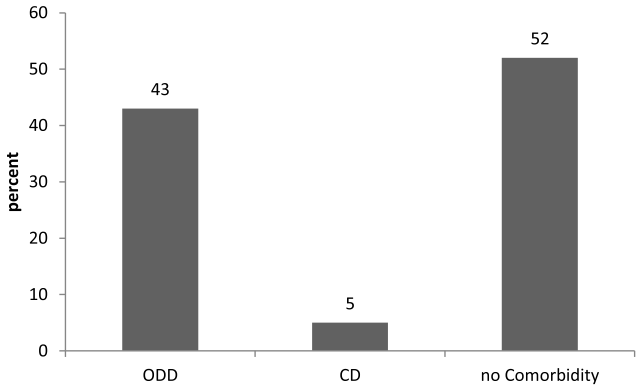

Fig. 5. Distribution of comorbid disorders with ADHD in the case group.

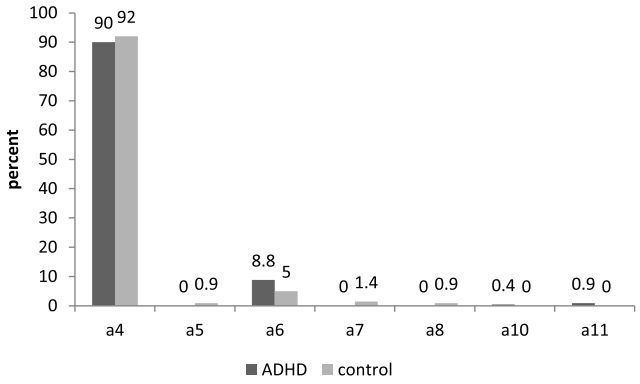

Fig. 6. Distribution of DRD4 gene polymorphisms based on the allele types between the two groups.

transporter and dopamine receptor genes have been more considered than other genes in ADHD disorder. Most genes that have been studied include dopamine transporter gene (DAT1), dopamine receptor gene4 (DRD4), and dopamine receptor gene 5 (DRD5).[14] Hence the DRD4 gene has been more considered than others associated in ADHD in patients due to its stronger association with this disorder. Based on the metaanalysis which was conducted in 2001 by Faraone, it was shown that among the three genes, most association was obtained in DRD4 and alleles 7.[15] Accordingly, the presence of repeat 7 allele of the DRD4 gene has increased the likelihood of developing ADHD by as much as 1.45 (95%, 1.65–1.27) and 1.16 in case-control studies (95% against 1.31–1.03) that was obtained in family-based studies. According to Li et al. meta-analysis through data collection from 33 studies of pooled odds ratio for ADHD in people who have had repeat allele 7 was 1.34 (with a confidence interval of 1.45–1.23) was calculated.[16] In other words, a person with ADHD without allele 7 of DRD4 gene is equal to 0.58. While if the person have a copy of the allele the probability reaches to 0.73. For those with two have two 7 alleles for DRD4 gene (homozygous) they all are diagnosed with ADHD.

The most frequent allele of DRD4 gene in the general population is allele 4 repeats.[10] In a study conducted on 1327 people of 36 different nationalities reported that the distribution of alleles in different populations is different.[17] However, the most frequent allele is associated with allele 4 repeats at 77.47% (the African) to 43.8% (in the American population) is different (global average pf DRD4 allele4 repeat is 64.3%).[2,16,17] DRD4 alleles distribution has been studied in a few studies in the Middle East. In a study in 2008 in Pakistan which was conducted on 474 volunteers, it was shown that the most common allele in the Persian breed is allele 4 repeat with frequency of 70.1%.[18] Allele7 repeat in the Persians group was obtained as 8.5% that was second after allele4. Fig. 1 shows the *DRD4* repeat polymorphism.

In this study, allele4 repeat was found as the most frequent allele of DRD4 gene that was obtained in ADHD and normal groups as 89.9% and 92.9% respectively that shows a high frequency compared with global distribution of allele 4repeat of DRD4 gene. Interestingly, in the present study a total of 235 subjects were examined and only 3 people of healthy subjects (1.2% of study participants) demonstrated allele 7 repeat that shows lowest abundance compared with the reported study. Additionally, allele6 that has rarely been reported in various studies was reported in this study, in the group with ADHD with the frequency of 8.8% and 5% in healthy subjects. So that in this study it was shown for the first time that the presence of allele6 increases the probability of suffering from ADHD to 16.809 (95% equal to 3.871–0.845) (Figs. 2–6).

5. Conclusions

Our data confirmed the relationship between polymorphism of DRD4 and ADHD. Further studies with larger population of other groups will be required to explain the relationship between DRD4 polymorphism and the risk of ADHD.

Conflicts of interest

There are no conflicts of interests among the authors.

Funding/Support

This work was financially supported by grant: (27) for research Affairs of Hamadan University of Medical Sciences.

Acknowledgments

Dear Deputy of Research Affairs of Hamadan University of Medical Sciences, Hamadan is grateful for the approval of a funded thesis with the code 27, as well as the patients and nurses participating in this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cegh.2019.08.016.

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