

The Correlation of Attention Deficit Hyperactivity Disorder with DRD4 Gene Polymorphism in Turkey

Esra Guney¹, Elvan Iseri², Sezen Guntekin Ergun³, Emriye Ferda Percin^{3*},
Mehmet Ali Ergun^{3#}, Ozhan Yalcin⁴ and Sahnur Sener^{2*}

¹Ankara Pediatric & Pediatric Hematology Oncology Training and Research Hospital,
Child and Adolescent Psychiatry Department, Ankara, Turkey, 06110

²Gazi University Medical Faculty, Child and Adolescent Psychiatry Department,
Ankara-Turkey, 06500

³Gazi University Medical Faculty, Department of Medical Genetics Ankara, Turkey, 06500,

⁴Bakirkoy Research and Training Hospital for Psychiatry, Neurology and Neurosurgery,
Child and Adolescent Psychiatry Department, Istanbul-Turkey, 34140,

Email: ¹<dresraguney@gmail.com>, ²<elvaniseri@yahoo.com>,
³<nsezenguntekin@yahoo.com>, ^{3*}<ferdaep@yahoo.com>,
^{3#}<maliergun@gmail.com>, ⁴<cpozhan@gmail.com>, ^{2*}<sahnur@gazi.edu.tr>

Cell: ¹<+90505 640 6895>, ²<+90312 202 5426>, ³<+90312 202 4707>,
^{3*}<+90312 202 4644>, ^{3#}<+90312 202 4637>, ⁴<+90532 523 1550>^{2*}<+90312 202 5426>

KEYWORDS Attention Deficit Hyperactivity Disorder. Genetic Factors. DRD4. Repeat Alleles

ABSTRACT Attention Deficit Hyperactivity Disorder (ADHD) is a disorder with a strong genetic background, and genetic factors are thought to play a crucial role in its aetiology and developmental course. In this study the researchers investigated the correlation of ADHD with the dopamine receptor D4 (DRD4) gene. Fifty patients (6–10 years of age) diagnosed between 1994 and 2001 and followed up 7–14 years until their adolescence and young adulthood (16–25 years of age) were included in the study. Fifty healthy individuals of the same age were included as the control group. DRD4 gene analysis of patients was performed after detailed clinical evaluation. The researchers found that 88% of patients continued to meet the criteria of ADHD in adolescence and young adulthood. The most frequent DRD4 gene alleles among the ADHD and control groups were 4-, 8- and 2-repeat alleles. While the frequency of the 8-repeat allele was higher than reported global estimations, none of the three alleles were found to be significant for ADHD. However, in the presence of the 2-repeat allele for the combined subtype of ADHD diagnosed in childhood, the persistence ratio was found to be statistically significant in adolescence and young adulthood. The DRD4 gene may play a role in the developmental course of ADHD in the Turkish population.

INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is a neurobehavioral disorder that begins during early childhood and negatively affects the functionality at various levels (Goldman et al. 1998). ADHD has a strong genetic background, and many different factors play a crucial role in its aetiology and course (Faraone and Mick 2010).

The dopamine D4 receptor (DRD4) gene is the most extensively studied gene located on the

11p15.5 chromosome (Thapar et al. 2005). The DRD4 gene contains a 48-base pair (bp) variable number of tandem repeats (VNTR) in exon 3, with lengths varying from 2 to 11 repeats; three of these have common variants of 2, 4, and 7 repeats (Van Tol et al. 1992; Rohde and Halpern 2004). In terms of binding of adenylyl cyclase, the DRD4 7-repeat receptor is two to three times less potent than 4-repeat or 2-repeat receptors (Oak et al. 2000). After in vitro studies demonstrated that the 7-repeat allele of the 48-bp repeat polymorphism leads to a blunted response to dopamine, studies focused on this VNTR polymorphism (Van Tol et al. 1992; Asghari et al. 1995).

The majority of studies investigating an association between a dopamine-4 receptor polymorphism and ADHD in children and adults have shown a significant correlation between ADHD and the 7-repeat allele (Holmes et al. 2000; Tahir et al. 2000; Curran et al. 2001; Roman et al. 2001). However, some studies have

Address for correspondence:

Dr. Esra Guney
Ankara Cocuk Sagligi ve
Hastaliklari Hematoloji Onkoloji Egitim
Arastirma Hastanesi, Ziraat Mah.,
Irfan Bastug Caddesi,
Kurtdereli Sokak, No:10, Diskapi,
Ankara, 06110, Turkey
Telephone: +90505 6406895
Fax: +90312 441 41 10
E-mail: dresraguney@gmail.com

not found any relationship between the DRD4 gene and ADHD (Castellanos et al. 1998; Marino et al. 2003; Johansson et al. 2008; Ribasés et al. 2012; Ji et al. 2013). It is not known whether the negative results stem from the differences in sample groups, genetic or diagnostic heterogeneities, or statistical weakness or whether they reflect real differences between the communities.

It is important to determine the genetic factors affecting the outcome of ADHD in order to provide comprehensive intervention and take the necessary measures from the early stages of development. Studies to determine the role of genetic factors on the developmental course of ADHD have frequently focused on the effect of the 7-repeat allele. In addition to studies that indicate the 7-repeat allele is related to a better clinical outcome of ADHD (Gornick et al. 2007), some studies have shown that the clinical outcome is worse in these patients (Langley et al. 2009). Barkley et al. (2006), however, reported no differences between the 7-repeat allele carriers and non-carriers in terms of the severity of behavioural symptoms, academic or work performance, and the results of neuropsychological tests in adulthood.

While the results of studies that have assessed the role of genetic factors on the developmental course of ADHD are controversial, it has been claimed that the effect of risky genes is more prominent in adolescent and adulthood periods compared to childhood (Langley et al. 2009).

Objectives

As the frequency of repeat alleles of VNTR in the DRD4 gene varies among different ethnic groups, the first aim of this study is to investigate the frequency of these repeat alleles in the Turkish population.

The second aim of this study is to explore the relationship between DRD4 alleles and ADHD and investigate the effect of the DRD4 gene on progression and outcome of ADHD in the Turkish population.

MATERIAL AND METHODS

Sampling of Patients and Controls

Fifty adolescent and young adults who were diagnosed with ADHD in childhood were in-

cluded in this study. The diagnosis of ADHD was based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) ADHD criteria between the years 1994 and 2001. Patients were followed in the Child and Adolescent Psychiatry Department of Gazi University Medical Faculty for 7–14 years, and they completed this follow-up period. The control group consisted of 50 adolescent and young adults who were healthy physically and mentally.

Informed consent was obtained from the adolescents and young adults and from parents of adolescents under the age of 18 years.

Exclusion criteria were as follows: presence of pervasive developmental disorder, psychotic disorder, neurological or genetic disorder, and an intelligence quotient (IQ) level below 80, evaluated between 1994 and 2001.

Genetic Analysis

Polymerase Chain Reaction (PCR)

Ten millilitres of blood was taken from each participant. DNA isolation was performed with the high salt concentration method. Regarding VNTR polymorphism, located at the third exon of the DRD4 gene, 5' GGTCTGCGGTGG-AGTCTG 3' and 5' GCGACTACGTGG-TCTACT 3' primers were used (Curran et al. 2001).

The PCR results were evaluated as follows: 2T, 3T, 4T, 5T, 6T 7T, 8T, and 10T alleles were visualized at 323 bp, 371 bp, 419 bp, 467 bp, 515 bp, 563 bp, 611 bp, and 707 bp, respectively, after running on 2% agarose gel electrophoresis. An agarose gel image of a 48-bp VNTR polymorphism is shown in Figure 1.

Statistical Analysis

Statistical Packages for the Social Sciences (SPSS) for Windows 11.5 was used for the statistical analysis. Categorical variables were analysed with chi-square and Fisher-exact tests, while the independent *t*-test was used for continuous variables for two-category variables and one-way analysis of variance was used for more than two categorical variables. Numbers and percentages were used in categorical variables, and mean $\pm$ standard deviation for continuous variables as descriptive data. Statistical significance was accepted as a *p* value of <0.05 .

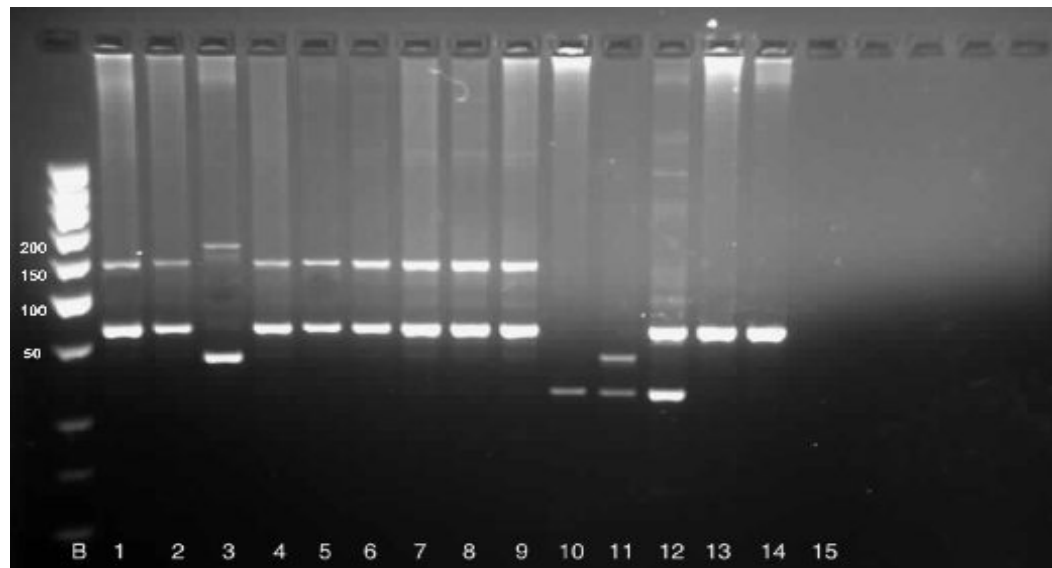


Fig. 1. Agarose gel image of a 48 base-pair VNTR polymorphism

B: marker (a 50 base-pair of DNA Leader);

1, 2, 4, 5, 6, 7, 8, 9=4/8; 3= 3/8; 10=2/2; 11=2/3; 12=2/4; 13,14=4/4; 15= negative control

RESULTS

Socio-demographic Features

The researchers evaluated 50 adolescents and young adults (39 male, 11 female) who were diagnosed with ADHD during childhood (age range at the time of diagnosis 6–10 years, mean age 7.98 years) and 50 healthy adolescents and young adults (33 male, 13 female). In the ADHD group, the mean age of the adolescents and young adults was 17.52 years (2.22 SD, range 16–25 years), while the control group's mean age was 18.22 years (2.18 SD, 16–25 years). There was no difference between groups for mean age ($t = -1.58$, $p=0.116$) or gender ($\chi^2=1.786$, $p=0.181$).

During childhood of the ADHD group, the most common subtype of ADHD was the combined subtype ($n=38$, 76%), followed by inattention ($n=9$, 18%) and hyperactivity/impulsivity ($n=3$, 6%). A diagnosis of ADHD remained in 44 (88%) cases, whereas six (12%) had remission after the 7–14-year follow-up. Among the ADHD sustained cases, 15 (34.1%) were diagnosed as combined subtype ADHD, whereas 29 (65.9%) fulfilled the criteria for inattention subtype.

Relationship of the DRD4 Gene with Clinical Findings in the ADHD Group

The frequency of the genotype and alleles of the DRD4 exon 3 VNTR polymorphism is summarized in Table 1.

Table 1: The frequency of genotypes and alleles of DRD4 in ADHD and control group

DRD4	ADHD ($n=50$)	Control ($n=50$)
<i>Genotypes</i>	<i>n, %</i>	<i>n, %</i>
4/4	26 (52%)	27 (54%)
4/8	16 (32%)	9 (18%)
2/4	5 (10%)	9 (18%)
2/2	1 (2%)	1 (2%)
2/3	1 (2%)	- (0%)
2/8	- (0%)	1 (2%)
3/4	- (0%)	1 (2%)
3/10	1 (2%)	- (0%)
4/6	- (0%)	1 (2%)
4/10	- (0%)	1 (2%)
<i>Alleles</i>	<i>%</i>	<i>%</i>
4T	73	75
8T	16	10
2T	8	12
3T	2	1
6T	-	1
10T	1	1

There was no statistically significant difference between groups in terms of the frequency

of 4/4, 4/8, 2/4, or other genotypes ($\chi^2=3.622$, $p=0.30$). There was also no statistical significance between ADHD and control groups in terms of the frequency of the 4-, 8-, 2-, 3-, 6-, and 10-repeat alleles of the DRD4 gene ($\chi^2=3.93$, $p=0.56$).

Both ADHD and control groups were classified according to the carried long allele (6-10 repeat alleles) since all subjects were carriers of at least one short allele (2-4 repeat alleles). The frequency of carrying the long allele in both groups is shown in Table 2. There was no difference between ADHD and control groups in terms of the frequency of the long allele ($\chi^2=1.214$, $p=0.18$).

Table 2: The comparison of frequency of long allele in ADHD and control groups

DRD4	ADHD (n=50) (n, %)	Control (n=50) (n, %)
Long allele -	33 (66%)	38 (76%)
Long allele +	17 (34%)	12 (24%)

The distribution of genotypes belonging to the DRD4 gene in terms of persistence or remission of ADHD in the ADHD group is shown in Table 3.

Table 3: The distribution of the genotypes of DRD4 gene in terms of persistence or remission of ADHD diagnosis in ADHD group

DRD4	Adolescent and adults with ADHD (n=44)	Remission group (n=6)	Total
4/4	21 (80.8%)	5 (19.2%)	26 (100%)
4/8	15 (93.8%)	1 (6.3%)	16 (100%)
2/4	5 (100%)	0 (0%)	5 (100%)
Others	3 (100%)	0 (0%)	3 (100%)

There was no statistical significance between genotypes, determined in the ADHD group as 4/4, 4/8, 2/4, and other rare genotypes, and the diagnostic status during adolescence or adult-

hood ($\chi^2=2.879$, $p=0.41$). Nonetheless, 83.3% of patients (n=5) in remission during adolescence or adulthood had the 4/4 genotype, 16.6% of these patients (n=1) had the 4/8 genotype. In the ADHD group, there was no significant difference between homozygous, heterozygous carriers and non-carriers of the 4-repeat allele in terms of diagnostic status during adolescence or adulthood ($\chi^2=2.738$, $p=0.25$). There was no significant difference between the long-allele carriers and non-carriers in terms of diagnostic status during adolescence or adulthood ($\chi^2=0.913$, $p=0.32$). The distribution of remission over the adolescence and early adulthood period of patients with ADHD carrying each allele of the DRD4 gene is summarized in Table 4.

In the ADHD group, there were no significant differences between 4-repeat allele carriers (n=47, 94%) and non-carriers (n=3, 6%) ($\chi^2=0.435$, $p=0.67$), 8-repeat allele carriers (n=16, 32%) and non-carriers (n=34, 40%) ($\chi^2=0.737$, $p=0.36$), 2-repeat allele carriers (n=7, 14%) and non-carriers (n=43, 86%) ($\chi^2=1.110$, $p=0.38$), 3-repeat allele carriers (n=2, 4%) and non-carriers (n=48, 96%) ($\chi^2=0.284$, $p=0.77$), or 10-repeat allele carriers (n=1, 2%) and non-carriers (n=49, 98%) ($\chi^2=0.139$, $p=0.88$) in terms of persistence of ADHD or status of remission during adolescence or early adulthood.

When the relationship between each allele and subtype of ADHD diagnostic status over the adolescence-adulthood period was investigated, the researchers found that the incidence of the combined subtype of ADHD was higher than the inattention subtype in patients who were carriers of the 2-repeat allele ($\chi^2=6.849$, $p=0.033$). Five (71.4%) children who were carriers of the 2-repeat allele and diagnosed with the combined subtype of ADHD during childhood met the criteria of the combined subtype of ADHD over adolescence and adulthood; 2 (28.6%) children met the criteria for the inat-

Table 4: The distribution of remission over the adolescence and early adulthood period in patients with ADHD carrying each repeat allele of DRD4 gene

DRD4	Adolescent and adults with ADHD (n=44)	Remission group (n=6)	Total
4 allele -4 allele +	3 (100%)41 (87.2%)	0 (0%)6 (12.8%)	3 (100%)47 (100%)
8 allele -8 allele +	29 (85.3%)15 (93.8%)	5 (14.7%)1 (6.3%)	34 (100%)16 (100%)
2 allele -2 allele +	37 (86 %)7 (100%)	6 (14%)0 (0%)	43 (100%)7 (100%)
3 allele -3 allele +	42 (87.5%)2 (100%)	6 (12.5%)0 (0%)	48 (100%)2 (100%)
10 allele -10 allele +	43 (87.8%)1 (100%)	6 (12.2%)0 (0%)	49 (100%)1 (100%)

tention subtype of ADHD. In the non-carrier group of the 2-repeat allele, 64.5% of the children diagnosed with the combined subtype of ADHD fulfilled the criteria of the inattention subtype of ADHD during adolescence or adulthood and 32.2% were diagnosed with the combined subtype of ADHD over the adolescent or adulthood period.

Children diagnosed with the combined type of ADHD who were carriers of the 2-repeat allele had a significantly higher rate of persistence of the combined type of ADHD during the adolescence and adulthood period than those who were not carriers of the 2-repeat allele ($\chi^2=7.213$, $p=0.02$).

There was no significant relationship between ADHD subtype during adolescence-adulthood and the 4-repeat allele ($\chi^2=2.148$, $p=0.34$), 8-repeat allele ($\chi^2=2.813$, $p=0.24$), 3-repeat allele ($\chi^2=5.510$, $p=0.75$) and 10-repeat allele ($\chi^2=0.739$, $p=0.69$).

DISCUSSION

ADHD is one of the most common disorders in childhood (Adler et al. 2007) and so it is important to determine what causes the disorder, to what extent the disorder continues into adolescence or adulthood, and the risk factors that directly or indirectly affect the outcome. Genetic factors have generally been acknowledged in the aetiology and course of ADHD.

Since DRD4 is commonly located in the frontal-subcortical networks of the brain that have been implicated in ADHD, studies have focused on the DRD4 gene and a 48-bp VNTR polymorphism in exon 3 because of its high variability (Van Tol et al. 1992; Asghari et al. 1995). The most common variants, 4-, 7-, and 2-repeat alleles, have been reported worldwide with frequencies of 64.3%, 20.6%, and 8.2%, respectively (Chang et al. 1996). The frequency of the 4-allele is highly variable between 0.16 and 0.96 and that of the 7-allele is between 0.01 and 0.78. The frequency of both alleles shows large variations among different ethnic groups and between communities (Chang et al. 1996; Qian et al. 2004; Swanson et al. 2007). In studies of populations in the United States and Europe, the most frequent allele is the 4-repeat allele, while the second most frequent is the 7-repeat allele despite the fact that the frequency of alleles is different in children with ADHD compared to

healthy controls (Szekely et al. 2004; Shaw et al. 2007). In Asia, the frequency of the 7-repeat allele is much lower (Chang et al. 1996). So far, there is no accepted exact explanation for this variability at the DRD4 locus (Turic et al. 2010).

Studies on ADHD have focused on the 7-repeat allele after showing its mediator effect on dopamine, which causes blunted receptor responses in invitro studies (Asghari et al. 1995). In some studies carried out with both adults and children, ADHD and the 7-repeat allele were shown to be interrelated (Holmes et al. 2000; Tahir et al. 2000; Curran et al. 2001; Roman et al. 2001), while in other studies no such relationship was shown (Castellanos et al. 1998; Marino et al. 2003; Johansson et al. 2008). Most studies reporting a relationship between ADHD and the 7-repeat allele have been conducted in the United States and Europe (Faraone et al. 1999; Holmes et al. 2000; Curran et al. 2001). In studies carried out in Asian populations (such as China, Korea, Taiwan), the frequency of the 7-repeat allele was found at a fairly low level, and no relationship was reported between ADHD and the 7-repeat allele (Qian et al. 2004; Brookes et al. 2005; Kim et al. 2005; Leung et al. 2005). In most of these Asian studies, the presence of a 7-repeat allele was not shown (Qian et al. 2004; Kim et al. 2005; Leung et al. 2005). Thus, the alleles were divided into two groups, long and short repeats, and were analysed, which also yielded negative results (Qian et al. 2004). The researchers found no 7-repeat allele during this study. Although this result is consistent with studies carried out in Asian populations (Qian et al. 2004; Kim et al. 2005; Leung et al. 2005), it is in contradiction with studies carried out in the United States or Europe, which reported the 7-repeat allele as a risk factor for ADHD (Comings et al. 1999; Holmes et al. 2000). The lack of a 7-repeat allele in this study can be explained by ethnic diversity or the limited sample size. Comings et al. (1999) reported that if the role of the DRD4 gene is limited to the 7-repeat allele, the effects of other repeat alleles or genotypes can be neglected and thus a significant proportion of the phenotypic effect of the DRD4 gene could be lost. Therefore differences between studies have been attributed to ethnicity and methodological differences.

Leung et al. (2005) reported a case-control study that showed a significant relationship between the 2-repeat allele and ADHD. Wang et al. (2004) argued that the 2-repeat allele, like the 7-repeat allele, may be responsible for the blunted receptor response to dopamine; they found the level of response located between the 7-repeat allele and the 4-repeat allele. Consequently, Leung et al. (2005) claimed that the increased frequency of the 2-repeat allele in Chinese children is compatible with the 7-repeat allele hypothesis. Several researchers have hypothesised that an increase in the frequency of any allele, except for the 4-repeat allele, would explain the relationship between the DRD4 gene and ADHD, which would have a pattern of variability across ethnic groups and continue through ethnic origins (Wang et al. 2004; Leung et al. 2005). However, in some studies, a relationship between the 2-repeat allele and ADHD has not been detected (Qian et al. 2004; Cheuk et al. 2006).

In this study, the most common alleles were 4-, 8-, and 2-repeat alleles in the ADHD group. The frequencies of alleles were 73% for the 4-repeat, 16% for the 8-repeat, and 8% for the 2-repeat allele. In the control group, however, the frequency of alleles was 75%, 10%, and 12%, respectively. Similar to previous studies (Qian et al. 2004; Kim et al. 2005; Cheuk et al. 2006), the most commonly detected allele in our study was the 4-repeat allele. However, the researchers found the frequency of the 8-repeat allele, which was the second most common repeat allele, at a higher rate compared to other studies (Qian et al. 2004; Kim et al. 2005). In various Asian studies, the 8-repeat allele was not found (Kim et al. 2005; Cheuk et al. 2006; Ji et al. 2013). A study of a European population indicated that the frequency of the 8-repeat allele is less than 1% (Chang et al. 1996). The frequency of the 2-repeat allele in this study was less than that reported for Asian populations (15–33%) (Kim et al. 2005; Leung et al. 2005), whereas it was consistent with the rest of the world (Chang et al. 1996).

The researchers found no significant relationship between each determined allele and ADHD. Although the frequency of the 8-repeat allele was higher than that reported worldwide, there was no significant relationship with ADHD. Thus, the hypothesis of Leung et al. (2005) suggesting that the increased rate of any allele other

than the 4-repeat allele in different ethnic groups can be assumed to show that the DRD4 gene is associated with ADHD was not supported in this study. The researcher found no significant relationship between carriers of long alleles (6–10 repeats) and ADHD.

The course of ADHD from childhood to adolescence and early adulthood is assumed to have a highly genetic feature, and genes are equally responsible both for the development and the persistence of the symptoms in children with ADHD (Rietveld et al. 2004). While the researchers found no 7-repeat allele in this study, the effect of the 7-repeat allele on the developmental course and outcome of ADHD has been frequently studied when evaluating the role of the DRD4 gene. In this study they found no relationship with clinical outcome of ADHD when the 8-repeat allele was analysed alone or with long alleles. In case-control studies, the DRD4 4-repeat allele levels have been lower in children with ADHD compared to healthy controls. Thus, Li et al. (2006) proposed that the 4-repeat allele may have a protective effect. However, recent studies have indicated that the 4-repeat allele is significantly associated with a higher ADHD symptom severity (Bidwell et al. 2011; Grizenko et al. 2012). Although the effect on symptom severity and an association with ADHD risk are different issues, there is obviously a need for further research to address these topics.

The researchers found the presence of the 4-repeat allele to be ineffective on the clinical outcome of disorder. Nevertheless, 83.3% of patients who were in remission during adolescence and adulthood were found to have a 4/4 genotype, and 16.6% of these patients were found to have a 4/8 genotype. No patient who was a non-carrier for the 4-repeat allele was in remission. However, it is possible that this results were affected by the limited number of patients who were non-carriers for the 4-repeat allele and who were in remission. This finding may suggest that carrying the 4 repeat allele, especially in the case of homozygosity, can be related to positive clinical outcomes, but further studies with a larger sample size are needed. The researchers found that children with the 2-repeat allele had a greater frequency of the combined type of ADHD during their adolescence or adulthood. The presence of 2-repeat allele portended a significantly higher risk for permanent combined subtype of ADHD. In the

2-repeat allele carrier group, 71.4% of children diagnosed with the combined type of ADHD in childhood met the criteria of the combined type of ADHD, and 28.6% of them were diagnosed with the inattention subtype of ADHD during adolescence and adulthood. The corresponding rates for 2-repeat non-carriers were 32.2% and 64.5%, respectively. During the follow-up period, there was no remission in any 2-repeat allele carrier. These findings are important in terms of showing that the presence of the 2-repeat allele is related to worse clinical outcomes.

CONCLUSION

The most commonly detected alleles in the DRD4 gene have been reported as 2-, 4-, and 7-repeat alleles in different populations. In this study, the most commonly detected alleles were 4-, 8-, and 2-repeat alleles. Interestingly, the frequency of the 8-repeat allele was higher than that of previous studies, although in both ADHD and control groups no 7-repeat allele was detected.

No allele was significantly related to ADHD. The presence of the 2-repeat allele caused the childhood combined subtype of ADHD to persist into adolescence or adulthood at a significantly higher rate. During the follow-up period, there was no remission in any 2-repeat allele carrier. This result is important in terms of showing that 2-repeat alleles might be associated with a poor clinical outcome of ADHD.

LIMITATIONS OF THE STUDY

The small sample size is the major limitation of this study. As sample size is not sufficient, our results cannot be generalized to all ADHD children in Turkey. Future studies comparing clinical differences in a larger number of ADHD patients are required, along with taking into account gene–gene and gene–environment interactions.

ACKNOWLEDGEMENTS

We would like to thank the children and their families participating in the study on a voluntary basis. We also would like to thank Assoc. Prof. Dr. Kenan Kose for his contributions in statistical analysis of study data. This study was

supported by the project unit of Gazi University Medical Faculty.

REFERENCES

- Adler LA, Newcorn JH, Faraone SV 2007. The impact, identification and management of attention-deficit/hyperactivity disorder in adults. *CNS Spectrums*, 12: 1-19.
- Asghari V, Sanyal S, Buchwaldt S, Paterson A, Jovanovic V, Van Tol HH 1995. Modulation of intracellular cyclic AMP levels by different human dopamine D4 receptor variants. *J Neurochem*, 65: 1157–1165.
- Barkley RA, Smith KM, Fischer M, Navia B 2006. An examination of the behavioral and neuropsychological correlates of three ADHD candidate gene polymorphisms (DRD4 7+, DBH TaqI A2, and DAT1 40 bp VNTR) in hyperactive and normal children followed to adulthood. *Am J Med Genet B Neuropsychiatr Genet*, 141B: 487–498.
- Bidwell LC, Willcutt EG, McQueen MB, DeFries JC, Olson RK, Smith SD, Pennington BF 2011. A family based association study of DRD4, DAT1, and 5HTT and continuous traits of attention-deficit hyperactivity disorder. *Behav Genet*, 41: 165-174.
- Brookes KJ, Xu X, Chen CK, Huang YS, Wu YY, Asherson P 2005. No evidence for the association of DRD4 with ADHD in a Taiwanese population within-family study. *BMC Med Genet*, 6: 31.
- Castellanos FX, Lau E, Tayebi N, Lee P, Long RE, Giedd JN, Sharp W, Marsh WL, Walter JM, Hamburger SD, Ginns EI, Rapoport JL, Sidransky E 1998. Lack of an association between a dopamine-4 receptor polymorphism and attention-deficit/hyperactivity disorder: Genetic and brain morphometric analyses. *Mol Psychiatry*, 3: 431–434.
- Chang FM, Kidd JR, Livak KJ, Pakstis AJ, Kidd KK 1996. The worldwide distribution of allele frequencies at the human dopamine D4 receptor locus. *Hum Genet*, 98: 91-101.
- Cheuk DKL, Li SYH, Wong V 2006. Exon 3 polymorphisms of dopamine D4 receptor (DRD4) gene and attention deficit hyperactivity disorder in Chinese children. *Am J Med Genet B Neuropsychiatr Genet*, 141B: 907–911.
- Comings DE, Gonzalez N, Wu S, Gade R, Muhleman D, Saucier G, Johnson P, Verde R, Rosenthal RJ, Lesieur HR, Rugle LJ, Miller WB, MacMurray JP 1999. Studies of the 48 bp repeat polymorphism of the DRD4 gene in impulsive, compulsive, addictive behaviors: Tourette syndrome, ADHD, pathological gambling and substance abuse. *Am J Med Genet*, 88: 358–368.
- Curran S, Mill J, Sham P, Rijdsdijk F, Marusic K, Taylor E, Asherson P 2001. QTL association analysis of the DRD4 exon 3 VNTR polymorphism in a population sample of children screened with a parent rating scale for ADHD symptoms. *Am J Med Genet*, 105: 387–393.
- Faraone SV, Biederman J, Weiffenbach B, Keith T, Chu MP, Weaver A, Spencer TJ, Wilens TE, Frazier J, Cleves M, Sakai J 1999. Dopamine D4 gene 7-repeat allele and attention deficit hyperactivity disorder. *Am J Psychiatry*, 156: 768–770.
- Faraone SV, Mick E 2010. Molecular genetics of attention deficit hyperactivity disorder. *Psychiatr Clin North Am*, 33: 159-180.

- Goldman L, Genel M, Bezman RJ, Slanetz PJ 1998. Diagnosis and treatment of attention-deficit/hyperactivity disorder in children and adolescents. *J Am Med Assoc*, 279(14): 1100-1107.
- Gornick MC, Addington A, Shaw P, Bobb AJ, Sharp W, Greenstein D, Arepalli S, Castellanos FX, Rapoport JL 2007. Association of the dopamine receptor D4 (DRD4) gene 7-repeat allele with children with attention-deficit/hyperactivity disorder (ADHD): An update. *Am J Med Genet B Neuropsychiatr Genet*, 144B: 379-382.
- Grizenko N, Fortier ME, Zadorozny C, Thakur G, Schmitz N, Duval R, Joobers R 2012. Maternal stress during pregnancy, ADHD symptomatology in children and genotype: Gene-environment interaction. *J Can Acad Child Adolesc Psychiatry*, 21: 9-15.
- Holmes J, Payton A, Barrett JH, Hever T, Fitzpatrick H, Trumper AL, Harrington R, McGuffin P, Owen M, Ollier W, Worthington J, Thapar A 2000. A family-based and case-control association study of the dopamine D4 receptor gene and dopamine transporter gene in attention deficit hyperactivity disorder. *Mol Psychiatry*, 5: 523-530.
- Ji HS, Paik KC, Park WS, Lim MH 2013. No association between the response to methylphenidate and DRD4 gene polymorphism in Korean attention deficit hyperactivity disorder: A case control study. *Clin Psychopharmacol Neurosci*, 11: 13-17.
- Johansson S, Halletland H, Halmøy A, Jacobsen KK, Landaas ET, Dramsdahl M, Fasmer OB, Bergsholm P, Lundervold AJ, Gillberg C, Hugdahl K, Knappskog PM, Haavik J 2008. Genetic analyses of dopamine related genes in adult ADHD patients suggest an association with the DRD5-microsatellite repeat but not with DRD4 or SLC6A3 VNTRs. *Am J Med Genet B Neuropsychiatr Genet*, 147B: 1470-1475.
- Kim YS, Leventhal BL, Kim SJ, Kim BN, Cheon KA, Yoo HJ, Kim SJ, Badner J, Cook EH 2005. Family-based association study of DAT1 and DRD4 polymorphism in Korean children with ADHD. *Neurosci Lett*, 390: 176-181.
- Langley K, Fowler TA, Grady DL, Moyzis RK, Holmans PA, van den Bree MB, Owen MJ, O'Donovan MC, Thapar A 2009. Molecular genetic contribution to the developmental course of attention-deficit hyperactivity disorder. *Eur Child Adolesc Psychiatry*, 18: 26-32.
- Leung PW, Lee CC, Hung SF, Ho TP, Tang CP, Kwong SL, Leung SY, Yuen ST, Lieh-Mak F, Oosterlaan J, Grady D, Harxhi A, Ding YC, Chi HC, Flodman P, Schuck S, Spence MA, Moyzis R, Swanson J 2005. Dopamine receptor D4 (DRD4) gene in Han Chinese children with attention deficit/hyperactivity disorder (ADHD): Increased prevalence of the 2-repeat allele. *Am J Med Genet (Neuropsychiatr Genet)*, 133: 54-56.
- Li D, Sham PC, Owen MJ, He L 2006. Meta-analysis shows significant association between dopamine system genes and attention deficit hyperactivity disorder (ADHD). *Human Molecular Genetics*, 15: 2276-2284.
- Marino C, Giorda R, Vanzin L, Molteni M, Lorusso ML, Nobile M, Baschiroto C, Alda M, Battaglia M 2003. No evidence for association and linkage disequilibrium between dyslexia and markers of four dopamine-related genes. *Eur Child Adolesc Psychiatry*, 12: 198-202.
- Oak JN, Oldenhof J, Van Tol HH 2000. The dopamine D(4) receptor: One decade of research. *Eur J Pharmacol*, 405: 303-327.
- Qian Q, Wang Y, Zhou R, Yang L, Faraone SV 2004. Family-based and case-control association studies of DRD4 and DAT1 polymorphisms in Chinese attention deficit hyperactivity disorder patients suggest long repeats contribute to genetic risk for the disorder. *Am J Med Genet B Neuropsychiatr Genet*, 128B: 84-89.
- Ribasés M, Ramos-Quiroga JA, Hervás A, Sánchez-Mora C, Bosch R, Bielsa A, Gastaminza X, Lesch KP, Reif A, Renner TJ, Romanos M, Warnke A, Walitza S, Freitag C, Meyer J, Palmason H, Casas M, Bayés M, Cormand B 2012. Candidate system analysis in ADHD: Evaluation of nine genes involved in dopaminergic neurotransmission identifies association with DRD1. *World J Biol Psychiatry*, 13: 281-292.
- Rietveld MJ, Hudziak JJ, Bartelsor M, Van Beijsterveldt CE, Boomsma DI 2004. Heritability of attention problems in children: Longitudinal results from a study of twins age 3 to 12. *J Child Psychol Psychiatry*, 45: 577-588.
- Rohde LA, Halpern R 2004. Recent advances on attention deficit/hyperactivity disorder. *Journal de Pédiatrie*, 80 Suppl 2: 61-70.
- Roman T, Schmitz M, Polanczyk G, Eizirik M, Rohde LA, Hutz MH 2001. Attention-deficit hyperactivity disorder: A study of association with both the dopamine transporter gene and the dopamine D4 receptor gene. *Am J Med Genet*, 105: 471-478.
- Shaw P, Gornick M, Lerch J, Addington A, Seal J, Greenstein D, Sharp W, Evans A, Giedd JN, Castellanos FX, Rapoport JL 2007. Polymorphisms of the dopamine D4 receptor, clinical outcome and cortical structure in attention-deficit/hyperactivity disorder. *Arch Gen Psychiatry*, 64: 921-931.
- Swanson JM, Kinsbourne M, Nigg J, Lanphear B 2007. Etiologic subtypes of attention deficit hyperactivity disorder: Brain imaging, molecular genetic and environmental factors and dopamine hypothesis. *Neuropsychol Rev*, 17: 39-59.
- Szekely A, Ronai Z, Nemoda Z, Kolmann G, Gervai J, Sasvari-Szekely M 2004. Human personality dimensions of persistence and harm avoidance associated with DRD4 and 5 HTTLPR polymorphisms. *Am J Med Genet B Neuropsychiatr Genet*, 126B: 106-110.
- Tahir E, Yazgan Y, Cirakoglu B, Ozbay F, Waldman I, Asherson PJ 2000. Association and linkage of DRD4 and DRD5 with attention deficit hyperactivity disorder (ADHD) in a sample of Turkish children. *Mol Psychiatry*, 5: 396-404.
- Thapar A, O'Donovan M, Owen MJ 2005. The genetics of attention deficit hyperactivity disorder. *Human Mol Genetics*, 14: 275-282.
- Turic D, Swanson J, Sonuga-Barke E 2010. DRD4 and DAT1 in ADHD: Functional neurobiology to pharmacogenetics. *Pharmacogenomics Pers Med*, 3: 61-78.
- Van Tol HH, Wu CM, Guan HC, Ohara K, Bunzow JR, Civelli O, Kennedy J, Seeman P, Niznik HB, Jovanovic V 1992. Multiple dopamine D4 receptor variants in the human population. *Nature*, 358: 149-152.
- Wang E, Ding YC, Flodman P, Kidd JR, Kidd KK, Grady DL, Ryder OA, Spence MA, Swanson JM, Moyzis RK 2004. The genetic architecture of selection at the human dopamine receptor D4 (DRD4) gene locus. *Am J Hum Genet*, 74: 931-944.