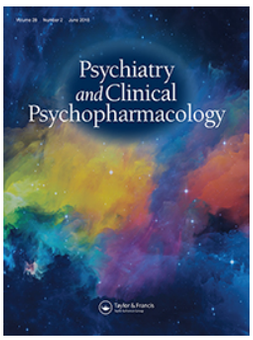

Psychiatry and Clinical Psychopharmacology

ISSN: 2475-0573 (Print) 2475-0581 (Online) Journal homepage: https://www.tandfonline.com/loi/tbcp21

Allele frequencies of dopamine D4 receptor gene (DRD4) and Catechol-O-methyltransferase (COMT) Val158Met polymorphism are associated with methylphenidate response in adolescents with attention deficit/hyperactivity disorder: a case control preliminary study

Aynur Pekcanlar Akay, Çiğdem Eresen Yazıcıoğlu, Sevay Alşen Güney, Handan Özek Erkuran, Sefa Kızıldağ, Burak Baykara, Gonca Özyurt, Şebnem Yıldırımcan Kadıçeşme, Süha Miral & Neslihan İnal Emiroğlu

To cite this article: Aynur Pekcanlar Akay, Çiğdem Eresen Yazıcıoğlu, Sevay Alşen Güney, Handan Özek Erkuran, Sefa Kızıldağ, Burak Baykara, Gonca Özyurt, Şebnem Yıldırımcan Kadıçeşme, Süha Miral & Neslihan İnal Emiroğlu (2018) Allele frequencies of dopamine D4 receptor gene (DRD4) and Catechol-O-methyltransferase (COMT) Val158Met polymorphism are associated with methylphenidate response in adolescents with attention deficit/hyperactivity disorder: a case control preliminary study, Psychiatry and Clinical Psychopharmacology, 28:2, 177-184, DOI: 10.1080/24750573.2017.1418134

To link to this article: https://doi.org/10.1080/24750573.2017.1418134

Published online: 03 Jan 2018.

PSYCHIATRY AND CLINICAL PSYCHOPHARMACOLOGY, 2018
VOL. 28, NO. 2, 177–184
https://doi.org/10.1080/24750573.2017.1418134

Taylor & Francis
Taylor & Francis Group

Allele frequencies of dopamine D4 receptor gene (DRD4) and Catechol-O-methyltransferase (COMT) Val158Met polymorphism are associated with methylphenidate response in adolescents with attention deficit/hyperactivity disorder: a case control preliminary study

Aynur Pekcanlar Akay[a], Çiğdem Eresen Yazıcıoğlu[b], Sevay Alşen Güney[a], Handan Özek Erkuran[c], Sefa Kızıldağ[b], Burak Baykara[a], Gonca Özyurt[d], Şebnem Yıldırımcan Kadıçeşme[b], Süha Miral[a] and Neslihan İnal Emiroğlu[a]

[a]Department of Child and Adolescent Psychiatry, Dokuz Eylul University, Izmir, Turkey; [b]Department of Molecular Biology and Genetics, Dokuz Eylul University, Izmir, Turkey; [c]Deparment of Child and Adolescent Psychiatry, Child Psychiatry Specialist, Dr. Behçet Uz Pediatrics and Pediatric Surgery Training Hospital, Izmir, Turkey; [d]Department of Child and Adolescent Psychiatry, Katip Çelebi University Medical Faculty, Izmir, Turkey

ABSTRACT

OBJECTIVES: In this study, it was aimed to analyse the relationship between clinical improvement in adolescents with attention deficit/hyperactivity disorder (ADHD) and the presence of allele frequencies of dopamine D4 receptor (*DRD4*), and Val158Met polymorphism of catechol-O-methyltransferase (*COMT*) genes.

METHODS: Thirty-four adolescents (age range, 13–18 years) with ADHD participated in this study. Thirty-two patients were males and two were females. Du Paul ADHD Rating Scale-Clinician version (ARS) and Clinical Global Impression-severity of impairment (CGI-S) were used for the evaluation of symptom severity. Fifty healthy age-matched adolescents were recruited as controls.

RESULTS: When the groups with ($n = 9$) and without ($n = 25$) 7-repeat alleles for *DRD4* were considered, there was a statistically significant decrease of DuPaul ARS total and hyperactivity scores in those treated with OROS-methylphenidate. When the Val/Met allele-positive group for *COMT* gene ($n = 17$) was compared with the Val/Val allele-positive group ($n = 13$) and Met/Met allele-positive group ($n = 4$), there was a statistically significant decrease of ARS total scores, ARS attention scores, and CGI scores in adolescents with ADHD treated with OROS-MPH.

CONCLUSIONS: Specific data from further studies with a larger sample sizes would provide more insights to replicate the current findings.

ARTICLE HISTORY
Received 30 September 2017
Accepted 13 December 2017

KEYWORDS
Attention deficit/hyperactivity disorder; adolescents; catechol-O-methyltransferase gene (COMT); dopamine D4 receptor gene (DRD4); methylphenidate; clinical global impression (CGI)

Introduction

Attention deficit/hyperactivity disorder (ADHD) is a heterogeneous neurodevelopmental disorder with multiple causes and courses, with a wide range of symptom severity and frequency across individuals, and a number of comorbidities [1]. It is the most prevalent neurodevelopmental disorder with a prevalence rate of 5.29% in children and adolescents worldwide [2], and frequently persists into adulthood [3].

In terms of dopaminergic system abnormalities in the pathophysiology of ADHD, molecular genetic studies declared at least four candidate genes that were related to ADHD (dopamine D4, D5, dopamine transporter, and serotonin transporter) [4]. According to studies regarding both the etiological point of view and treatment response of methylphenidate, the dopamine D4 receptor (*DRD4*) gene is especially emphasized in the dopaminergic system, and studies found the catechol-O-methyltransferase (*COMT*) gene may be crucial for response to treatment [5–7]. Due to current knowledge in Turkish sample, there was no study which investigated the relation of both these genes and methylphenidate treatment response.

The *DRD4* gene is been mapped in the chromosomal region 11p15.5, and it includes a 48 base pair variable number tandem repeat polymorphism in exon 3. Two to 11 repeats may be present in this locus, 4 and 7 repeats have been shown as the most common repeats. *DRD4* is mostly situated in the frontal lobe, orbitofrontal, and anterior cingulate cortex [8,9], and it is crucial because of the acceptance of this receptor's relationship with cognitive and attentional brain functions and the 7-repeat (7R) polymorphism [6,8,10,11]. *DRD4* with the 7-repeat (7R) polymorphism was found to decrease sensitivity to dopaminergic stimuli [12]. *DRD4* 7R is reported as moderately associated with

CONTACT Gonca Özyurt goncaenginozyurt@gmail.com Department of Child and Adolescent Psychiatry, Katip Çelebi University Medical Faculty, Izmir, Turkey

ADHD [13]. Although there are many supportive publications for this relationship, there are some publications that provided contrary findings [6].

The *COMT* gene is located in chromosomal region 22q11.1–q11.2. *COMT*, a metabolism enzyme, is the main determining factor for external cell levels of dopamine in the prefrontal cortex. A single-nucleotide polymorphism was found in the *COMT* gene that causes a methionine change of valine 158 and is unique to humans [8]. Previous studies showed the association between the Val/Met functional polymorphism in *COMT* and prefrontal cortex information processing [14–16], which has important roles in psychiatric disorders [15,17,18]. The prefrontal cortex and dopaminergic pathway are significant in ADHD [19–23], and the Val/Met polymorphism of *COMT* has been investigated in the etiology of ADHD. *COMT* enzyme has three forms distribution, low ($COMT_{LL}$), intermediate ($COMT_{LH}$), and high ($COMT_{HH}$) activities [24,25]. Met/Met homozygote enzyme activity is three- to four-fold slower than that of Val/Val homozygotes, and Val/Met heterozygotes' enzyme activity level is intermediate [26]. According to a study Val/Val genotype is more common in ADHD population than healthy population and it plays a positive role in response to medication [8].

Our steps for reaching our objectives are presented below:

(1) To determine whether there are meaningful changes in allele frequencies in terms of *DRD4* and *COMT* polymorphisms of genes in adolescents with ADHD compared with healthy controls.
(2) To analyse the clinical improvement in adolescents with ADHD and the presence of a relationship with allele frequencies of *DRD4* and polymorphisms of *COMT* genes.
(3) To examine the relationship between comorbid conditions and subtypes of ADHD and *DRD4* and *COMT* polymorphisms of genes in adolescents with ADHD.

Methods

The study was performed in Dokuz Eylul University, School of Medicine, Department of Child and Adolescent Psychiatry and Department of Molecular Biology and Genetics. Thirty-four adolescents (age range, 13–18 years) with ADHD participated in this study. All adolescents were recruited from the ADHD outpatient clinic at the Department of Child and Adolescent Psychiatry. Parents and adolescents provided written informed consent and assent to participate. This investigation was approved by the Republic of Turkey Ministry of Health Review Board. Thirty-two patients were males and three were females. None had a comorbid neurologic disease or any other significant clinical disease. All patients were drug naive. Both the adolescents and parents were interviewed during the diagnostic process. Du Paul ADHD Rating Scale-Clinician version (ARS) [27] and the Clinical Global Impression-Severity of Impairment (CGI-S) [28] were used for the evaluation of symptom severity. For inclusion of the patients to this study, Schedule for Affective Disorders and Schizophrenia for School Aged Children Kiddie-SADS-lifetime Version (K-SADS-PL) [29] semi-structured clinical interviews were conducted and ADHD diagnoses were confirmed [29] Exclusion criteria were intelligence quotient (IQ) being <70, history of head trauma or neurologic illness, developmental delay, any mood disorder or pervasive developmental disorder. We performed baseline neurologic and physical examinations, and electroencephalography (EEG) to exclude neurologic problems. An estimated full-scale IQ was obtained using the Wechsler Intelligence Scale for Children-Revised (WISC-R) and the Wechsler Adult Intelligence Scale (WAIS) for children older than 16 years. Blood analysis of the patients was undertaken for liver functions, thyroid functions, complete blood count (CBC) and genetic evaluations. ECG was performed before MPH treatment. The controls were healthy adolescents who were matched by age. The controls underwent genetic evaluations once at baseline and the parents of the controls completed a clinical interview and the K-SADS-PL. Potential controls with evidence of ADHD or comorbid psychiatric conditions were excluded. Blood was taken for genetic analysis from all 50 healthy controls. No significant deviations from Hardy-Weinberg equilibrium were detected for *COMT* Val158Met polymorphism. Homozygote reference allele (Met/Met) was more common among control (71.4%) while heterozygote allele (Val/Met) was more common among cases. The difference of the three polymorphisms among cases and controls were not significant ($p = .52$). *DRD4* genotype of our cases and control were categorized into four groups based on their four and seven genotypes. Cases had more only seven genotypes (57.1%) and control had more both genotypes (80.0%). There was no statistically significant difference based on four genotype categories among cases and controls (Fisher's exact test, $p = .12$). Clinical improvements were assessed using the ARS and CGI on two occasions (before beginning OROS-MPH and at the end of the study).

Due to power analysis investigation at least 34 cases were planned for 0.05 alpha error margin and 80% power by taking middle effect size power.

OROS-methylphenidate treatment consisted of oral daily doses. Each subject's dose was individually titrated in accordance with the clinical response in CGI and ARS scales. The starting dose was 18 mg and it was titrated up to 54–72 mg over four weeks (average dosage: 1 mg/kg/day). Treatment was administered orally once a day at

breakfast. Study compliance was assessed by self-report, mother's report, and through pill counting. The patients were seen weekly at the dose adjustment phase, and monthly thereafter. Height, weight, pulse, blood pressure, adverse effects, and CGI and ARS scales were assessed by a child psychiatrist who was blinded to the genotypes and imaging data. After two months, all patients were free to continue using OROS-MPH.

At the beginning of the study, we had 37 ADHD cases. However, one male and two female adolescents were excluded from the study because the failed to present to their follow-up visit and they did not use their medication properly. Robust improvement was defined as an improvement for the ADHD group: 50% reduction in ARS scale scores and 2-point reduction in CGI severity scores [30].

Kiddie Schedule for Affective Disorders and Schizophrenia for School-Age Children - Present and Lifetime Version (K-SADS-PL): K-SADS-PL is a widely used semi-structured diagnostic interview tool [29]. It inquires about current and past episodes of child and adolescent psychiatric disorders and allows a diagnosis to be made. The Turkish version of the K-SADS-PL was reported to have good test-retest and inter-rater reliability [31]. In the current study, K-SADS-PL was used to make the diagnosis of ADHD along with allowed comorbidities such as enuresis and tic disorders.

Wechsler Intelligence Scale for Children-Revised (WISC-R): The WISC-R was designed to measure the IQ of children aged between 6 and 16 years [32]. Standardization of the WISC-R for Turkish children was conducted by Savasir and Sahin [33]. The WISC-R along with impaired functioning was used to rule out mental retardation in the present study.

Clinical Global Impression (CGI) Scale: The CGI is a 7-item Likert-type scale that allows physicians to evaluate the severity of the disorder at the time of assessment, relative to the physician's past experience with patients who have the same diagnosis. The scores range from 1 (normal, not at all ill) to 7 (extremely ill) [28].

DuPaul ARS: This scale is composed of 18 items that tap into symptoms of ADHD listed in the DSM-IV criteria. Each item has a 4-point scale of 0–3. Nine items are related to inattention and the remainder is related to hyperactivity and impulsivity. This scale was previously used in Turkish studies of ADHD [27].

Genetic assays

DNA was extracted from frozen peripheral blood using a DNA isolation kit (Roche). The exon III repeat region of the *DRD4* receptor was characterized as follows. The primers used were 5′-CTTCCTACCCTGCCCGCTCA TGCTGCTGCTCTACTG-3′ and 5′-ACCACCACC GGCAGGACCCTCATGGCCTTGCGCTC-3′ [34]. The reaction mixture contained the following components: 100 ng DNA; 0.2 mM dNTP mix, 20 pmol of each primer, 2.5 U Fast Start Taq polymerase (Roche), 1x Fast Start Taq buffer, and 1x GC rich solution in a total volume of 25 µL. The polymerase chain reaction (PCR) conditions consisted of an initial denaturing step at 95°C for 5 min, followed by 30 cycles that included a 45 sec denaturing step at 95°C, a 45 sec annealing step at 58°C, and a 90 sec extension step at 68°C. Cycling was followed by a final extension at 68°C for 5 min. The PCR products were separated using electrophoresis through a 2% agarose gel, stained with ethidium bromide, using a Thermo Scientific horizontal-gel apparatus. Fifty base pair and 100 bp ladders were used to score the various repeat alleles. The gel was imaged using a digital imaging system. Each sample was provided for *COMT* polymorphism (Val/Met) by amplification of a 169 base pair fragment. The forward primer was 5′–ACTGTGGCTACTCAGCTGTG–3′ and the reverse primer was 5′–CCTTTTTCCAGGTCTGAC AA–3′ [35]. The reaction mixture consisted of the following components: 40 pmol of each primer; 200 ng DNA; 0.2 mM dNTP mix, 1.4 mM $MgCl_2$, 5 units TAQ polymerase (recombinant), and 1x TAQ buffer in a total volume of 25 µL. The PCR conditions consisted of an initial denaturing step at 94°C for 4 min followed by 32 cycles that included a 30 sec denaturing step at 94°C, a 30 sec annealing step at 55°C, and a 30 sec extension step at 72°C. Cycling was followed by a final extension at 72°C for 5 min. The obtained amplification products were sent for DNA sequence analysis (Macrogen, Europa). Sequence data were compared with homosapien catechol-O-methyltransferase (*COMT*), Ref Seq Gene on chromosome 22 NCBI Reference Sequence: NG_011526.1

Statistical analyses

Statistical evaluations were performed using SPSS 15.0 program (SPSS Inc., Chicago, IL., U.S.A). The 34 subjects with ADHD and 50 male healthy controls' results were compared in our study. The Chi-square test and when needed, Fisher's exact Chi-square test were used for the comparison of categorical variables. Continuous variables were compared using the *t*-test. Continuous variables are reported as means (with standard deviations) and categorical variables are identified as percentages. Pairwise comparisons were used to determine the source of differences. Statistical significance was determined at $p < .05$. The difference between the two-month and baseline ARS and CGI scores was calculated using the paired-samples *t*-test. The Mann–Whitney U test was used to compare ARS and CGI scores between the subjects with and without 7-repeat allele in *DRD4*, and between subjects with and without a robust response to OROS-MPH treatment. The Kruskal–Wallis test was used to compare ARS and CGI scores between the subjects with Val/Val, Val/Met, and Met/Met repeat alleles in *COMT*, and between

subjects with and without a robust response to OROS-MPH treatment. Allele frequencies in terms of *DRD4* and *COMT* polymorphisms of genes in adolescents with ADHD and improvement were compared using the Chi-square test. Differences in mean ARS and CGI scores between ADHD subtypes and comorbidities were analysed using the Mann–Whitney U test.

Results

Thirty-four patients were included in the study. Adolescents with ADHD ranged in age from 13 to 18 years with a mean of 14.20 ± 1.53 years. Healthy Adolescents ranged in age from 13 to 18 years with a mean of 14.28 ± 1.60 years ($p = .897$). There was a strong male predominance in this sample due to the fact that 94% ($n = 32$) of the patients were male in study group. Ninety percent ($n = 45$) of the healthy controls were male ($p = .404$).

With regard to ADHD subtype, 32.4% ($n = 11$) met criteria for ADHD-Predominantly Inattentive Type, and 67.6% ($n = 23$) were diagnosed as having ADHD-Combined Type. In addition, 64.7% ($n = 22$) of the patients had comorbid diagnosis (14 had oppositional defiant disorder, 3 had oppositional defiant disorder + anxiety disorders, 2 had comorbid anxiety disorders, 1 had adjustment disorder, 1 had tic disorder, and 1 had conduct disorder). All participants were drug naive. The number of cigarette smokers was 3. Patients used non-steroid analgesic and penicillin- group antibiotics during the study procedures. One patient received an influenza vaccination injection. The adverse effects were loss of appetite, headache, agitation, nausea, insomnia, fatigue, and tachycardia at mild-to-moderate levels.

The mean WISC-R verbal, WISC-R performance, and WISC-R total scores of the patients with ADHD were 92.12 ± 15.98, 95.61 ± 17.30, and 91.31 ± 13.95, respectively.

Genotype frequencies for 34 adolescents for the *DRD4* gene were as follows: 9 (26%) were positive for the *DRD4* 7-repeat allele, 25 (74%) were negative for the *DRD4* 7-repeat allele, 29 (85%) were positive for the *DRD4* 4-repeat allele, and 5 (15%) were negative for the *DRD4* 4-repeat allele.

The genotype frequencies of the 34 adolescents for *COMT* gene were as follows: 13 (38%) were homozygous for the *COMT* Valine-repeat allele, 17 (50%) had one copy of the Valine-repeat and one copy of Methionine-repeat allele, and 4 (12%) were homozygous for the *COMT* Methionine-repeat allele. Case–control analyses of two polymorphisms (*DRD4* 48 bp VNTR, and *COMT* Val158Met) were conducted on the 34 adolescents with ADHD and 50 similar aged control subjects; there were no statistically significant differences in the allele frequencies of the investigated VNTRs of genes between the ADHD group and healthy controls (Table 1).

Table 1. Allele frequencies of the *DRD4*, and *COMT* polymorphisms in patients with ADHD and controls.

Polymorphic sites	*DRD4* 48 bp VNTR				*COMT* Val158Met		
Genotype	7+	7−	4+	4−	Met/Met	Met/Val	Val/Val
ADHD							
n (total 20)	9	25	29	5	4	17	13
Frequency (%)	26	74	85	15	12	50	38
Control							
n (total 50)	16	34	47	3	10	20	20
Frequency (%)	32	68	94	6	20	40	40

The mean score on the CGI was 5.18 ± 0.72 at baseline. There was a statistically significant improvement in behaviour after eight weeks, 3.45 ± 1.09 ($p < .001$). There were statistically significant differences in ARS for total scores ($p < .001$), ARS inattention/hyperactivity ($p < .001$) and impulsivity/hyperactivity ($p < .001$) subscales (Table 2).

When the group with and without 7-repeat allele for *DRD4* was considered, no significant differences were found between children with ADHD with ($n = 9$) and without ($n = 25$) 7-repeat alleles in the *DRD4* gene in terms of age and IQ. When the baseline ARS scores were compared, the mean ARS total of the 7-repeat allele-positive group (31.44 ± 7.67) was significantly lower than 7-repeat allele-negative group (37.76 ± 7.93) for *DRD4* ($p = .027$). However, there was a statistically significant decrease of ARS total scores ($p = .008$), ARS hyperactivity scores ($p = .029$) in those treated with OROS-MPH in the 7-repeat allele-negative group for the *DRD4* gene.

Due to improvement indicators of at least 50% reduction in ARS scores and a 2-point reduction in CGI (robust improvement), there were significant relationships between improvement and *DRD4* 7-repeat allele presence ($p = .018$). In the present study, the 7-repeat allele-positive group for *DRD4* gene had no response to OROS-MPH.

When the group with and without the 4-repeat allele for *DRD4* was considered, no significant differences were found between adolescents with ADHD with ($n = 29$) and without ($n = 5$) the 4-repeat allele in the *DRD4* gene in terms of age, IQ, ARS, and CGI scores.

No significant differences were found between adolescents with ADHD with ($n = 13$) and without ($n = 21$) homozygosity for the Val/Val allele in the *COMT* gene regarding age and IQ. Due to improvement indicators, there were no significant relationships between improvement and Val/Val allele positivity in the

Table 2. ARS and CGI Scales at pre-treatment and rating scales after two months OROS-methylphenidate treatment.

	Pre-treatment	Post-treatment	*p*
ARS total	36.09 ± 8.25	18.57 ± 8.89	**<.001**
ARS inattention/hyperactivity	20.73 ± 3.89	11.21 ± 5.27	**<.001**
ARS impulsivity /Hyperactivity	15.12 ± 6.18	7.36 ± 4.62	**<.001**
CGI	5.18 ± 0.72	3.45 ± 1.09	**<.001**

Notes: *p* values determined by paired sample *t*-test. Bold values indicate statistically significant results. ARS: DuPaul Rating Scale; CGI: Clinical Global Impression.

ADHD group. When the Val/Val allele-positive group for the *COMT* gene ($n = 13$), Met/Met allele-positive group for the *COMT* gene ($n = 4$), and Val/Met allele-positive group for *COMT* gene ($n = 17$) were analysed, there were statistically significant decreases in ARS total scores ($p = .029$), ARS attention scores ($p = .019$), and CGI scores ($p = .020$) in those treated with OROS-MPH. There were statistically significant decreases of ARS total scores ($p = .008$), ARS attention scores ($p = .005$), and CGI scores ($p = .008$) in those treated with OROS-MPH in the Val/Met allele-positive group for the *COMT* gene ($n = 17$).

Some 67.6% ($n = 23$) of the patients with ADHD were of the combined type and ODD was the most frequent comorbidity (64.7%). No significant relationships were found between adolescents with ADHD who were diagnosed as having mixed subtype and predominantly inattentive subtype ($n = 11$) in age, IQ, CGI, and ARS scores. No significant relationships were found between adolescents with ADHD with ($n = 22$) and without comorbidities ($n = 12$) in terms of age, IQ, CGI, and ARS scores.

There were no relationships between the *DRD4* gene and ADHD subtypes and comorbidities. There was no relationship between the *COMT* gene and ADHD subtypes. However, there were significant relationships between comorbidities and the *COMT* gene. Adolescents with homozygosity for the Val/Val allele in the *COMT* gene ($n = 13$) had fewer comorbidities ($p = .035$).

Discussion

The case–control analyses of two genes(*DRD4* 48 bp VNTR, and *COMT* Val158Met) were performed on 34 adolescents with ADHD and 50 similar-aged control subjects in this 2-month, single centre, prospective study. There were no statistically significant differences in the allele frequencies of the investigated VNTRs of *DRD4* genes between the ADHD group and healthy controls. However, the results of this study showed that while treatment response to OROS-MPH was poor in adolescents with ADHD with 7-repeat alleles for the *DRD4* gene, adolescents with ADHD without 7-repeat alleles for the *DRD4* gene showed a good response to OROS-MPH treatment. In addition, there were statistically significantly decreased ARS total scores and ARS hyperactivity scores in those treated with OROS-MPH in the 7-repeat allele-negative group for the *DRD4* gene. However, no significant differences were found between adolescents with ADHD with and without 4-repeat alleles in the *DRD4* gene in ARS, CGI scores, and improvement. Hamarman et al. (2004) found that patients carrying the 7-repeat allele required a higher dose of MPH for symptom improvement [36]. 7-repeat allele of *DRD4* was found as related with impaired attention [37]. A recent study from Korea reported that no significant difference was seen between the *DRD4* genetic type, allele distribution, and CGI drug response [38], whereas another Korean ADHD population with the *DRD4* 4-repeat allele was associated with good response to MPH [38]. An additional two studies resulted in negative findings [5,39]. Although the mean ARS total for the 7-repeat allele-positive group was significantly lower than the 7-repeat allele-negative group for *DRD4* gene at baseline, the results of the present study support the findings of previous studies that the presence of the 7-repeat allele for the *DRD4* gene is related to poor response to MPH treatment for ADHD.

DRD4 and *COMT* genes play important roles in development of psychiatric disorders not only ADHD but also anxiety disorders and depression [40].

In the present study, there was no isolated role for the *DRD4* gene in ADHD susceptibility in a sample of adolescents with ADHD. No evidence was found for an association between the *DRD4* 7-repeat allele and ADHD when our ADHD sample was compared with a control sample. Several studies that focused on the *DRD4* gene found an association of the 7-repeat allele with ADHD [6,21,41]. The negative findings for the *DRD4* gene in the current study are in agreement with the studies of Einsenberg et al. (2000) and Hawi et al. (2000) [42,43].

When the Val/Val allele-positive group for *COMT* gene, Met/Met allele-positive group for *COMT* gene, and Val/Met allele-positive group for *COMT* gene were considered, there were statistically significant decreases of ARS total scores, ARS attention scores, and CGI scores in adolescents treated with OROS-MPH. There were statistically significant decreases of ARS total scores, ARS attention scores, and CGI scores in adolescents treated with OROS-MPH in the Val/Met allele-positive group for the *COMT* gene ($n = 17$). In Kereszturi et al.'s (2008) study, a higher MPH response was reported in the Val/Val group; the Val/Met response was better in the current study [5]. In Hong et al.'s study (2015) it was found that a network of white matter (connections linking 18 different brain regions) was significantly weakened in youth with ADHD who were COMT Met-carriers compared to those who were Val-homozygous ($p < 0.05$, family-wise error-corrected) [44]. This situation may be related with these results. In Kabukçu et al.'s study (2016) first, an association COMT val158met polymorphism and ADHD in the right (R) cingulum (cingulate gyrus) (CGC) was found which affect brain development [45]. In Yatsuga et al.'s study (2014), no significant difference was found in the Val/Val genotype according to disorder, and WISC and ADHD rating scale scores; similar to Yatsuga et al.'s study, WISC scores and genotypes were not associated in the present study, although there were statistically significant decreases of ARS scores and CGI scores in adolescents

treated with OROS-MPH in our study [46]. In the studies of Tahir et al. (2000) and McGough et al. (2009), no relation was found between *COMT* genotype and ADHD symptom severity [47,48]. Caspi et al. (2008) showed an association between Val/Val genotype and increased aggression and conduct problems [49]. However, recent studies reported a lack of association between the *COMT* genotype and ADHD. In a meta-analysis, no association between the *COMT* genotype and ADHD was found. Different results may be considered due to the multifactorial nature of ADHD. The *COMT* gene Val allele was more frequent in ADHD groups in the studies of Kereszturi et al. (2008) and Yatsuga et al. (2014) [5,46]. However, no significant difference was found between the groups in the present study. The sample size of the current study might be too small to prove meaningful differences. In a recent meta-analysis (2015) no relation was found between ADHD and the *COMT* Val158Met polymorphisms in all study participants (OR = 1.078, 95% CI = [0.962, 1.207], $p = .196$) [50].

This present study should be interpreted in the context of certain limitations. First, because our sample size was small, it would be difficult to generalize the results to a hypothesis on predicting treatment response to MPH in ADHD. Regression to the mean and ceiling effects may also be responsible for the study findings. There may be one additional limitation. Although comorbid psychiatric conditions were screened with the K-SADS-PL. Nevertheless, our findings provide pharmacogenetics on stimulant treatment of ADHD worthy of further investigation. Larger and more varied treatment arms with IR MPH and atomoxetine may also have enriched our results.

Disclosure statement

Aynur Pekcanlar Akay is Advisory Board Member of Janssen, Sanofi and Lilly Pharmaceutical Companies. Other authors reported no conflicts of interest related to this article.

Funding

This work was supported by Dokuz Eylül Üniversitesi.

References

[1] American Psychiatric Association Diagnostic and statistical manual of mental disorders (DSM-5). American Psychiatric Pub 2013.
[2] Polanczyk G, de Lima MS, Horta BL, et al. The worldwide prevalence of ADHD: a systematic review and metaregression analysis. Am J Psychiatry. 2007;164:942–948.
[3] Kessler RC, Adler L, Barkley R, et al. The prevalence and correlates of adult ADHD in the United States: results from the national comorbidity survey replication. Am J Psychiatry. 2006;163:716–723.
[4] Bobb AJ, Castellanos FX, Addington AM, et al. Molecular genetic studies of ADHD: 1991 to 2004. Am J Med Genet B Neuropsychiatr Genet. 2005;132:109–125.
[5] Kereszturi E, Tarnok Z, Bognar E, et al. Catechol-*O*-methyltransferase Val158Met polymorphism is associated with methylphenidate response in ADHD children. Am J Med Genet Part B: Am J Med Genet. 2008;147B:1431–1435.
[6] Roman T, Schmitz M, Polanczyk G, et al. Attention-deficit hyperactivity disorder: A study of association with both the dopamine transporter gene and the dopamine D4 receptor gene. Am J Med Genet. 2001;105:471–478.
[7] Szobot C, Roman T, Cunha R, et al. Brain perfusion and dopaminergic genes in boys with attention-deficit/hyperactivity disorder. Am J Med Genet Part B (Neuropsychiatric Genetics). 2005;132B:53–58.
[8] Gizer IR, Waldman ID. Double dissociation between lab measures of inattention and impulsivity and the dopamine transporter gene (*DAT1*) and dopamine D4 receptor gene (*DRD4*). J Abnorm Psychol. 2012;121:1011–1023.
[9] Noain D, Avale ME, Wedemeyer C, et al. Identification of brain neurons expressing the dopamine D4 receptor gene using bac transgenic mice. Eur J Neurosci. 2006;24:2429–2438.
[10] Johnson KA, Kelly SP, Robertson IH, et al. Absence of the 7-repeat variant of the *DRD4* VNTR is associated with drifting sustained attention in children with ADHD but not in controls. Am J Med Genet B Neuropsychiatr Genet. 2008;147B:927–937.
[11] Kieling C, Roman T, Doyle AE, et al. Association between *DRD4* gene and performance of children with ADHD in a test of sustained attention. Biol Psychiatry. 2006;60:1163–1165.
[12] Asghari V, Sanyal S, Buchwaldt S, et al. Modulation of intracellular cyclic AMP levels by different human dopamine D4 receptor variants. J Neurochem. 1995;65:1157–1165.
[13] Faraone SV, Mick E. Molecular genetics of attention deficit hyperactivity disorder. Psychiatr Clin North Am. 2010;33:159–180.
[14] Tunbridge EM, Harrison PJ, Weinberger DR. Catechol-O-methyltransferase, cognition, and psychosis: Val158Met and beyond. Biol Psychiatry. 2006;60(2):141–151.
[15] Egan MF, Goldberg TE, Kolachana BS, et al. Effect of *COMT* Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. Proc Natl Acad Sci USA. 2001;98(12):6917–6922.
[16] Winterer G, Egan MF, Kolachana BS, et al. Prefrontal electrophysiologic 'noise' and catechol-O-methyltransferase genotype in schizophrenia. Biol Psychiatry. 2006;60(6):578–584.
[17] Karayiorgou M, Gogos JA, Galke BL, et al. Identification of sequence variants and analysis of the role of the catechol-O-methyl-transferase gene in schizophrenia susceptibility. Biol Psychiatry. 1998;43(6):425–431.
[18] Kotler M, Barak P, Cohen H, et al. Homicidal behavior in schizophrenia associated with a genetic polymorphism determining low catechol O-methyltransferase (*COMT*) activity. Am J Med Genet. 1999;88(6):628–633.
[19] Faraone, SV, Biederman, J. Neurobiology of attention-deficit hyperactivity disorder. Biol Psychiatry. 1998;44(10):951–958.

[20] Barr CL, Xu C, Kroft J, et al. Haplotype study of three polymorphisms at the dopamine transporter locus confirm linkage to attention-deficit/hyperactivity disorder. Biol Psychiatry. 2001;49(4):333–339.
[21] LaHoste GJ, Swanson JM, Wigal SB, et al. Dopamine D4 receptor gene polymorphism is associated with attention deficit hyperactivity disorder. Mol Psychiatry. 1996;1(2):121–124.
[22] Cook EJ, Stein MA, Krasowski MD, et al. Association of attention-deficit disorder and the dopamine transporter gene. Am J Hum Genet. 1995;56 (4):993–998.
[23] Faraone SV, Biederman J, Chen WJ, et al. Genetic heterogeneity in attention-deficit hyperactivity disorder (ADHD): gender, psychiatric comorbidity, and maternal ADHD. J Abnorm Psychol. 1995;104 (2):334–345.
[24] Mannisto PT, Kaakkola S. Catechol-O-methyltransferase (*COMT*): biochemistry, molecular biology, pharmacology, and clinical efficacy of the new selective *COMT* inhibitors. Pharmacol Rev. 1999;51(4):593–628.
[25] Weinshilboum RM, Raymond FA. Inheritance of low erythrocyte catechol-o-methyltransferase activity in man. Am J Hum Genet. 1977;29(2):125–135.
[26] Lachman HM, Papolos DF, Saito T, et al. Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. Pharmacogenetics. 1996;6(3):243–250.
[27] Du Paul GJ, Power TJ, Anastopoulos AD. ADHD rating scale-IV:checklists, norms, and clinical interpretations. New York: Guilford Press; 1998.
[28] Guy W. ECDU assessment manual for psychopharmacology, revised. Bethesda (MD): US Department of Health, Education and Welfare; 1976.
[29] Kaufman J, Birmaher B, Brent D, et al. Schedule for affective disorders and schizophrenia for school-Age children-present and lifetime version (K-SADSPL): initial reliability and validity data. J Am Acad Child Adolesc Psychiatry. 1997;36(7):980–988.
[30] Kemner JE, Starr HL, Ciccone PE, et al. Outcomes of OROS methylphenidate compared with atomoxetine in children with ADHD: a multicenter, randomized prospective study. Adv Ther. 2005;22(5):498–512.
[31] Gokler B, Unal F, Pehlivanturk B, et al. Reliability and validity of schedule for affective disorders and schizophrenia for school age children present and lifetime version – Turkish version (K-SADSPL-T). Turk J Child Adolesc Ment Health. 2004;11(3):109–116. (Turkish).
[32] Wechsler D. WISC-R Manual for the Wechsler intelligence scale for children – revised. New York: Published by Psychological Corporation; 1974.
[33] Savasir I, Sahin N. Manual for the Wechsler intelligence scale for children – revised (WISC-R). Ankara, (Turkish): Published by Turkish Psychology Association Publication; 1995.
[34] Kotler M, Manor II, Sever Y, et al. Failure to replicate an excess of the long dopamine D4 exon III repeat polymorphism in ADHD in a family-based study. Am J Med Genet (Neuropsychiatric Genetics). 2000;96:278–281.
[35] Eisenberg J, Mei-Tal GI, Steinberg A, et al. Haplotype relative risk study of Catechol-O-methyltransferase (*COMT*) and attention deficit hyperactivity disorder (ADHD): association of the high-enzyme activity Val allele With ADHD impulsive-hyperactive phenotype. Am J Med Genet (Neuropsychiatric Genetics). 1999;88:497–502.
[36] Hamarman S, Fossella J, Ulger C, et al. Dopamine receptor 4 (*DRD4*) 7-repeat allele predicts methylphenidate dose response in children with attention deficit hyperactivity disorder: a pharmacogenetic study. J Child Adolesc Psychopharmacol. 2004;14 (4):564–574.
[37] Gorlick MA, Worthy DA, Knopik VS, et al. DRD4 long allele carriers show heightened attention to high-priority items relative to low-priority items. J Cogn Neurosci. 2015 Mar;27(3):509–521. doi:10.1162/jocn_a_00724.
[38] Ji HS, Paik KC, Park WS, et al. No association between the response to methylphenidate and *DRD4* gene polymorphism in Korean attention deficit hyperactivity disorder: a case control study. Clin Psychopharmacol Neurosci. 2013;11(1):13–17.
[39] Winsberg BG, Comings DE. Association of the dopamine transporter gene (DAT1) with poor methylphenidate response. J Am Acad Child Adolesc Psychiatry. 1999;38:1474–1477.
[40] Lacerda-Pinheiro SF, Pinheiro Junior RF, Pereira de Lima MA, et al. Are there depression and anxiety genetic markers and mutations? A systematic review. J Affect Disord. 2014 Oct;168:387–398. doi:10.1016/j.jad.2014.07.016.
[41] Holmes J, Payton A, Barrett J, et al. Association of *DRD4* in children with ADHD and comorbid conduct problems. Am J Med Genet Part A. 2002;114(2):150–153.
[42] Eisenberg J, Zohar A, Mei-Tal G, et al. A haplotype relative risk study of the dopamine D4 receptor (*DRD4*) exon III repeat polymorphism and attention deficit hyperactivity disorder (ADHD). Am J Med Genet Part A. 2000;96(3):258–261.
[43] Hawi Z, McCarron M, Kirley A, et al. No association of the dopamine *DRD4* receptor (*DRD4*) gene polymorphism with attention deficit hyperactivity disorder (ADHD) in the Irish population. Am J Med Genet Part A. 2000;96(3):268–272.
[44] Hong SB, Zalesky A, Park S, et al. COMT genotype affects brain white matter pathways in attention-deficit/hyperactivity disorder. Hum Brain Mapp. 2015 Jan;36(1):367–377. doi:10.1002/hbm.22634.
[45] Kabukcu Basay B, Buber A, Basay O, et al. White matter alterations related to attention-deficit hyperactivity disorder and COMT val(158)met polymorphism: children with valine homozygote attention-deficit hyperactivity disorder have altered white matter connectivity in the right cingulum (cingulate gyrus). Neuropsychiatr Dis Treat. 2016 Apr 21;12:969–981. doi:10.2147/NDT.S104450.
[46] Yatsuga C, Toyohisa D, Fujisawa TX, et al. No association between catechol-O-methyltransferase (*COMT*) genotype and attention deficit hyperactivity disorder (ADHD) in Japanese children. Brain and Development. 2014;36:620–625.
[47] Tahir E, Yazgan Y, Cirakoglu B, et al. Association and linkage of *DRD4* and DRD5 with attention deficit hyperactivity disorder (ADHD) in a sample of Turkish children. Mol Psychiatry. 2000;5(4):396–404.
[48] McGough J, McCracken J, Loo SK, et al. A candidate gene analysis of methylphenidate response in attention-deficit/hyperactivity disorder. J Am Acad Child Adolesc Psychiatry. 2009;48:1155–1164.

[49] Caspi A, Langley K, Milne B, et al. A replicated molecular genetic basis for subtyping antisocial behavior in children with attention-deficit/hyperactivity disorder. Arch Gen Psychiatry. 2008;65:203–210.

[50] Lee YH, Song GG. BDNF 196 G/A and COMT Val158Met polymorphisms and susceptibility to ADHD: a meta-analysis. J Atten Disord. 2015 Feb 17: pii. doi:1087054715570389.